Crop Improvement

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CONTENTS

About the PBEA Series

Background

The Plant Breeding E-Learning in Africa (PBEA) e-modules were originally developed as part of the Bill & Melinda Gates Foundation Contract No. 24576.

Building on Iowa State University's expertise with online plant breeding education, the PBEA e-modules were developed for use in curricula to train African students in the management of crop breeding programs for public, local, and international organizations. Collaborating with faculty at Makerere University in Uganda, University of KwaZulu-Natal in South Africa, and Kwame Nkrumah University of Science and Technology in Ghana, our team created several e-modules that hone essential capabilities with real-world challenges of cultivar development in Africa using Applied Learning Activities. Our collaboration embraces shared goals, sharing knowledge and building consensus. The pedagogical emphasis on application produces a coursework-intensive MSc program for Africa.

PBEA Project Director: Walter Suza

Original Module Coordinator: Asheesh Singh, Jessica Barb, Arti Singh

Collaborating Faculty and Experts in Africa: Richard Akromah, Stephen Amoah, Maxwell Asante, Ben Banful, John Derera, Richard Edema, Paul Gibson, Sadik Kassim, Rufaro Madakadze, Settumba Mukasa, Margaret Nabasirye, Daniel Nyadanu, Thomas Odong, Patrick Ongom, Joseph Sarkodie-Addo, Paul Shanahan, Husein Shimelis, Julia Sibiya, Pangirayi Tongoona, Phinehas Tukamuhabwa.

The authors of this textbook series adapted and built upon the PBEA modules to develop a series of textbooks covering individual topic areas. It is our hope that this project will facilitate wider dissemination and reuse of the PBEA modules' content.

Explore the Series

- Crop Genetics

- Quantitative Methods for Plant Breeding
- Molecular Plant Breeding
- Quantitative Genetics for Plant Breeding
- Crop Improvement
- Cultivar Development

Chapter 1: Basic Principles of Plant Breeding

Arti Singh; Jessica Barb; Asheesh Singh; and Anthony A. Mahama

Africa produces a diversity of crops including cereal, pulse, oilseed, root, and tuber species (Table 1), but contributes less than a quarter of the world production of root and tuber crops (Ngopya, 2003). In East Africa, bananas (especially cooking bananas) are an extremely important crop. In Uganda, for example, bananas serve as the largest only source of calories. Even though crop production is mostly for subsistence needs, there is tremendous business potential in local and international markets.

Table 1 Some major food crops of Africa

Cereal	Pulse	Oilseed	Root and Tuber
Maize	Dry beans	Seed cotton	Cassava
Sorghum	Groundnut	Sesame	Yams
Millet	Cowpeas	Palm	Sweet Potatoes
Rice	Soybean	n/a	Potatoes
Wheat	Cocoa beans	n/a	n/a
Barley	n/a	n/a	n/a

Learning Objectives

- Provide an overview of major crops and production challenges in Africa
- Review or introduce basic breeding principles
- Understand the concepts of setting plant breeding objectives in a program
- Review plant reproductive systems

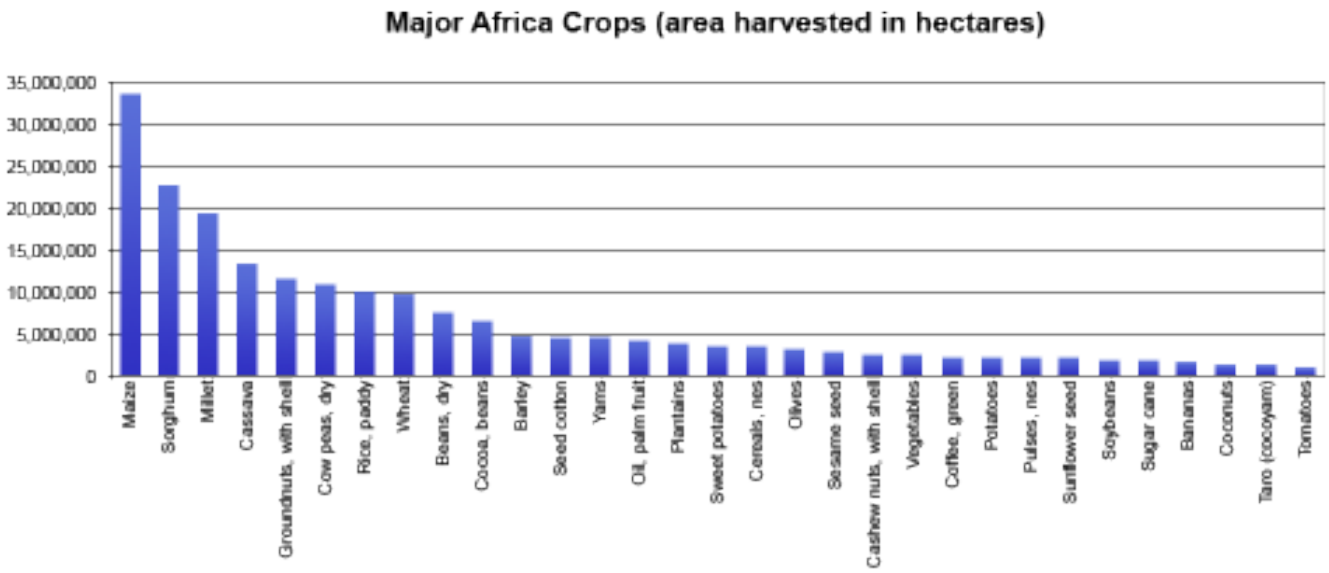

Fig. 1 Harvested area in hectares of major foods crops in Africa. Click the image to see a larger version. Data from FAOSTAT, 2014.

Production Challenges for African Crops

Production of African crops is dependent on yield potential and on protection of yield (that is, realization of yield potential). Numerous factors which reduce production include:

1. Biotic constraints to production (i.e., living biological organisms such as disease causing organisms and insects)
2. Abiotic constraints to production (i.e., non-living factors such as heat, drought, poor soil fertility)
3. Lack of incentive to increase production due to poor marketing opportunities, the lack of infrastructure (such as roads, storage facilities, etc.), and policies that fail to encourage increased production.
4. Lack of capital to invest in increasing production

Breeding efforts only address the first two constraints of biotic factors and abiotic factors. The ability to store a crop after harvest is also affected by biotic and abiotic factors. In order to improve food security and provide income to farmers, continuous efforts by plant breeders are needed to increase production per unit area of land while maintaining crop quality.

Systems of Reproduction in Crops

Asexual Reproduction

Plant reproductive systems (or mating systems) fall into three main categories: asexual, autogamous (self-fertilizing), and allogamous (cross-fertilizing). These topics are covered in greater detail in **Crop Genetics** on Reproduction in Crop Plants.

Asexual reproduction generates individuals that are genetically identical to the mother parent plant and are referred to as clones. The two main forms of asexual reproduction/propagation are *vegetative* and *apomictic*. Vegetative propagation is the creation of clones from stem cuttings, suckers (similar to tillers), tubers, runners (stolons), rhizomes, bulbs, scions, and other plant parts. Cassava, sweet potato, and sugarcane are propagated via stem cuttings. Bananas are typically propagated by suckers, while potatoes are propagated by tubers. Elephant grass (Napier grass, *Pennisetum purpureum*) is propagated by rhizomes, sets (suckers), and stem cuttings. Apomictic reproduction is the asexual propagation of a plant via clonal seeds formed by one of several means that either bypass meiosis or result in a failure of meiosis. Examples of apomictic crops include *Citrus* and many perennial forage species.

Sexual Reproduction (Self- and Cross-Fertilization)

Sexual reproduction involves the union of a male sperm with a female egg cell or ovary. This process is called fertilization. There are two types of pollination: self-pollination and cross-pollination. When the pollen of a plant pollinates a flower on the same plant the process is called self-pollination (Fig. 2A). Pollination is the transfer of male sperm carried in pollen to the female part of a flower called the stigma (Fig. 2B). When the pollen of a plant pollinates a flower on another plant of the same species the process is called cross-pollination. In nature, cross-pollination requires wind, water, insects, birds, or other animals to transfer the pollen. Most cultivars (i.e., cultivated varieties) are created by a process that involves at least one generation of cross-pollination by plant breeders; including both self- and cross-pollinated species. (See Reproduction in *Crop Genetics*).

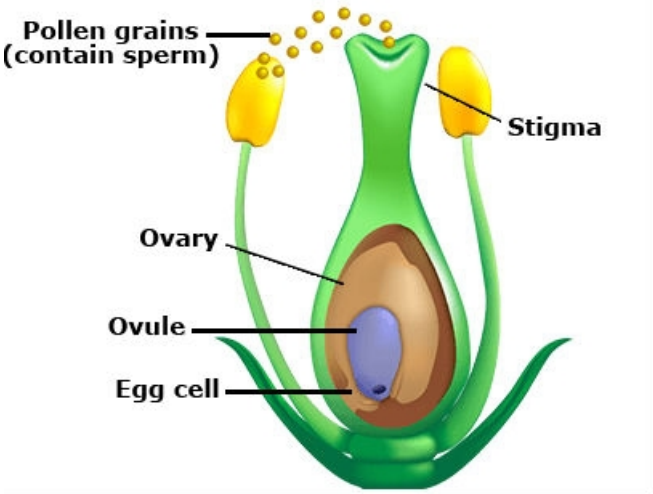

Fig. 2A A perfect flower of self-fertilizing plant showing reproductive parts (anthers, stigma, ovary, ovule and egg cell and released pollen).

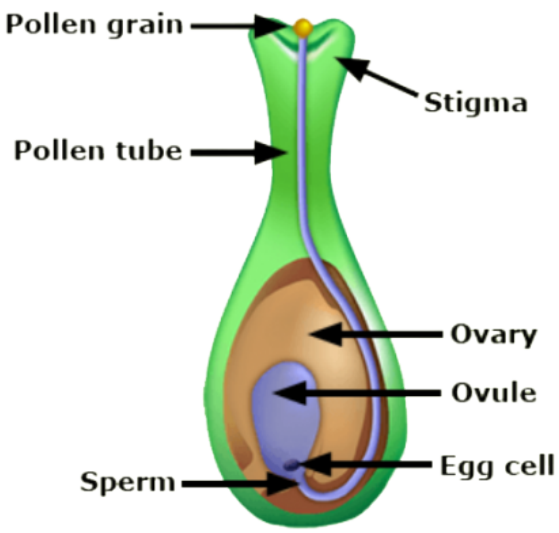

Fig. 2B The pollen tube grows down style and delivers the male gamete to the ovule for union with the egg cell.

Both the sperm and the egg are haploid, meaning they contain a single set of chromosomes from the male or female parent, respectively. Fertilization unites the single set of chromosomes in the sperm nucleus with the single set of chromosomes in the egg nucleus to produce a complete pair of chromosomes (diploid) in the zygote. Several crop species, for example, banana, sweet potato, potato, and many grasses have three or more sets of chromosomes and therefore they are, in general, called polyploids (e.g., triploids, tetraploids, pentaploids, hexaploids, octaploids).

Flowering plants have a unique process called **double-fertilization** in which the embryo and the endosperm are fertilized separately. Each pollen grain contains two pollen nuclei; one pollen nucleus fuses with the egg cell to form a diploid zygote and the second pollen nucleus fuses with the two polar nuclei in the ovule, eventually developing into a triploid endosperm. The zygote begins to divide by mitosis forming a multicellular embryo within the ovule. The endosperm provides the energy source that is used by the embryo prior to formation of true leaves that begin photosynthesis. Following fertilization the ovule (with embryo and endosperm) develops into a seed.

Genetic Information Transfer

Genetic information (i.e., DNA) from both the male and female parents is present in a seed produced by fertilization. It is this union of sperm and egg cell that results in the creation of genetic variation (if the male and female gametes possess different genetic information).

Offspring that result from the union of gametes from male and female plants with dissimilar genotypes are known as hybrids. Plant breeders select genotypes (i.e., male and female parents) that complement each other to combine the positive (or desirable) traits from each parent in the hybrid offspring.

Genetic information is transferred from generation to generation through seed, which typically consists of an embryo, an endosperm, and a seed coat. The endosperm includes the beginnings of a radicle, a hypocotyl, and one or two cotyledons (seed leaves), which support the formation of roots, stems, and true leaves, respectively. Monocot plant species have a single cotyledon (i.e., mono = one) and dicot plant species have two cotyledons (di = two). The seed coat serves as a protective coat around the seed.

Plant Reproductive Systems

Autogamous Mating System

Crops are capable of both self- and cross-pollination. These crops are classified as either autogamous (self-pollinated) or allogamous (cross-pollinated) depending on the relative frequency of self- or cross-pollination that is observed in the species.

Self-fertilization occurs if male and female gametes derived from the same plant unite. Self-fertilization also refers to the union of gametes from the same genotype. One trait, i.e., plant characteristic, that virtually ensures self-pollination is cleistogamy, where pollen shed occurs before the flower opens (i.e., anthesis). Cleistogamy promotes self-pollination and severely limits cross-pollination. Cleistogamy is observed in some legumes (e.g., groundnut, peas, some beans, soybean). In some cereals (e.g., rice, wheat, and barley) the majority of self-pollination occurs before flowers open, but some cross-pollination can occur after the flowers open, even if only partially. This allows for some cross-pollination compared to relatively little or no cross-pollination in cleistogamous species. Most self-fertilizing species undergo a small amount of outcrossing: for example in soybean natural outcrossing of 0.03% to 2% or higher has been observed in some conditions (Caviness, 1966; Ray et al., 2003). Thus, it is critical to understand the mating system of the crops you are working with and how it is affected by different environmental conditions.

Examples of important crops with an autogamous mating system include: sorghum, millet, rice, wheat, barley, groundnuts, cocoa, and major pulse crops like cowpeas, and dry beans.

Allogamous Mating System

Fertilization in cross-pollinated plants occurs via the union of male and female gametes from different plants, which are often different genotypes.

A crop is classified as allogamous when it has a higher percentage of pollination and fertilization with different individuals than with itself.

There are several traits/plant characteristics that promote cross-pollination:

1. **Male sterility:** Male sterility is caused by the formation of non-functional pollen grains, which prevents self-pollination and promotes cross-pollination. Two types of male sterility are: *cytoplasmic male sterility* (CMS), which is caused by mitochondrial genes interacting with nuclear genes; and *genic male sterility* (GMS), which is caused by nuclear genes alone. These phenomena can be exploited for hybrid seed production.
2. **Self-incompatibility:** Self-incompatibility refers to the inability of viable pollen to fertilize flowers of the same or similar genotype. Self pollen is rejected on the surface of the stigma or in the style while foreign pollen is unaffected and can germinate, grow, and fertilize the egg cell. Self-incompatibility prevents self-pollination and enforces cross-pollination. More details on self-incompatibility is found in the **Crop Genetics – Controlled Hybridization Module**.
3. **Imperfect flowers:** Imperfect flowers are missing either stamens or pistils (i.e., unisex flowers). Unisex flowers either occur on the same plant (i.e., monoecious) or different plants (i.e., dioecious).

Monoecious plants have separate male (i.e., staminate) and female (i.e., pistillate) flowers, although they occur on the same plant. In some crops, the male and female flowers are present in the same inflorescence such as in banana (Fig. 4). In some cases, they are on separate inflorescences, as in maize (Fig. 5).

Dioecious plants have separate staminate and pistillate flowers present on different plants. Dioecious plants are diclinous (i.e., having flowers of only one sex). Examples of dioecious crops include papaya, date palm, and spinach.

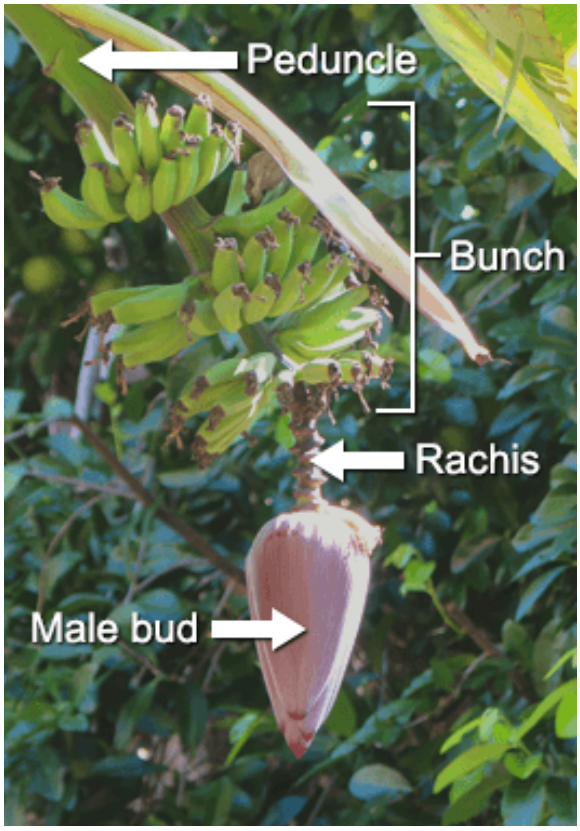

Fig. 4 Monoecious banana flower with male flower bud and female flowers developing into fruits. Photo by Iowa State University.

Fig. 5 Maize is a monoecious species with both staminate(male) and pistillate (female) flowers on the same plant. Photo by Iowa State University. (Male and female maize flowers on the same plant)

Protandry/Protogyny

4. **Protandry/Protogyny**: Cross-pollination is often observed in crop species that show protogyny (i.e., the pistils/stigmas of a plant mature and become receptive before the anthers of that plant) and protandry (i.e., stamens/anthers of a plant develop and the pollen grains mature and are shed before the pistils/stigma of that plant mature and become receptive). Protogyny is typical in cassava (although this crop is not typically seed propagated) (Fig. 6), and it has also been used to make hybrids in pearl millet (Andrews et al., 1993). Sunflower and coconut are examples of crops that display protandry. Mechanical obstructions such as a membrane around the anthers in alfalfa (also called Lucerne) flowers may also limit normal dehiscence of pollen and limit self-pollination.

Fig. 6 Cassava plant and tubers. Plant photo by Iowa State University. Tubers photo by David Monniaux, licensed under CC-SA 3.0 via Wikimedia Commons. (Cassava seedling on the left and harvested cleaned tubers on the right)

Understanding Reproductive Systems

Breeders must understand the reproductive system of the crop they are working on to make knowledgeable decisions about which breeding methods (i.e., crossing techniques, population maintenance, isolation distances, line and population development) are suitable and which type of cultivar (i.e., hybrid, pure-line, synthetic, clone) is appropriate. The modes of pollination and reproduction of some major crops are shown in Table 2. More information on the reproductive systems of crops is found in Allard (1960; pp. 40-41).

Table 2 Methods of pollination and reproduction of crop examples

Mode of pollination and reproduction	**Some examples of crop plants**
A. Autogamous Species	
1. Seed Propagated	Rice, wheat, finger, millet, barley, oats, common bean, cowpea, tomato, groundnut, soybean
2. Vegetatively Propagated	Potato
B. Allogamous Species	
1. Seed Propagated	Maize, rye, pearl millet, sunflower, oil palm
2. Vegetatively Propagated	Sugarcane, coffee, cocoa, banana, cassava, yam, rubber
C. Autogamous species with cross pollination	Sorghum, cotton, pigeon pea

Breeding Populations and Cultivar Types

Populations

Genetically speaking, a population is a group of individuals that share a common gene pool. If all individuals within the population have the same genotype the population is *homogeneous*; if the individuals have different genotypes the population is *heterogeneous*. For example, gene A has alleles A_1 and A_2 (assuming a diploid). If a population is homogenous then all individuals are the same; all are A_1A_1 (homozygous), or all are A_1A_2 (heterozygous), or all are A_2A_2 (homozygous). If a population is heterogeneous then some individuals have different genotypes; a combination of A_1A_1, A_1A_2, and/or A_2A_2.

The genotype of a population and individuals within a population varies depending on the reproductive system of a species. A natural population of a cross-pollinated species consists of a heterogeneous mixture of individuals some or most of which will be heterozygous (A_1A_2) for individual loci. A natural population of a self-pollinated species will usually also consist of a heterogeneous mixture of individuals, but each individual will be mostly homozygous ($A_1A_1$and/or A_2A_2) at individual loci. Populations of an asexually reproducing species may be homogeneous or heterogeneous and individuals will likely be heterozygous (A_1A_2) at many loci.

Cultivars

Some examples of populations are (1) a commercial maize hybrid cultivar (allogamous) which is homogeneous (single cross hybrid) and heterozygous, (2) a commercial soybean pure line cultivar (autogamous) which is homogeneous and homozygous, (3) a commercial maize synthetic cultivar which is heterogeneous and heterozygous, and (4) a commercial potato cultivar (clonal), which is homogeneous and heterozygous.

Clonal, synthetic, and hybrid cultivars are heterozygous. Pure-line cultivars are homozygous. Self-pollination is used to achieve homozygosity in an autogamous species. In allogamous species self-pollination is used to develop inbred lines that are used as parents to create hybrids

Clonal Cultivars

Clonal Cultivars: Most commercial crops are propagated through seed. However, a significant number of agriculturally important species are propagated by using plant parts other than seed which include: stem cuttings, suckers, tubers, and stolons (Fig. 7). As the term 'clone' implies, offspring are identical to the mother parent clone plant and are therefore homogenous in the absence of pollination and mutation. Clonal cultivars, although homogenous, are typically heterozygous, therefore 'hybrid vigor' is fixed and maintained, unlike a maize hybrid, which is propagated through seed, and loses hybrid vigor with each generation of selfing or sib-mating.

The steps to create/develop a clonal cultivar are:

1. develop a genetically variable base/source population;
2. evaluate and select superior clones from the population; and
3. multiply the new cultivar for commercial use.

Examples of clonal cultivars include: cassava, sweet potato, potato, cacao, and yam. The cultivars of these crops are homogeneous and heterozygous

Fig. 7 Examples of vegetative tissue used for the propagation of clonal cultivars. Photos A and B from htttp://www.freeimages.com. Photo C and D by Shui-zhang Fei, Iowa State University

Synthetic Cultivars

Synthetic cultivars: Synthetic cultivars are produced by intermating a population of purposefully selected inbred lines, clones, hybrids, strains, or other populations of cross-pollinated plants. Synthetic cultivars are highly heterozygous and heterogeneous. Inbreeding depression is severe and plants that develop from self-pollinated seed lack the vigor of those obtained by cross-pollination. In a heterogeneous population, each plant is genetically different from another.

The components (clones, inbred lines, etc.) of a synthetic cultivar are maintained in their original form so that the cultivar can be reconstituted as needed. A synthetic cultivar is different from an open-pollinated variety because the components are maintained in their original form while with an open-pollinated variety the components are not maintained. Clonally propagated plants or inbred lines with desirable characteristics (traits) are selected and then isolated and allowed to cross-pollinate, randomly or in a structured format in a polycross nursery (Fig. 8). Seed is harvested from the clones or inbred lines and planted in progeny rows for evaluation. The best clones or inbred lines are then selected both for superior plant traits and on the performance of their progeny rows, which measures their general combining ability with the rest of the population. These selected parents are then replanted and permitted to cross pollinate in isolation. Open-pollinated seed harvested from these parental clones or inbred lines (after one or more cycles of intermating) is then sold as a synthetic cultivar.

Cultivar Development Example

Fig. 8 An example of a polycross in bentgrass breeding. Selected bentgrass clones are planted and random pollination occurs. Photo by Iowa State University

An example of the development of maize synthetics is the development of the maize population HIS1 (Brewbaker, 2009).

The development of synthetic cultivars of maize and other crops differs from that described for turfgrass and forage species because of several important biological differences.

1. It is possible to inbreed maize because inbreeding depression is much less than in turfgrass and forage species.
2. There is no self-incompatibility in maize and some self-pollination can occur, even though it is a monoecious species that is wind pollinated.
3. It is an annual crop.

Pure-Line Cultivars

Pure-line cultivars: Pure-line cultivars are developed for self-pollinated species. Self-pollination leads to homozygosity and homogeneity.

Improved self-pollinated cultivars are obtained through three basic approaches.

- **Introductions:** a breeder assembles cultivated varieties currently grown in other regions (domestically or internationally) and identifies lines that exhibit desirable characteristics and are adapted to the new target area. A breeder must be aware of licensing and material transfer agreement (MTA) issues (see next module) before acquiring lines from different sources. Introductions are typically received as pure-lines, but they may contain mixtures of different genotypes or off-types and may require that the breeder rogues these plants out to obtain clean and uniform seed that can be released commercially. [**Note:** problem of mixture or off-types is common for self-pollinated, cross-pollinated or clonal crops; MTA are required for most if not all crops (self- and cross-pollinating and clonal) when the seed or explant is sent or acquired by breeders].
- **Selection:** a breeder assembles landraces, identifies the best genotypes, and releases one or more for commercial production. This approach has applicability for orphan crops but has not been widely used for major world crops.
- **Hybridization:** a breeder makes hybridizations between genotypes with desirable characteristics, evaluates, and selects superior genotypes for commercial production. This is the most common approach for developing new cultivars.

Cultivar Examples

Examples: beans, cowpea, rice, finger millet, tobacco, and wheat. Cultivars are homogeneous and homozygous.

Fig. 9 Examples of pure-line cultivars. Rice photo by Gary Cramer, USDA NRCS; Tobacco -photo from www.freeimages.com; Wheat photo by Jeff Vanuga, USDA NRCS.

Hybrid Cultivars

Hybrid cultivars: Hybrid cultivars are produced by crossing inbred lines, typically two inbred lines in the case of a two-way/single-cross hybrid. Inbred lines are chosen for their combining ability to achieve maximum expression of hybrid vigor (i.e., the F_1 trait mean is higher than the mid-parent mean and higher than the trait values for the individual inbred parents). Hybrid vigor (i.e., the visible measure of heterosis) is more important in allogamous crop species as the expression is typically lower in autogamous species. Pollen control via the use of mechanical tools, chemicals, or genetic male sterility is necessary to create hybrid seed.

Heterosis (or hybrid Vigor) is defined as the difference between the hybrid and the mean of the two parents (Falconer and Mackay, 1996). This definition is also described as midparent heterosis. High-parent heterosis is the superiority of a hybrid over the better parent (Bernardo, 2014).

Examples of hybrid cultivars include: commercial single-cross maize hybrids, commercial three-way cross maize hybrids, and sunflower hybrids. Hybrid cultivars are usually utilized for allogamous species but some hybrids are produced for some autogamous species (e.g., sorghum, tomato). Single-cross hybrid cultivars are homogeneous and heterozygous (Fig 10). Three-way hybrids are both heterogeneous and heterozygous.

Fig. 10 Single cross maize hybrid field. Photo by Iowa State University (A field of maize plants with tassels fully extend and shedding pollen).

Seed Law

Definition of a Cultivar According to UPOV

According to seed law a cultivar or cultivated variety is *distinct*, *uniform*, and *stable*.

- **Distinct:** a cultivar is distinct if it can be differentiated by one or more identifiable characteristics from all other cultivars currently available or previously developed. Distinctiveness can involve morphological, physiological, molecular, or other characteristics.
- **Uniform:** a cultivar is uniform if no variation among individuals exists for the distinguishing characteristics that make it distinct from other cultivars.
- **Stable:** a cultivar is stable if plants remain the same from generation to generation. If there are multiple generations in a cycle of propagation the plant characteristics should be the same at the end of each such cycle.

These concepts come out of 'The International Union for the Protection of New Varieties of Plants' (UPOV), which is an intergovernmental organization based in Geneva, Switzerland. UPOV was established in 1961 by the International Convention for the Protection of New Varieties of Plants (the "UPOV Convention").

Below is an excerpt from The International Union for the Protection of New Varieties of Plants (UPOV):

> "The mission of UPOV is to provide and promote an effective system of plant variety protection, with the aim of encouraging the development of new varieties of plants, for the benefit of society."

International Union for the Protection of New Varieties of Plants (UPOV) Criterion

Distinctness (Article 7)

Criterion: A variety is deemed to be distinct if it is clearly distinguishable from any other variety whose existence is a matter of common knowledge at the time of filing of the application.

Uniformity (Article 8)

Criterion: A variety is deemed to be uniform if, subject to the variation that may be expected from the particular features of its propagation, it is sufficiently uniform in its relevant characteristics. The uniformity requirement within the Convention has been established to ensure that the variety can be defined as far as is necessary for the purpose of protection. Thus, the criterion for uniformity does not seek absolute uniformity and takes into account the nature of the variety itself. Furthermore, it relates only to the relevant characteristics for the protection of the variety.

Stability (Article 9)

Criterion: A variety is deemed to be stable if its relevant characteristics remain unchanged after repeated propagation or, in the case of a particular cycle of propagation, at the end of each such cycle. As with the uniformity requirement, the criterion for stability has been established to ensure that the identity of the variety, as the subject matter of protection, is kept throughout the

period of protection. Thus, the criterion for stability relates only to the relevant characteristics of a variety."

Exercise

Read the UPOV Overview online and go through the table of contents (under FAQs) to familiarize yourself with plant variety protection and plant breeder's rights:

- What is UPOV?
- What is a plant variety?
- Why do farmers and growers need new plant varieties?
- How are new plant varieties of benefit to society?
- What is Plant Variety Protection?
- Who can protect a plant variety?
- What are the Exceptions to the Breeder's Right?
- What are the conditions for obtaining protection?
- What information is there on the impact of PVP?

Visit the main UPOV website for more details on UPOV.

Setting Breeding Objectives

Plant breeding objectives will depend on geographical adaptation, prevalent biotic and abiotic factors that influence production, uses of a cultivar, crop reproductive system (for example, pureline or hybrid), and factors that are important to farmers, and end-users. It is fundamental that genetic variation exists for the trait that you are attempting to improve and that it is transmissible. Plant breeding programs need to be adequately set up for screening breeding material for the traits that are being improved based on the objectives. Applied learning activity #1 will be used to better understand the considerations involved in setting plant breeding objectives.

References

Andrews, D.J., B. Kiula, and J.F. Rajewski. 1993. The use of protogyny to make hybrids in pearl millet. p. 122-126. In: J. Janick and J.E. Simon (eds.), New crops. Wiley, New York.

Allard, R.W. 1960. Principles of plant breeding. John Wiley and Sons, New York.

Bernardo, R. 2014. Essentials of Plant Breeding. Stemma Press, Minnesota.

Brewbaker, J.L. 2009. Registration of nine maize populations resistant to tropical diseases. Journal of Plant Registrations 3:10–13.

Caviness, C.E. 1966. Estimates of natural cross pollination in Jackson soybeans in Arkansas. Crop Sci. 34:376–378.

DePauw, R.M., R.E. Knox, F.R. Clarke, H. Wang, M.R., Fernandez, J.M. Clarke, and T.N. McCaig. 2007. Shifting undesirable correlations. Euphytica 157:409–415.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. 4th ed. Longman, Essex, England.

FAO 2014. Major African crops. Statistics Division (FAOSTAT, 2014).

Ngopya, F. 2003. Importance of root crops in Africa. In Proceedings of the Expert Consultation on Root Crop Statistics – Volume II: Invited Papers. Harare, Zimbabwe, 3-6 December 2002. Food and Agriculture Organization of the United Nations, FAO, Rome.

Ray, J.D., T.C. Kilen, C.A. Abel, and R.L. Paris. 2003. Soybean natural cross-pollination rates under field conditions. Environ. Biosafety Res. 2:133–138.

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Chapter 2: Pedigree Naming Systems and Symbols

Asheesh Singh and Anthony A. Mahama

Understanding the symbols used to describe the progeny generated following hybridization and self-pollination is critical for clear communication between/among researchers in the breeding community. The two symbols used by plant breeders to enable such communication are F and S, where F is derived from the word filial, defined as the sequence of generations after the mating of two parents. S is the symbol used to denote generations of self-pollination. Different systems exist in the use of the F and S symbols to describe breeding groups – populations, individuals, and inbred lines – making it the more crucial to avoid misleading outcomes by clearly stating the meaning of symbols, and thus underscores that advantages and disadvantages exist in the use of each system.

Learning Objectives

- Demonstrate the relationship between the "F" and "S" symbols used to designate generations of selfing or sib-mating.
- Describe how breeding lines are designated by "F#:#" or "S#:#" according to the generation that they were derived.
- Demonstrate pedigree writing.
- Demonstrate how selection history is recorded using a Breeder's Cross Identification (BCID) designation.

Using F# and S#

Symbology

A. Using "$F_{\#}$" and "$S_{\#}$" to designate the number of generations of selfing or sib-mating:

In plant breeding, the 'F' symbol is used to denote the filial (i.e., family) generation of offspring

following a cross between two or more parents. The subscript (#) represents the specific generation ($F_{\#}$). F_1 is the first generation following a cross and subsequent generations are designated F_2, F_3, F_4, etc., based on the number of generations the offspring are self-pollinated or sib-pollinated (i.e., pollinated by a sibling plant in the same progeny row). Pollination can occur naturally or artificially if it is imposed by the breeder. If both of the parents of a cross are homozygous then the F_1 offspring will be homogeneous (i.e., all plants will be uniform and genetically the same) and heterozygous at individual loci. If either or both of the parents are heterozygous then the F_1 offspring will be heterogeneous (non-uniform). In a complex cross involving more than two parents, even if the parents are homozygous), the F_1 generation will be heterogeneous and heterozygous.

Single Gene Example With Two Homozygous Parents

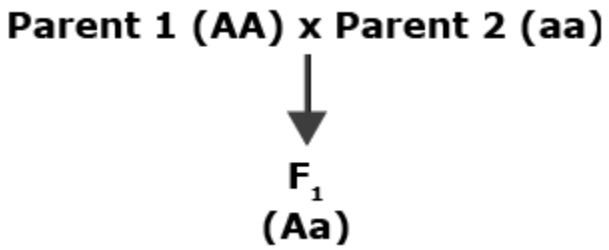

Fig. 1 Homozygous biparental cross to create heterozygous F1 progeny

In the example above (Fig. 1) , both parents were developed by the breeder after undergoing several generations of selfing so that they are homozygous at all loci. The cross between these two unrelated parents produces F_1 progeny that are all uniformly heterozygous (Aa), and F_1 progeny population (all F_1 from this cross) will be homogeneous since each F_1 will be 'Aa' type at this locus.

Self-Pollination

When an F_1 plant is self-pollinated or when two F_1 plants are crossed with each other, F_2 seed is produced. If the parents were homozygous then the F_2 generation is the first generation when the offspring are heterogeneous (i.e., segregating for different parental alleles). The F_2 generation is typically the generation when selection for simple traits begins. Self-pollination

of F_2 plants produces F_3 plants, self-pollination of F_3 plants produces F_4 plants, and so on as shown in Fig. 2.

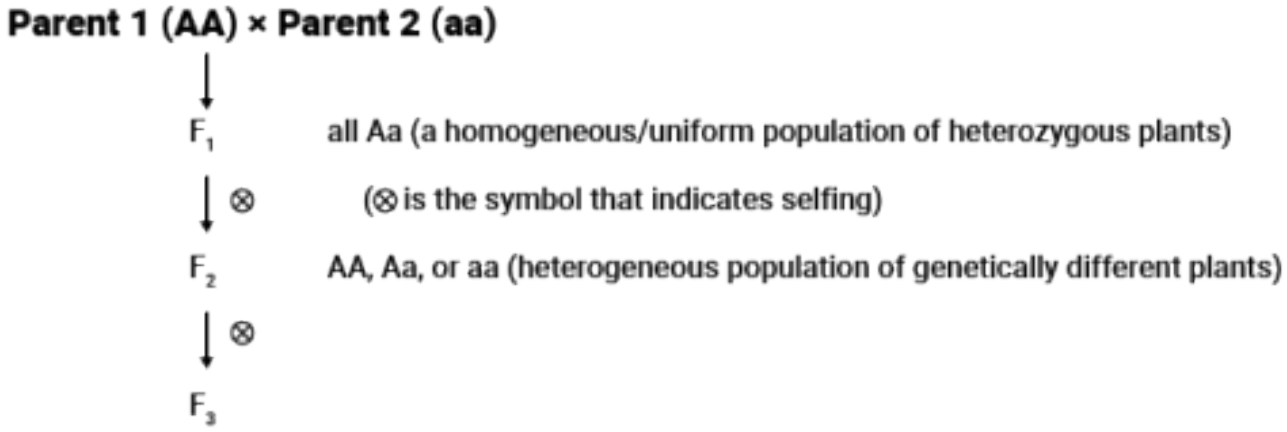

Fig. 2 Production of F_3 progeny from a biparental cross by self pollination of the F_1 and F_2 progeny.

Cross-Pollinated Species

In cross-pollinated species, '$S_{\#}$' is used instead of '$F_{\#}$'. The symbol S_0 can be used to describe the progeny from a single cross between two homozygous parents as either:

1. Similar to F_1 (in self-pollinated) which indicates that the plant was not derived from self-pollination or
2. Similar to F_2 (in self-pollinated) which indicates that the population is formed by random mating and is therefore heterogeneous and heterozygous

Therefore, it is important that the breeder clearly describes what she/he is referring to in a particular situation and then be consistent in usage.

Using $F_{x:y}$ and $S_{x:y}$

Breeding Lines

B. Using "$F_{x:y}$" or "$S_{x:y}$" to describe breeding lines according to the generation they were derived:

Breeding lines (or genotypes) are derived from individual plants at various generations. An $F_{2:4}$ line refers to an F_4 line that was derived from a single F_2 plant. The F_2 plant was selfed to produce F_3 seeds, which were then grown in a single F_3 progeny row, self-pollinated, and then harvested as a F_4 bulk of many or all of the F_3 plants in this row. Based on this scheme each individual F_2 plant gives rise to a genetically distinct $F_{2:4}$ line. These lines are also described as "F_2-derived lines in the F_4 generation", or simply "F_2-derived F_4 lines".

Summary

An $F_{3:4}$ line refers to an F_4 line (or progeny row) created from a single F_3 plant growing in an F_3 progeny row that was produced from the seed of a self pollinated single F_2 plant. An individual plant was selected from an F_3 row to produce F_4 seed and this seed when grown represents an $F_{3:4}$ line. These are also described as "F_3-derived lines in the F_4 generation", or simply "F_3-derived F_4 lines". The difference from $F_{2:4}$ lines is because the first subscript designates the generation of the last individual-plant selection (Fig. 3).

To summarize:

$F_{x:y}$ or $S_{x:y}$, describes 'x' as the generation where single plant was harvested separately to give rise to the derived line, and y represents the current generation of inbreeding of the plants within this derived line.

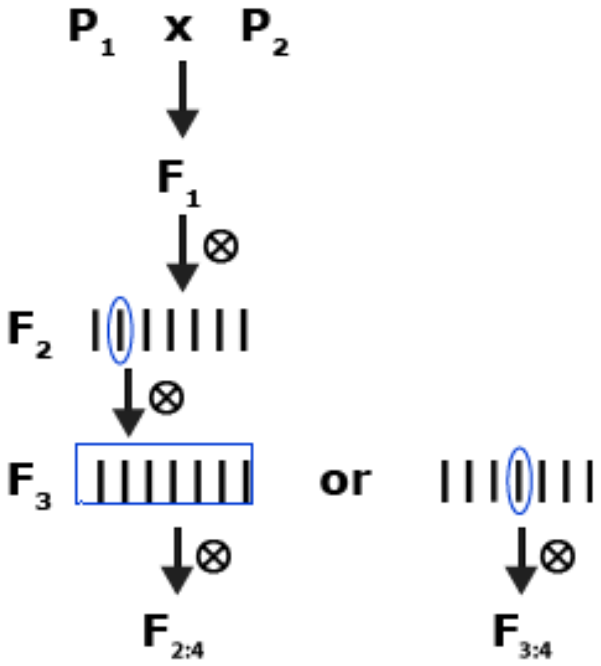

Fig. 3 Schematic of development of F_2-derived F_4 line ($F_{2:4}$) or F_3-derived F_4 line ($F_{3:4}$); lines are kept separate and selfed to inbreed to desired generation.

Writing a Standard Pedigree

Symbology

C. Example of writing a standard pedigree:

Each organization follows a different standardized system for recording pedigrees. In this section, we will describe the system adapted from Purdy et al (1968) modified and used by wheat breeders at CIMMYT (CGIAR institute). Depending on the crop you work on and where you are employed you may use a modified system.

The female parent is designated by listing it first (starting from the left) followed by the male parent (on the right). For example, A is the female parent and B is the male parent in an (A x B) cross. An (A x B) cross can also be written as A/B.

If an F_1 (A/B) plant is pollinated with parent C, and the F_1 is used as the female and C as the

male, the resulting three-way cross would be designated as A/B//C. Subsequent crosses with parental materials D, E, F, and G used sequentially (all as males) are indicated using a number to record the cross order in the following way: A/B//C/3/D/4/E/5/F/6/G.

Example

If the example above is changed to use D & F as female parents, with E and G remaining as males, the cross would be recorded as follows:

- Step 1: A/B is the first cross,
- Step 2: A/B//C is the second cross, where A/B is the female.
- Step 3: **D/3**/A/B//C is the third cross, with D as female, and A/B//C as male.
- Step 4: D/3/A/B//C/**4**/**E**, with E as male, and the 4-parent cross as the female. NOTE: bold and underline text is for information and instructional purpose only. In writing a pedigree, you will not have to bold text. One will simply write the pedigree as D/3/A/B//C/4/E

The inclusion of "5/F" as the female and 6/G as a male completes the pattern.

Backcross Pedigree

In multiple backcrosses, the sequence of these letters from left to right corresponds to the sequence in which the backcrosses are made. Backcross pedigrees include an asterisk (*) and a number indicating the dosage of the recurrent parent. The asterisk and the number are placed next to the crossing symbol (/) that divides the recurrent and donor parents. The following are examples of pedigree formats involving backcrosses:

- A is the recurrent parent: A*2/B of the initial cross and has been used as a parent two times. Therefore, A*2/B indicates one backcross or a BC_1 cross.
- B is the recurrent parent: A/3*B, and has been used as a parent three times. Therefore, A/3*B indicates a BC_2 cross.

A*2/B is therefore A//A/B and indicates that A was used as a female in both F_1 and BC_1.

A/3*B could be B/3/A/B//B and indicates that A was used as a female, F_1 was then used as female, and BC_1 was used as male.

A/3*B could be A/B//B/3/B and indicates that A was used as a female, F_1 was then used as female, and BC_1 was used as a female.

The F#: derived symbols as previously described for regular crosses will follow the $BC_{\#}$ designation. For example, $BC_1F_{2:4}$ or $BC_2F_{2:4}$.

Identity Number

Assigning an Identity Number to Each Cross or Backcross

Every cross should receive a unique ID number that will allow everyone in the breeding group to recognize the year the cross was made (e.g., 2014), a cross number (e.g., 1001), and perhaps the target purpose of the cross (e.g., HO for high-oil, or abbreviation for another specific trait or market segment).

Using BCID

Recording Selection History

D. Recording selection history using a Breeder's Cross IDentification (BCID) designation:

Every F_1 plant, segregating line, or advanced line in a program is assigned a Breeders' Cross IDentification (BCID) and a selection history. This selection history records the process of selection, which describes where and how the initial cross was made and where and how subsequent selection steps occurred for each generation of selection.

Example

An example of this system is provided below (using CIMMYT's wheat breeding program).

Each BCID begins with a letter designation for the origin of the cross (e.g., CM = crusa Mexicana; Spanish for 'Mexican cross'). This is followed by an indication of the kind of cross (e.g., BW = bread wheat x winter wheat, SS = spring x spring wheat; SW = spring x winter wheat), an abbreviation of the year when the cross was made (e.g., 00 = 2000), an abbreviation of the

location where the cross was made (e.g., Y = Yaqui Valley), and finally a sequential number representing the order in which that cross was made within the crossing cycle (e.g., 0124). The table below shows the letter codes used to indicate the locations in Mexico where crosses were made and the different environmental conditions where CIMMYT breeders carry out selection in wheat. Note that more than abbreviations than shown in table are used to describe locations or nurseries.

Table 1 Location and selection environment code in CIMMYT wheat breeding

Code	Description of Location and/or Environmental Condition
B	El Batan
M	Toluca ("M" stands for the State of Mexico)
Y	Cd. Obregon full irrigation (Cd. = Cuidad = city; "Y" stands for Yaqui valley region in Mexico)
KBY	Cd. Obregon Karnal bunt
HY	Cd. Obregon Heat (heat, late planting)
SY	Cd. Obregon Semi-arid (reduced irrigation)
PR	Poza Rica
PZ	Patzcuaro
SJ	Sierra de Jalisco (El Tigre)
AL	Selection for tolerance to low pH and aluminum toxicity in laboratory test (El Batan)
YDB	Selection for BYDV tolerance in El Batan (BYDV = barley yellow dwarf virus)

Location Codes

Location codes for other countries were determined by the cooperators/breeders in those countries to ensure that everyone was aware and compliant. Hypothetical examples of BCIDs and selection histories are presented below.

Table 2 Examples of BCIDs and selection histories for a simple cross using the pedigree method, the modified pedigree/bulk method, and the selected bulk method.

Example #	Type of cross	Breeder's Cross ID (BCID)	Selection history (by generation)						
n/a	n/a	n/a	F_1	F_2	F_3	F_4	F_5	F_6	F_7
1	A/B	CMBW08Y0199	n/a	35Y	15M	7Y	5M	12Y	0M
2	A/B	CMBW08Y0124	n/a	81Y	010M	010Y	010M	15Y	0M
3	A/B	CMSS07Y051	n/a	030Y	030M	030Y	53Y	0M	n/a

- ***Example 1:*** BCID = CMBW08Y0199, a single cross was made in Mexico ("CM") in 2008 between a bread and winter wheat at location 'Y' and this was cross# 0199 that year. The pedigree method of selection was followed for the development of the genotype. The selection history for this example indicates that in the F_2, the 35^{th} plant was selected at location "Y", and in the F_3 generation this was the 15^{th} plant selected at location "M", etc. Finally, in the F_7 the "0M" indicates that a single plot was grown at location "M" and harvested in bulk (i.e., all plants in the plot were harvested into one bag or packet). This created the genotype CMBW08Y0199-35Y-15M-7Y-5M-12Y-0M.
- ***Example 2:*** BCID = CMBW08Y0124, a single cross was made in Mexico ("CM") in 2008 at location "Y", with 0124 designating that this cross was number 0124 in the series of crosses made at that location and year. The selection history reflects that a modified pedigree/bulk selection method was used. In the F_2 the "81Y" indicates that this genotype was the 81st individual plant among those selected at location "Y." The F_3 designation of "010M" indicates that 10 plants were selected and harvested in bulk from the F_3 progeny row grown at location "M." Seed from the bulked F_3 progeny row was planted at location "Y" in the F_4 and 10 plants were selected and harvested in bulk. Similar scheme was used in F_5. In the F_6 a single plant was selected from this genotype (15th plant) at location "Y" and constituted the seed for the next generation. In the F_7 (or more appropriately, $F_{6:7}$) all plants in the progeny row at location "M" were harvested in bulk, as shown by the designation "0M". This created the genotype CMBW08Y0124-81Y-010M-010Y-010M-15Y-0M.
- ***Example 3:*** BCID = CMSS07Y051, which describes that a single cross was made in Mexico ("CM") in 2007 at location "Y", with 051 designating that this cross was number 051 in the series of crosses made at that location and year. The cross was of type "SS", spring wheat x spring wheat. The selection history indicates four generations (F_2-F_4) of selection in which

30 plants were bulked from the progeny row (or plot) for each season at either the "M" or "Y" locations. In the F_5 generation the genotype selected was the 53rd plant from the bulk plot at the "Y" location. In the F_6 a complete plot bulk was harvested at location "M". This lead to the creation of the genotype CMSS07Y051-030Y-030M-030Y-53Y-0M.

Additional Notes

After the BCID, the selection history is presented in which the numbers identify the number of individual plant(s) selected and the letter indicates the location where selection took place and/or under what specific conditions selection was conducted.

The zero-letter combinations (e.g., 0Y, 0M, etc.) are reserved for populations harvested in bulk during that generation (i.e., the entire plot was cut and threshed as one unit). A zero followed by a number (e.g., 05..., 010...) and then by a letter indicates that the modified pedigree/bulk selection method was used in which a certain number (e.g., 5 or 10) of selected heads are bulk (0) harvested. The location where the selection was made and, in some cases, the special type of selection performed, is indicated by a letter code.

References

Purdy, L.H., W.Q. Loegering, C.F. Konzak, C.J. Peterson, and R.E. Allan. 1968. A Proposed Standard Method for Illustrating Pedigrees of Small Grain Varieties. Crop Science 8:405-406.

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Chapter 3: Genetic Variation and Germplasm Usage

Asheesh Singh; Arti Singh; Jessica Barb; and Anthony A. Mahama

The presence of genetic variation is a key prerequisite for genetic improvement in plant breeding and plays a pivotal role in germplasm usage in breeding programs. Therefore plant breeders and students in plant breeding can benefit immensely from an understanding of sources of genetic variation present, and ways of creating genetic variability where it is limited. The source of genetic material in a breeding program may come from one's own breeding program, a colleague's breeding program with the same or different organizations, or gene banks, among others. Good stewardship needs to be followed by plant breeders to utilize the genetic material.

Learning Objectives

- Know processes that create genetic variation
- Gain an understanding of the concepts of types and origin of genetic variation
- Become familiar with plant genetic resources and working with variability in hybridizations
- Know the legal issues with germplasm usage and exchange

Relationship of Plant Breeding to Natural Selection

Creating Genetic Variability

Natural selection requires three main processes to function:

A. **Processes that create genetic variability: gene mutation, recombination, chromosomal segregation, gene flow are some of the ways to create genetic variability.** This provides the potential to change the composition of individuals in the population. Mutations are considered random as they are not created to address a "need" of the organism. Therefore

mutations can be neutral, harmful, or beneficial. Somatic mutations (occurring in the non-reproductive cell) are not useful to genetic variability. Gene flow can be an important source of genetic variation if genes are carried to a population where those genes did not previously exist (Fig 1).

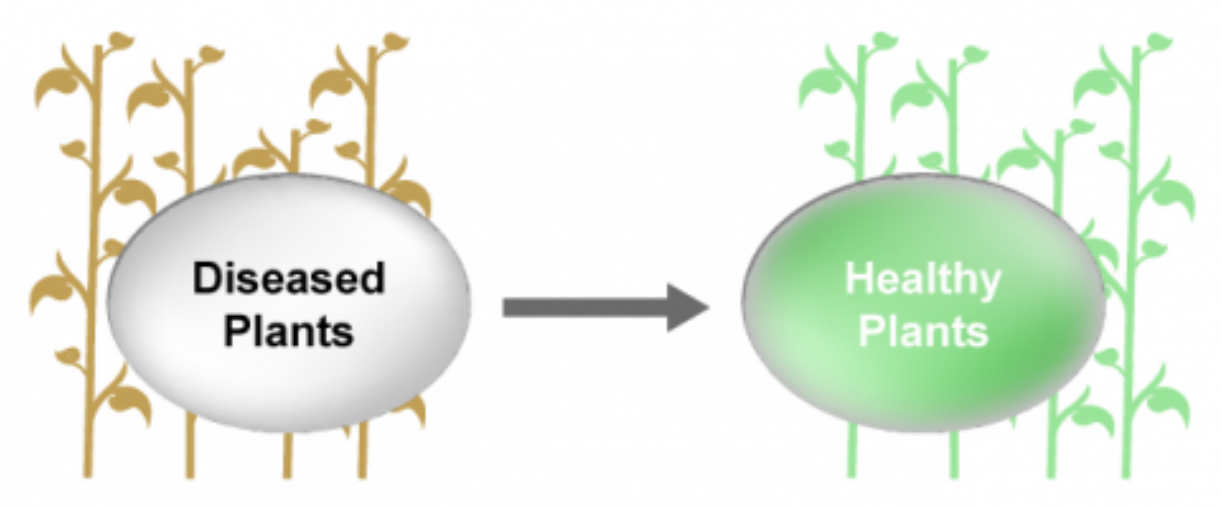

Fig. 1 Genetic flow from diseased plants to healthy plants.

B. **Processes that rearrange genetic variability: natural selection or random genetic drift.** These processes will lead to a change in population, due to the favoring of reproduction of certain individuals over others, thus causing a change in the gene pool (genes possessed by the mating population). *Natural selection* operates through reproductive fitness (the ability to produce offspring that contribute to the gene pool of the next generation).

As the term implies, *random genetic drift* is random and uncontrollable. For example, in a population, some individuals may leave more offspring by chance than other individuals. Let us consider a hypothetical situation in a forest where there are 50% each of two tree species. Species A is predominant in the western part of the forest and species B is predominant in the eastern part. If fire destroyed 80% of trees in the western part of the forest, species A will be significantly reduced in number, and so species B will leave more offspring, leading to a genetic drift. It is important to note that preponderance of offspring of species B is due to the chance destruction of species A, and not necessarily because species B is healthier or more productive. Unlike natural selection, genetic drift is neutral to adaptation. In the forest fire example above, if species B had wood properties that made them fire-resistant (remember this is a hypothetical example) then a fire will destroy species A and reduce the number of species A offspring in

the next generation compared to species B. Because this trait of fire protection is genetic, after repeated fires, species B will have more off-springs and will evolve due to natural selection. This example can be extended to a crop plant and disease.

C. **Processes that maintain the product (minimize disturbance).**

These processes or mechanisms serve to protect the integrity of a population's gene pool. This functions to maintain the genetic identity of the product, for example, due to reproductive isolating mechanisms. Examples of reproductive isolating mechanisms include sterility or failure of mating due to asynchrony (where males flower and shed pollen before the stigma of the females are receptive or vice versa).

Deliberate Choice

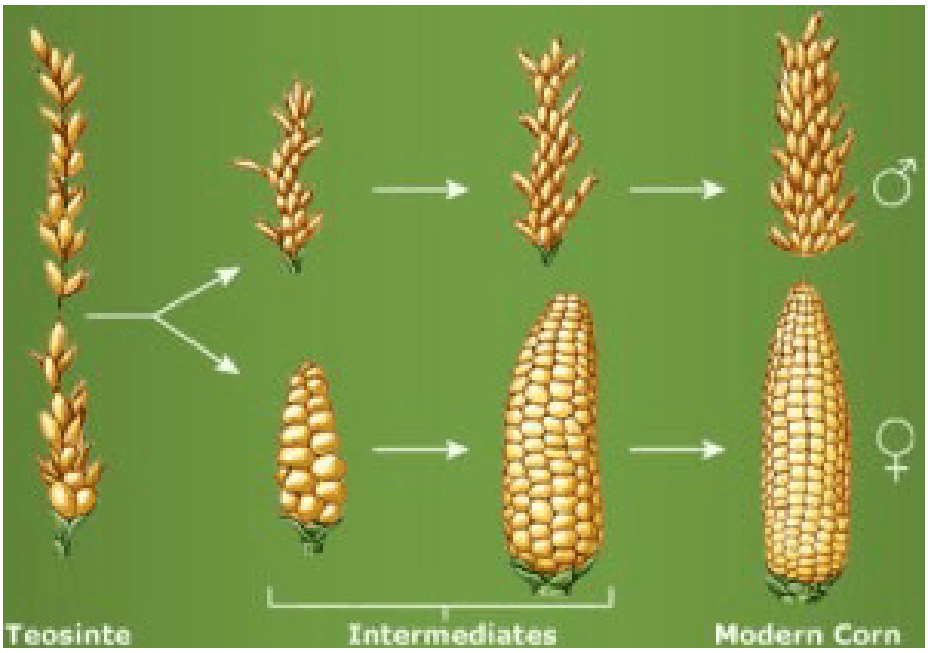

Fig. 2 Evolution of teosinte to modern maize.

Artificial selection describes the deliberate choice of individuals for breeding in each generation and the advancement of select individuals. Directional selection is a form of artificial selection in which phenotypically superior plants are chosen for breeding. Artificial selection has been practiced for thousands of years by humans to make improvements in plant species. For example, artificial selection led to the rise of modern maize from its progenitor, teosinte

(Fig. 2). Numerous studies show that teosinte (*Zea mays* ssp. *parviglumis*, a grass species) is a progenitor of maize (*Zea mays* L. ssp. mays). Very small differences in morphology (under genetic control) differentiate maize and teosinte. For example, teosinte has a cupulate fruit case protecting each kernel and the rachis segment (internode) and glume (modified bract) cover the kernel (Fig. 3). The cupule and glume are present in maize but they are significantly reduced in size and therefore do not surround the kernel. In maize, these organs form the cob. Ears of teosinte disarticulate at maturity such that the individual fruit cases become the units of seed dispersal. Ears of maize remain intact at maturity, which allows for easy harvest by humans. In teosinte, each cupulate fruit case holds a single-spikelet (kernel-bearing structure). In teosinte, the cupulate fruit cases are borne in two ranks on opposite sides of the longitudinal axis of the ear. In maize, the cupules are borne in four (or more) ranks.

Recreating Primitive Maize

Previous work by George Beadle has shown that primitive maize can be recreated by crossing teosinte and modern maize. While some of the changes between teosinte and maize may have happened naturally, the rest resulted from domestication and artificial selection as these differences made maize suitable for production for humans. Today we continue to improve the yield of maize using directional selection.

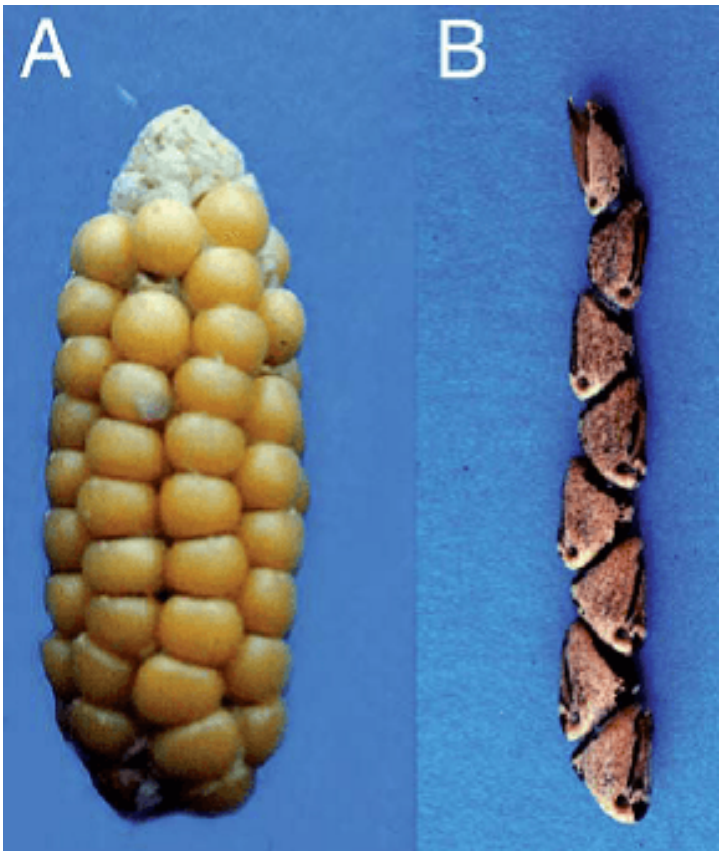

Fig. 3 (A) A 'Reconstructed' ear of primitive maize (left). This small-eared form of maize was bred by George Beadle by crossing teosinte with Argentine popcorn and then selecting the smallest segregants. (B) Ear of pure teosinte (*Zea mays* ssp. *parviglumis*) composed of eight cupulate fruit cases.

Making Progress

Artificial selection in genetically heterogeneous populations always leads to a successful outcome (i.e., mean change in population phenotype over generations in the direction of selection). This is true unless a biologically constraining limit is reached. The mean of a trait can be altered in both directions (i.e., an increase or a decrease in a trait's arithmetic mean value) if genetic variability exists in a population.

Genetic variation is ESSENTIAL for making progress using artificial selection.

Can we change the mean phenotype of a genetically uniform population (or completely inbred genotype) over generations?

What will happen if mutations occur in the genetically uniform population? Will the mean phenotype change over generation in the same genetically uniform population (now with mutations)?

Successful Maize Experiment

In 1896, C.G. Hopkins started long-term artificial selection experiments looking at oil (Fig. 4) and protein (Fig. 5) content in maize. The open-pollinated corn cultivar Burr's White was used as the founder population. Four strains were established: Illinois High Oil (IHO), Illinois Low Oil (ILO), Illinois High Protein (IHP), and Illinois Low Protein (ILP) with high and low referring to the direction of the selection. After 48 generations, reverse selection was started in each strain to establish the Reverse High Oil (RHO), Reverse Low Oil (RLO), Reverse High Protein (RHP), and Reverse Low Protein (RLP) strains. After seven generations of selection in RHO, selection was again reversed to create the Switchback High Oil strain (SHO) to study the effect of selection.

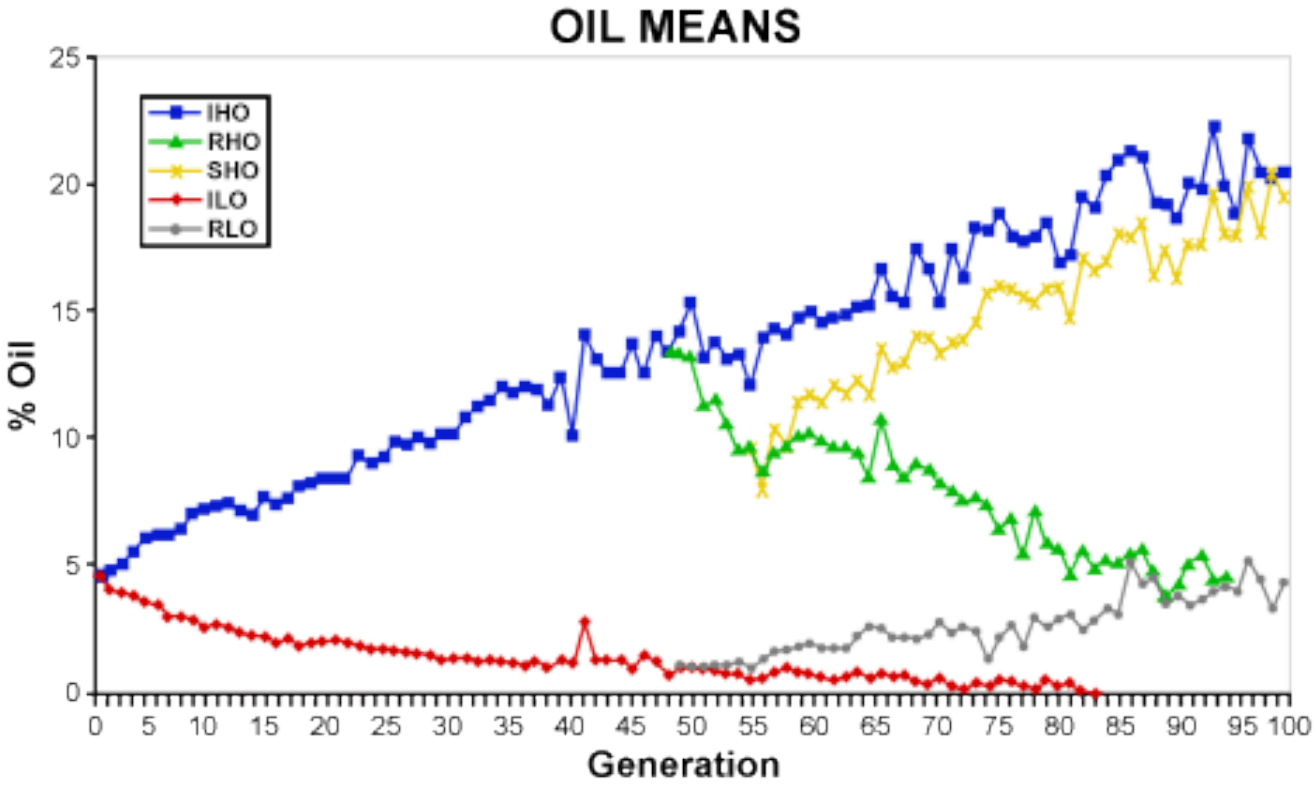

Fig. 4 Mean oil percentage over generations for Illinois High Oil (IHO), Illinois Low Oil (ILO), Reverse High Oil (RHO), Reverse Low Oil (RLO), Switchback High Oil strain (SHO). Illustration by Dudley and Lambert, 2004; used by permission.

Protein Content

The effects of selection on oil content ceased (i.e., Generation 85) in the ILO strain when the oil content reached a level that was no longer measurable with the analytical tools used in this experiment. Protein content reached a lower limit after approximately 65 generations, likely due to biological (i.e., physiological) limit in this crop species. An upper limit was not reached for oil content in IHO and SHO indicating that significant genetic variance still existed in these strains even after 100 generations of selection.

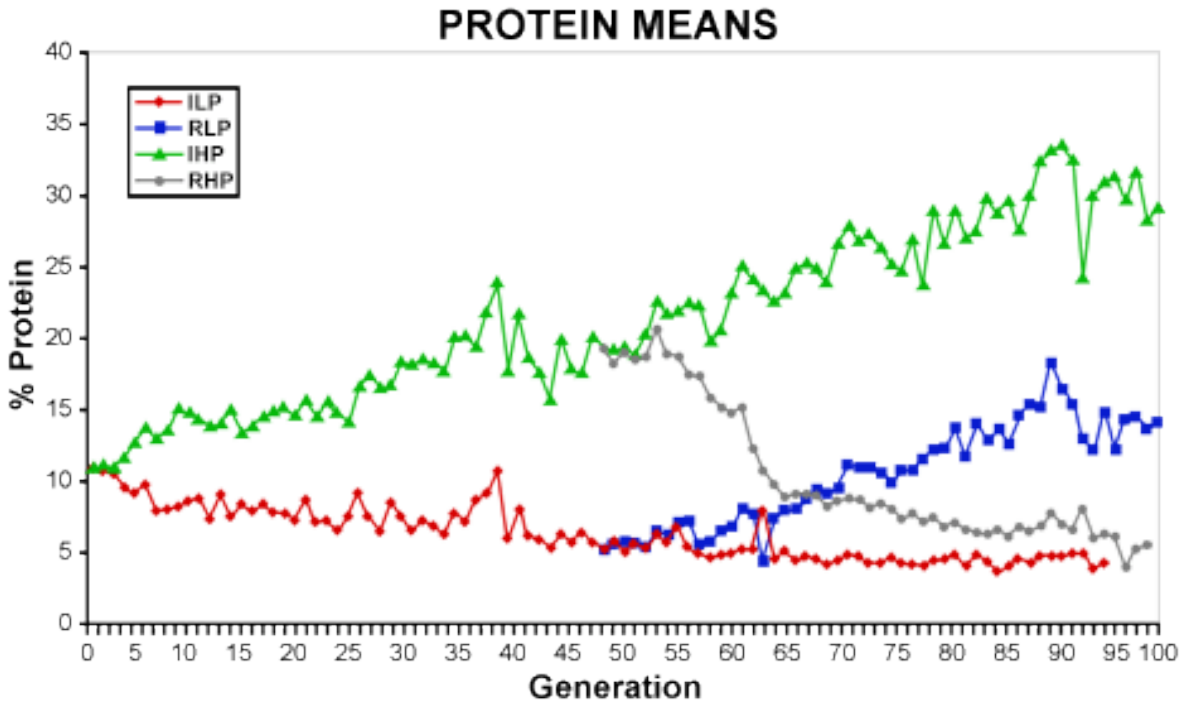

Fig. 5 Mean protein percentage over generations for Illinois High Protein (IHL), Illinois Low Protein (ILP), Reverse High Protein (RHP), and Reverse Low Protein (RLP). Illustration by Dudley and Lambert, 2004; used by permission.

Overview of the Plant Breeding Process

Flow Chart

Figure 6 presents a broad outline of plant breeding process. For a detailed flow chart on breeding process see Simmonds, 1979.

The three main phases of the plant breeding process are:

1. **Germplasm development**: Generally one trait is improved at a time. Crossed are made between wild accessions or related species and/or between elite breeding line or cultivar. Considerations on commercial suitability is low or non-existent. The intention of the work is to develop improved parental germplasm, not a cultivar. Genetic conservation and genetic variability is improved. Genebanks are more heavily relied on for parental stock material.
2. **Cultivar development**: Generally several traits are improved simultaneously. The finished product is a genotype or population that has desirable characteristics for release as a cultivar. Crosses are made between elite lines as parents and may include a germplasm line (see above) as one of the parents. In general, both parents are elite lines (See Steps in Cultivar Development). Considerations on commercial suitability are primary. The intention of the work is to develop an improved cultivar to be grown by farmer(s). Breeder will make phenotypic and genotypic selection decisions on multiple traits and in several generations (pure-bred and inbred lines). Wide adaptation and performance testing is done prior to commercialization.
3. **Technology Transfer:** For germplasm development, there are smaller components of technology transfer for scientists and breeders. For cultivar development, there is a larger component of technology transfer for scientists, breeders, agronomists, pathologists, entomologists, seed merchants, and extension scientists.

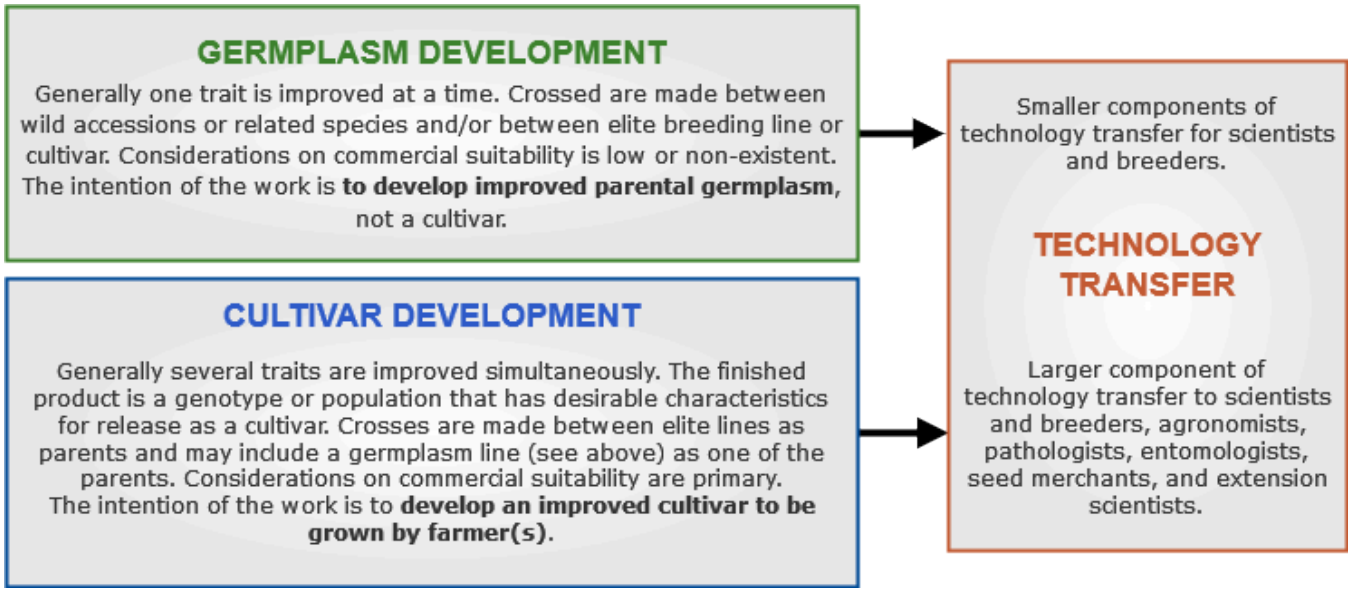

Fig. 6 An overview of the plant breeding process for development of new crop varieties.

Cultivar Development Strategies

In the public and private sectors, the same individual may be responsible for germplasm development and cultivar development phases. In other situations, two or more individuals may be engaged in these two phases independently but collaboratively within the same or different teams. Germplasm developers will be more interested in working on one or few traits (to transfer them from unadapted or wild relatives) and would not be as concerned about its overall suitability for a fit into a commercial release market. On the other hand, a cultivar development breeder has to consider the commercial requirements of his/her crop and its overall suitability.

In the cultivar development strategies, elements that are common in all programs are:

1. Setting objectives
2. Identifying available parents?
3. Creating breeding populations
4. Evaluating and selecting in these population in appropriate environments to meet the objectives
5. Identifying the most suitable genotype for commercial release

Setting Breeding Objectives

Definition

Breeding objectives are based on a mandate (market segment needs), organizational focus, farmer requirement, industry needs, profitability, and sustainability. Objectives need to be clearly defined and based on importance, feasibility and cost-effectiveness. It is not sufficient to set an objective as 'increase yield'. The breeder should put some quantifiable description, such as "increase yield by x% over check ABC", where the comparison has to be made head-to-head. Plant breeding is an expensive activity and careful consideration needs to be made prior to setting objectives. The breeding team needs to engage growers, industry, and consumers to decide on objectives. In a large company, this may be done by a different team and the results communicated to the breeder to help her/him define the objectives. A breeder may develop the highest yielding inbred or hybrid parents or population, but without growers' ability to grow it, this product (inbred, hybrid, population) will not be a commercial success. For example, if a very high yielding genotype has poor storability, growers and industry will not accept this genotype.

In plant breeding, multiple objectives are generally set and a prioritization made to decide on 'must to have' versus 'nice to have' trait. 'Must to have' are traits that absolutely need to be included in the product (pure line, or hybrid, or OPV) for it to be suitable for commercial release, whereas 'nice to have' are traits, which are not essential but may add value to the product.

Exercises

Pick three crops common to your agro-ecological zone, and list 2-3 traits that are 'must to have' and 'nice to have' for each crop.

Identifying Parents

An important consideration for setting breeding objectives is to identify parents for hybridization that have the necessary traits that the breeder will want in the cultivar to be developed. Sources of parental material will be genotypes or populations from your own program, your colleagues' programs (within or outside of your organization), international breeding centers, and gene banks. We will learn a little more about sources of parental material in the next few sections.

After setting of objectives, a breeder will create breeding populations (i.e., create genetic variability) by crossing two or more parents. In crop species with sexual reproduction, generation advancement is generally occurring in parallel with selection for traits as per defined objectives. Once a finished product (genotype) is ready, broader adaptation testing is performed prior to picking the most suitable cultivars for commercialization.

In the next section, we will learn about gene banks, which contain accessions that may be useful to a breeder as sources of genetic variability for use in breeding.

GeneBanks: Role, Procedures, Acquisition, and Stewardship

Roles of Gene Banks

For decades, local, regional, and international efforts have been attempting to preserve valuable agrobiodiversity for future generations by setting up collections of genetic resources, called genebanks. Genebanks contain 'landraces' or local varieties of cultivated and non-cultivated wild relatives. This serves to protect and preserve seed diversity as well as provide an accessible source to plant breeders to obtain seed of interest. There are currently about 1,750 institutional crop collections around the world, as well as a number of community-based seed bank initiatives. CGIAR Research Program for Managing and Sustaining Crop Collections is dedicated to maintaining the 706,000 samples of crop, forage, and agroforestry resources held in "genebanks" at 15 CGIAR research centers around the world. Species which include cereals, legumes, roots and tubers, trees, and other essential staple crops are stored in CGIAR international collections. All accessions within these collections are for the international public good, available under the terms and conditions negotiated by the International Treaty on Plant Genetic Resources for Food and Agriculture.

In the USA, the National Plant Germplasm System aids scientists and addresses the need for genetic diversity by:

- acquiring crop germplasm
- preserving crop germplasm
- evaluating crop germplasm
- documenting crop germplasm
- distributing crop germplasm

GRIN

For example, for the USDA's Germplasm Resources Information Network (GRIN), the steps are to search for genotypes that you are interested in and then place an order to receive seed:

- Access GRIN's Seach Query interface.

- Access GRIN's Order Form.

The breeder should determine which genebank has the collection of material in their crops, proceed to search the genebank and order seed. This process involves numerous paperwork (agreements, seed importing or exporting permits and customs documents) and planning ahead is critical to ensure that you receive seed on time.

[Note: Many times it is useful to contact the curator or other scientists at a genebank as they can sometimes help to make suggestions on a specific trait or accession you may be looking to obtain. However, one needs to do their groundwork first.]

Type of Variability and Sources of Genetic Material

Natural Variability – The Gene Pool Concept

For plant breeders, it is very important to be aware of available germplasm resources that will be useful to improve traits. The gene pool concept was proposed by Harlan and de Wet (1971) as an attempt to provide a practical guide to place existing classifications into genetic perspective. This information on the relatedness among crop plants and their relatives could be useful to breeders and geneticists wishing to make crosses among them. It is important to note that the gene pool concept did not attempt to change the taxonomy. Its purpose is to serve as a guide to plan breeding activities. Various genetic resources are assigned to different gene pools of a crop species based on ease of hybridization, i.e., ability to move genes between them. The three major gene pools are: *primary*, *secondary* and *tertiary*. Gene pools are not static but change as more information becomes available or as new technologies become available to manipulate genomes. For example, in their paper soybean was reported not to have a secondary or tertiary gene pool. However, we now consider that 26 perennial *Glycine* species are in tertiary gene pool and G. *tomentella* has now been used to transfer genes to *G. max* (Singh et al. 2014; R.J. Singh, USDA-ARS, IL, personal communication). Therefore breeders need to be aware of what is going on around them with the use of unique genetic material.

Primary Gene Pool

Species in primary gene pool can be cultivated, landraces, farmer developed or maintained population, ecotypes, and spontaneous races (wild or weedy). Among forms of this gene pool, crossing/gene transfer is easy; hybrids are generally fertile (i.e., no sterility issues) with normal chromosome pairing and gene segregation. Most breeders work exclusively within this gene pool which is also the major source of genetic variation for improvement programs. Remember that most breeding programs that are engaged in developing cultivars for commercial production work on elite material exclusively and would spend very little direct efforts on unadapted or wild relatives (because of undesirable linkage blocks, breaking of desirable linkage block and epistatic interactions with undesirable genes from wild relatives).

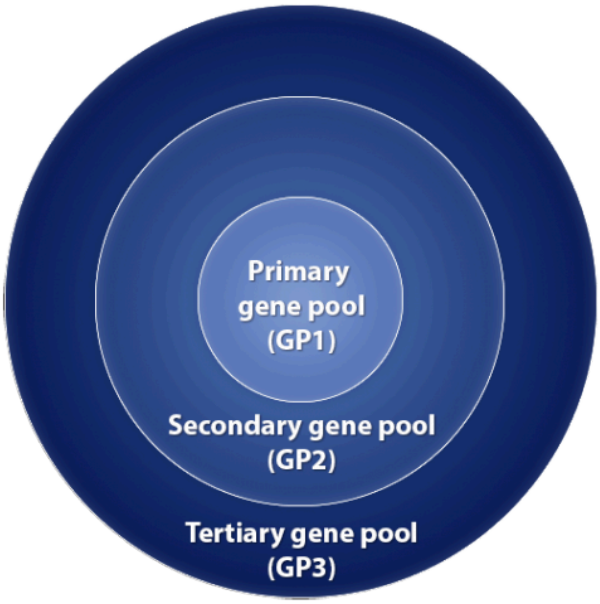

Fig. 7 Schematic diagram of primary gene pool (GP-1), secondary gene pool (GP-2) and tertiary gene pool (GP-3). Adapted from Harlan and De Wet, 1971.

Secondary gene pool

Crop's secondary gene pool will include species between which gene transfer is possible, but difficult. Hybrids tend to be sterile; chromosomes pair poorly during meiosis; F_1 plants are weak and develop to maturity with difficult; because of some sterility in F_1's , recovery of desired types in advanced generations is generally difficult. The secondary gene pool includes related species within the same genus, although all species within a genus won't be in the secondary gene pool and it is also possible that species outside the genus can be in this gene pool.

Tertiary Gene Pool

Gene transfer between a crop and a species in its tertiary gene pool is very difficult (will require embryo rescue, chromosome doubling, bridging species to obtain hybrids). This gene pool

includes distant relatives in other genera or distantly related species within the same species. Hybrid sterility is common, although chromosome doubling may restore fertility by providing homologues for each chromosome. The boundaries of this group are poorly defined and shift as new hybridization techniques are developed.

Hybridization

A bridging species is a third species that facilitates exchange of germplasm between the other crop species and tertiary gene pool species by developing complex hybrids. In their paper, Harlan and de Wet (1971) described a classic example of the use of bridging species where there was an interest to cross *Elymus* x *Triticum*. As expected, hybrid seed could not be obtained. When embryo rescue was used, very few hybrids were obtained and even then these were sterile. However, these researchers found out that if they used *Agropyron* x *Triticum* derivative as female parent and then crossed the hybrid to *Elymus*, introgression of *Elymus* alleles was possible without need for special technique (See Harlan and de Wet, 1971).

Wide Hybridization or Interspecific/Intergenetic Hybridization

'Wide cross' refers to crossing that involves individuals outside of cultivated species. This typically involves the secondary and/or tertiary gene pools. Even though it is difficult, it may be useful to transfer vitally important traits, including disease resistance, or other traits simply not found in cultivated genotypes. Many examples exist in wheat and rice.

Examples

Example 1: In wheat, the T1BL.1RS wheat (*Triticum aestivum* L.) – rye (*Secale cereal* L.) has been of particular interest and was widely used in bread wheat breeding programs worldwide. At one point, it was estimated that several million hectares of wheat were planted to cultivars possessing this **translocation** (tertiary gene pool: rye to wheat crop). This segment had disease resistance cluster for leaf rust, stem rust, stripe rust and powdery mildew, all of which are important diseases of wheat. Additionally, this segment was reported to possess genetic factors that improved grain yield and kernel weight. Resistance to specific genes in the translocation segment have been overcome in some parts of the world, which shows the continual nature of plant breeding where better genetic packages (cultivars) need to be developed.

Example 2: In rice, the first example of transfer of a useful gene from wild species was the introgression of a gene for grassy stunt virus resistance from *Oryza nivara* to cultivated rice. Other examples are transfer of Xa-21 for bacterial blight resistance from *O. longistaminata* to cultivated rice; CMS sources from *O. perennis* and *O. glumaepatula* into rice for hybrid rice production. In 1970's, grassy stunt virus epidemics were reported in several countries and this was transmitted by brown plant hopper (diseased rice plants produced no panicles or small panicles with deformed grains) leading to severe yield losses. Several thousand accessions of cultivated rice and wild species of *Oryz*a were screened for resistance, which identified one *O. nivara* accession as resistant. Plant breeders then successfully transferred grassy stunt virus resistance to improved varieties through a backcross breeding method and resistant varieties were released for cultivation. Other examples include, transfer from *O. officinalis* into elite rice genes for resistance to brown plant hopper, white backed plant hopper (WBPH) and bacterial blight. Some other examples are presented Table 1.

Table 1 Introgression of genes from wild Oryza species into cultivated rice. AA = cultivated rice diploid genome. Data from D.S. Brar and G.S. Kush, 1997.

Trait transferred to *O. sativa* (AA Genome)	**Donor *Oryza* Species**	
	Wild species	**Genome**
Grassy stunt resistance	*O. nivara*	AA
Bacterial blight Resistance	*O. longistaminata*	AA
	O. officinalis	CC
	O. minuta	BBCC
	O. latifolia	CCDD
	O. australiensis	EE
	O. brachyantha	FF
Blast resistance	*O. minuta*	BBCC
Brown planthopper resistance	*O. officinalis*	CC
	O. minuta	BBCC
	O. latifolia	CCDD
	O. australiensis	EE
	O. granulataa	GG
Whitebacked planthopper resistance	*O. officinalis*	CC
Cytoplasmic male sterility	*O. sativa f. spontanea*	AA
	O. perennis	AA
	O. glumaepatula	AA
Yellow stemborer resistance	*O. brachyanthaa*	FF
	O. ridleib	HHJJ
Sheath blight resistance	*O. minutia*	BBCC
Tungro tolerance	*O. rufipogona*	AA
	O. rufipogona	AA
Increased elongation ability	*O. officinalisb*	CC
Tolerance to acid sulfate soils	*O. rufipogona*	AA
	O. rufipogona	AA

Trait transferred to *O. sativa* (AA Genome)	**Donor *Oryza* Species**	
	O. rufipogona	AA

Artificially Created Variability: Mutation and Transgenes

Induced Mutation to Augment Genetic Diversity

Novel genes are produced by several methods, commonly through the duplication and mutation (Fig. 8) of an ancestral gene, or by recombining parts of different genes to form new combinations with new functions. Lethal mutations do not carry their germline forward, however, nonlethal mutations accumulate within the gene pool and increase the amount of genetic variation. The abundance of some genetic changes within the gene pool can be reduced by natural selection, while other "more favorable" mutations may accumulate and result in adaptive changes. A germline mutation gives rise to a *constitutional mutation* in the offspring, that is, a mutation that is present in every cell.

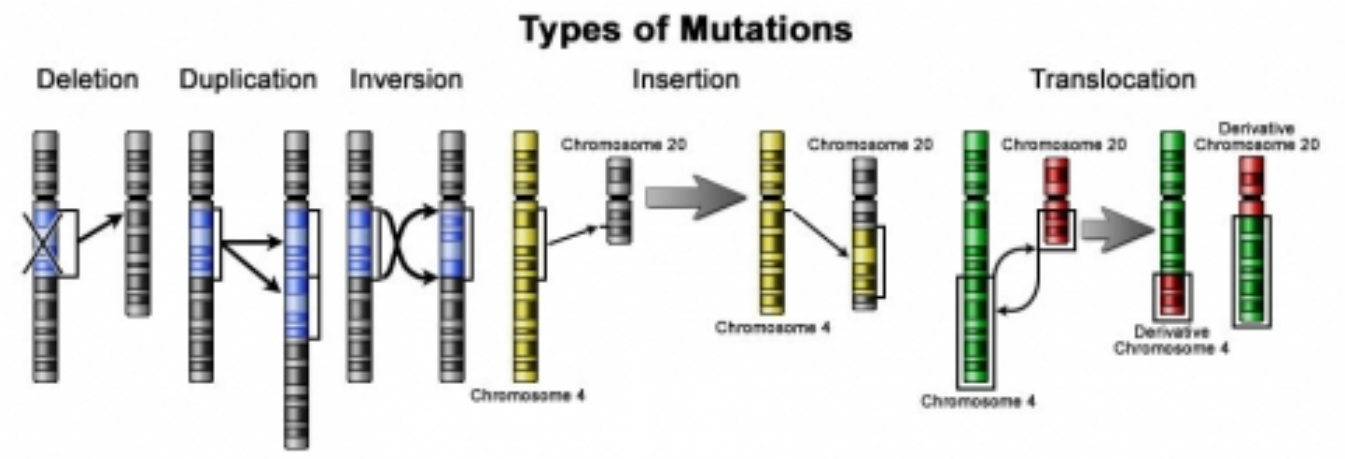

Fig. 8 Basic types of chromosomal mutation Illustration. Adapted from National Human Genome Research Institute.

Mutation Breeding

Deletions lead to loss of gene(s) and duplication can lead to an additive effect due to added gene(s). In inversion, linkage block changes occur and other genes in close proximity will co-segregate. In insertion and translocation, a gene moves to a new chromosome, and can have similar effect as duplication.

[Note: Mutations can be subdivided into germ line mutations, which are passed on to descendants through their reproductive cells, and somatic mutations, which involve non-reproductive system cells and are therefore not usually transmitted to descendants].

Examples of Success with Mutation Breeding

Quality Protein Maize (QPM). Maize endosperm protein is deficient in two essential amino acids, lysine and tryptophan. The opaque 2 mutant gene, together with endosperm and amino acid modifier genes, was used for the development of QPM varieties. Compared to regular maize, QPM has about twice as much lysine and tryptophan, and 30% less leucine, which makes it suitable and useful for human and animal nutrition. QPM varieties are now estimated to be grown on millions of hectares. The high protein content and better amino acid profile is achieved by "opaque-2" single gene mutation. In the early 1960s, a mutant maize with similar total protein content but double the amount of lysine and tryptophan was developed. Subsequent conventional breeding efforts generated numerous cultivars with improved agronomic characteristics, and these were referred to as QPM. Dr. Evangelina Villegas and Dr. Surinder Vasal were awarded the 'World Food Prize' in 2000 for their work on development and advancement of QPM cultivars in the world.

Approaches

If the goal of *mutation breeding* is to alter only a single trait, the plant breeder needs to be aware that other regions of the genome (i.e., other genes) may have been mutated and also that, that one change may alter other aspects of the plant. Hence extensive agronomic testing of that single mutant is required prior to commercialization or extensive use as a parent in the breeding program.

Traditionally, chemical or physical agents were used to induce mutations in crop genomes, and

included radiation (X-rays, gamma rays, fast neutrons, etc.), chemicals such as ethyl methane sulfonate (EMS) and others. These mutagens can disrupt chromosomes, causing deletions, insertions, breakage, etc., and will create genetic variation. Major disadvantage of this approach is the non-targeted mutation events. After receiving your M_1 seed (one has to send several thousand seed of the same cultivar) plant breeder has to increase the generation to achieve homozygosity (mutant allele will segregated initially) and constantly phenotype for the traits of interest. This can be very resource intensive depending on the cost to phenotype the trait of interest (field for morphological trait or lab for quality trait or chemical component). At low doses, chromosomal changes are not as dramatic (it is desirable not to use high doses as major chromosomal aberrations and lethality can occur) and the mutation frequency is low, therefore warranting large population sizes to be screened. This leads to high expenses to phenotype and sometimes very difficult to identify a target mutant event.

Some of the newer approaches include, space light ion irradiation, use of restriction endonucleases, Zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regulatory interspaced short palindromic repeat (CRISPR)/Cas-based RNA-guided DNA endonucleases. These techniques lead to genetic modifications by inducing DNA double-strand breaks that stimulate error-prone nonhomologous end joining or homology-directed repair at specific genomic locations leading to site targeted mutation.

Procedure

The procedure to create a mutant population is briefly as following: Seed (M_0) of an inbred (homozygous, homogenous) genotype is subjected to treatment (chemical, physical, etc). The treated seed are then grown and plants grown from these seeds (treated with a mutagen) form the M_1 population (where M stands for Mutant and '1' refers to first the generation in development similar to the concept of filial generation we learned in previous chapter). It is important to realize that although we started from an inbred line, after seed treatment the resultant M_1 generation will consist of plants that are heterogeneous, as each M_1 plant may have different mutation (or several mutations) in its genome. Each plant will also be heterozygous (or hemizygous) at numerous loci. This would mean that M_1 is similar to an F_1 developed from noninbred parents. The step includes, self-fertilization of the M_1 plants to develop the M_2 generation. This will allow the recessive mutants to be made homozygous in order to produce observable phenotypes. Identifying mutants generally requires large populations of M_1 plants. Other considerations are size of M_1 and M_2 family to recover the mutant type, ploidy

level, genetically effective cell (GEC) present in germline (these are those cells of the germline that contribute towards formation of gametes and so to offspring), frequency of chimeras. After several M generations, mutant phenotype is confirmed and established as stable (non-segregating). Mutant line then can be used in a forward or backcrossing program to transfer the favorable mutant to elite cultivars using multiple but separate crosses to ensure recovery of the elite phenotype together with the mutant trait.

Transgenic Approaches to Increase Genetic Diversity

Transgenic technology involves the transfer of cloned genes via transformation or particle bombardment so that the transformed plant expresses the foreign gene. Theoretically, the genes can come from virtually any living organism – from within the same primary gene pool to beyond the tertiary gene pool. Over the past few years, mainly only single genes have been transferred, such as herbicide tolerance in various crops, or corn borer resistance in corn.

Creating Breeding Populations – Types of Crosses

Types of Crosses

Once objectives are set and the breeder has done background investigation to pick the parents that possess the traits that will help meet the objectives, she/he will move to the next step in plant breeding process, which is to develop breeding populations. While some breeding population may start from landraces, most populations will be made by making planned hybridizations (crosses). The primary purpose of crossing is to expand genetic variability by bringing together genes from the parents in the cross to produce offspring that contain genes that will help meet the objectives. Sometimes, multiple crosses are made to generate the variability in the base population to begin the selection process in the program. In most self and cross pollinated species where the product is an inbred line or a hybrid, single crosses are made, while complex crosses are made in population improvement schemes. Parents are selected to have the maximum number of desirable traits and minimize undesirable traits (what is generally called an 'elite by elite' cross). This way, recombinants that possess both sets of desirable traits will occur in significant numbers in the F_2, the generation of maximum variability (in self-pollinating and inbred line development programs). Several factors will impact the population

size include number of genes differing among parents in a cross, number of alleles per locus, and linkage of the gene loci.

Major types of crosses made

1. **Single cross.** This is attempted when a breeder is making a cross between two elite lines. (Line A x Line B).
2. **Three-way cross.** If two lines are not sufficient to bring together all the necessary traits to meet the objectives, multiple cross with three parents can be used to provide an opportunity to obtain recombinants with all the desirable traits. Three way cross is (Line A x Line B) x Line C. If cultivar development is targeted in a three way cross, the third parent 'Line C' in our example, should be an elite and adapted genotype to get at least 50% of favorable genetics.
3. **Double cross.** A double cross is a cross of two single crosses [(Line A x Line B) x (Line C x Line D)]. The breeder should attempt to make the two single crosses in the same season and then cross the resultant F_1 in the next season crossing cycle to minimize the time to obtain F_1 from the double cross. More parents start to introduce more opportunities to break linkages (including favorable ones) and there is a low frequency of obtaining desirable recombinants in the early generation selection. The double-cross hybrid is more genetically broad-based than the single-cross hybrid.
4. **Diallel or Partial Diallel cross.** A diallel cross is one in which each parent is crossed with every other parent in the set (complete diallel), yielding n * (n – 1)/2 different combinations (where n is the number of entries) excluding reciprocal crosses where the female/male order is reversed. For example, where n equals 9, 36 crosses will be made.This method requires making a large number of crosses and it is more suitable for cross-pollinated species. This method is generally used for genetic studies and not for population development. Sometimes, a partial diallel is used in which only certain parent combinations are made. For n equals 9 example, not all 9 parents will be used in crosses.
5. **Back cross.** The primary goal of this crossing method is to incorporate a specific trait (from a donor parent) into an existing elite cultivar (referred to as recurrent parent in back crossing). The donor parent has one trait that a breeder desires to include into an elite cultivar, which is considered to possess all necessary traits except this one trait. The resultant product (after successive crossings to recurrent parent) is a cultivar that is similar to the recurrent parent with the additional trait from the donor parent. This methods is more efficient if the interest is to improve a current popular cultivar which has

an obvious deficiency. Molecular markers have improved the efficiency and reduced the time to develop cultivars through backcrossing.

Visual Depiction of Types of Crosses

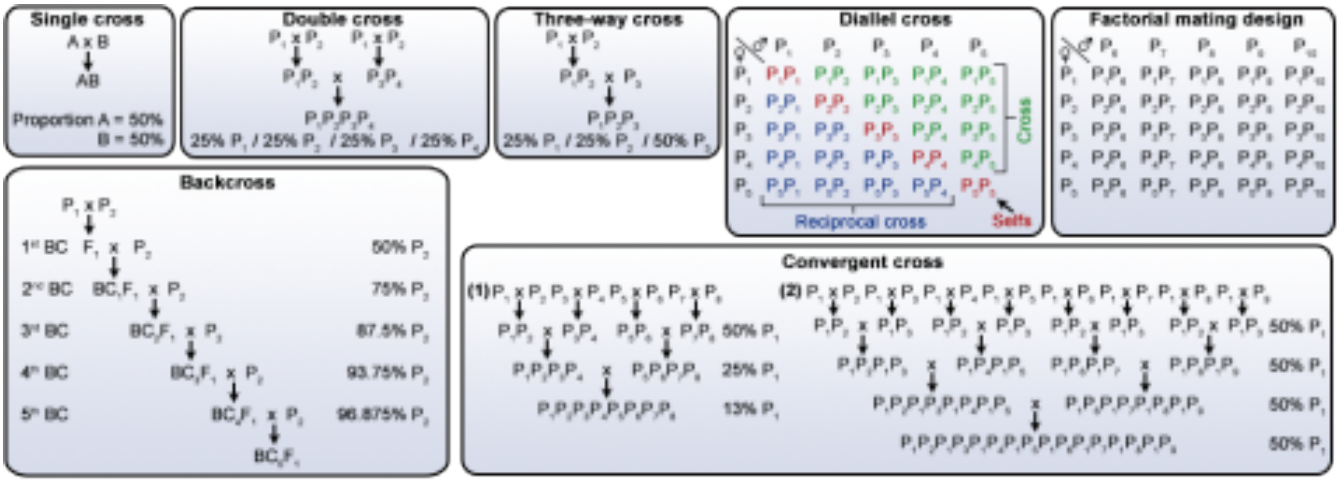

Fig. 9 Methods of creating breeding populations. Adapted from Acquaah, 2012.

Material Transfer Agreement (MTA)

Legal Considerations

Seed or plant part material transfer agreement (MTA) is an agreement that allows for transfer of seed or plant part without transfer of title. The agreement is between the provider (one who provides seed) and the recipient (one who receives seed). Provider maintains ownership of the seed transferred at all times during the agreement dates and beyond. Transferred seed is received and used by the recipient according to the terms listed in the legally binding contract. In the agreement, the provider may impose conditions of audit or term bound reports on the usage of material and the recipient has to bind to these conditions. When a plant breeder makes request for seed, she/he will work with their organizational designate in the office of intellectual Property and Commercialization or the office that manages Intellectual Property (IP) and Technology Transfer. Similarly, the plant breeder should discuss with the same office when she/he receives a request for seed/plant part and should **NEVER give out material without**

going through proper steps. MTA is signed by the sending breeder and his/her organizational representative, as well as recipient breeder and his/her organizational representative. Plant breeder or researcher utilizing the material (i.e., seed/plant part) is ultimately responsible for fulfilling the obligations of the MTA and therefore has to follow the regulations. Remember: MTA is a legal document.

MTA Sections

- *Introduction:* short text on the type of material or purpose
- *Parties:* describes the sender and recipient and their organizational affiliations
- *Definitions:* describes scientific terms such as material (seed and genotype etc.)
- *Description of use of the materials:* conditions on what can and cannot be done using the material
- *Confidential information:* lists any specific confidentiality clauses
- *Intellectual Property rights:* this is where licensing, royalties, inventions conditions are listed
- *Warranties:* This is to protect sender or provider that stipulates that material does not come with any warranties.
- *Liability and/or indemnification:* Recipient assumes all liability for damages that may arise from how/what recipient does to the material after transfer and sender is not liable.
- *Publication:* provides description on publication rights for receiver.
- *Governing law:* describes which jurisdiction laws will apply (state, country). In most cases where provider and recipient are from separate countries, the two parties may not define this section.
- *Termination:* date of termination of the agreement. It may also describe what the recipient has to do with leftover material from the agreement. Most likely original material sent by provider is expected to be destroyed.
- *Signatures:* Agreement is not considered executed until all necessary signatures are obtained and material should not be sent until MTA is signed and official. Signatories are the official organization representatives, provider breeder and recipient breeder. [note: provider and recipient may or may not be a breeder. For example, recipient or provider may be a geneticist]
- *Exhibits or appendices*: list of material, or data accompanying the material.

International Treaty on Plant Genetic Resources for

Food and Agriculture

SMTA

The Standard Material Transfer Agreement (SMTA) is a mandatory model for parties wishing to provide and receive material under the Multilateral System. It is the result of lengthy negotiations among the Contracting Parties to the Treaty and may not be varied or abbreviated in any way. However, as a template, it contains some paragraphs and sections that need to be completed for each use.

The material transfer agreements that use the standard template are private agreements between the particular providers and recipients, but the Governing Body, through FAO as the Third Party Beneficiary, is recognized as having an interest in the agreements. The standard template has been developed to ensure that the provisions of the Treaty regarding the transfer of PGRFA under the Multilateral System are enforceable on users."

Farmers' Rights

"*Farmers' Rights:* In its Article 9, the International Treaty recognizes the enormous contribution that the local and indigenous communities and farmers of all regions of the world, particularly those in the centers of origin and crop diversity, have made and will continue to make for the conservation and development of plant genetic resources which constitute the basis of food and agriculture production throughout the world. It gives governments the responsibility for implementing Farmers' Rights, and lists measures that could be taken to protect and promote these rights:

- The protection of traditional knowledge relevant to plant genetic resources for food and agriculture;
- The right to equitably participate in sharing benefits arising from the utilization of plant genetic resources for food and agriculture; and
- The right to participate in making decisions, at the national level, on matters related to the conservation and sustainable use of plant genetic resources for food and agriculture.

Importance

The International Treaty also recognizes the importance of supporting the efforts of farmers and local and indigenous communities in the conservation and sustainable use of plant genetic resources for food and agriculture, including through a funding strategy. In this strategy, priority will be given to the implementation of agreed plans and programs for farmers in developing countries, especially in the least developed countries, and in countries with economies in transition, who conserve and sustainably utilize plant genetic resources for food and agriculture."

It is important that breeders realize that if an MTA was signed to send or receive seed, it is a legal document and they are legally bound to follow the conditions.

Uses of germplasm: Methods to use germplasm in breeding programs include direct release as cultivars [less likely in species with breeding efforts, more likely in orphan crops where some selection may be done on a plant introduction or landrace prior to release as a cultivar]. The second and more appropriate use of germplasm is for introgression of single-gene traits from the wild species or unadapted germplasm into the elite cultivars.

References

Acquaah, G. 2012. Principles of Plant Genetics and Breeding, 2nd Edition. Wiley-Blackwell.

Brar, D.S., and G.S. Khush. 1997. Alien introgression in rice. Plant Molecular Biology 35: 35–47.

Harlan, J.R., and J.M.J. de Wet. 1971. Toward a Rational Classification of Cultivated Plants. Taxon, 20(4): 509-517.

Simmonds, N. 1979. Principles of Crop Improvement. Harlow, UK. Longman.

Singh, R.J., and R.L. Nelson. 2014. Methodology for creating alloplasmic soybean lines by using Glycine tomentella as a maternal parent. Plant Breeding. 133(5): 624-631.

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Chapter 4: Refresher on Population and Quantitative Genetics

Asheesh Singh and Anthony A. Mahama

The presence of genetic variation is a key prerequisite for genetic improvement in plant breeding. Creation of breeding populations with sufficient variability among individuals is key to success of breeding programs. Behind the visual variability in populations are underlying genetic variations and interactions, the understanding of which the plant breeders and students in plant breeding can benefit from to ensure successful use of the sources of genetic variation and their manipulation to maximize improvement of programs.

Learning Objectives

- Demonstrate understanding of the different populations in plant breeding
- Distinguish between qualitative and quantitative traits important in plant breeding
- Demonstrate understanding of the concept of variability, phenotype, genotype, and genotype x environment interactions
- Describe the concept of heritability and its importance in plant breeding
- Discuss selection theory and response to selection (breeder's equation)
- Distinguish between specific versus general combining ability and their calculations
- Describe the concept of heterosis and write the equation for estimating heterosis

Populations

Basic Principles

Population genetics deals with the prediction and description of the genetic structure of populations as it relates to Mendel's laws and other genetic principles. Fundamentals of population genetics were developed for natural outcrossing species, but it is important to note

that plant breeders utilize population genetic theories because the breeding methods they use are designed to increase the frequency (proportion) of desirable alleles in the population.

The difference between population and Mendelian genetics is that population genetics principles are applied to the total products of all matings that will occur in the population, not just to one specific mating (as is the case with Mendelian genetics).

Population refers to any group of individuals sharing a common gene pool.

Gene pool refers to the sum total of all genes present in a population.

Types of Populations in Breeding Program

Simple populations

Simple populations consist of two to four parents. The simplest type of population is created by developing a segregating population from a cross of two elite lines, i.e. a two-way cross population. An F_2 generation can be produced by self-fertilizing the F_1. F_2 and higher filial generations are developed (and selections made in these populations) in commercial breeding programs of self-fertilizing crops (such as wheat, rice, and soybean). In many cross-pollinated crops like maize, the commercially marketed products are single cross hybrids (F_1) from inbred parents developed following generations of selection and selfing.

Another type of segregating population is a backcross population, which can be made from the F_1 by crossing it to one of the parents, producing a BC_1F_1, which can be selfed to form a segregating population. The backcross population is particularly useful if one parent is superior to the other in most traits and the objective is to combine one or two genes from unadapted (or overall undesirable) parent into an elite line. Each backcross F_1 seed will be heterogeneous and therefore phenotypic testing or marker-assisted selection will be required to select the desirable plant to use in subsequent backcrossing to fix or enrich for favorable genes.

Single Cross

A cross involves two parents (Fig. 1), for example the crossing of two elite lines for F_1's from which an F_2 population can be produced by self-fertilizing the F_1. Parent 'A' and 'B' contribute 50% each (genetically)

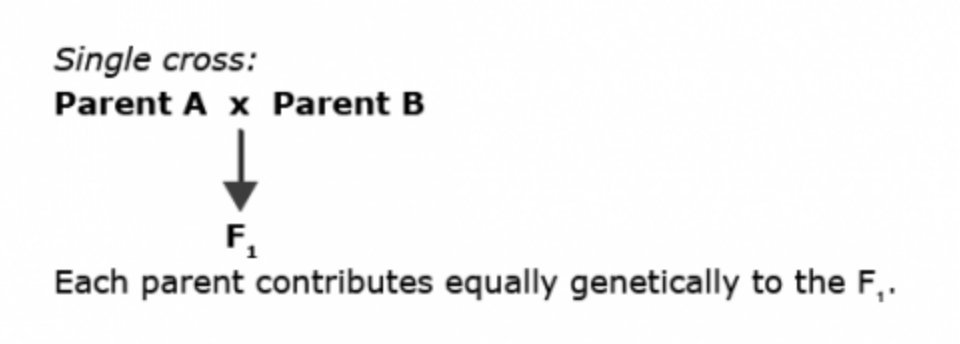

Fig. 1 Single cross between two parents for F1 progeny.

Three-Way Cross

Crossing the single cross F_1 to a third parent (inbred line) and self-pollinating the resulting three-way hybrid creates a three way cross population (Fig. 2). The generation resulting from the self-fertilization is generally called the F_2 population. Note that the third parent contributes 50% of the alleles to the final population therefore should generally always be one of the best parents. A three way cross is useful if one of the parents is less desirable and one or two are more desirable.

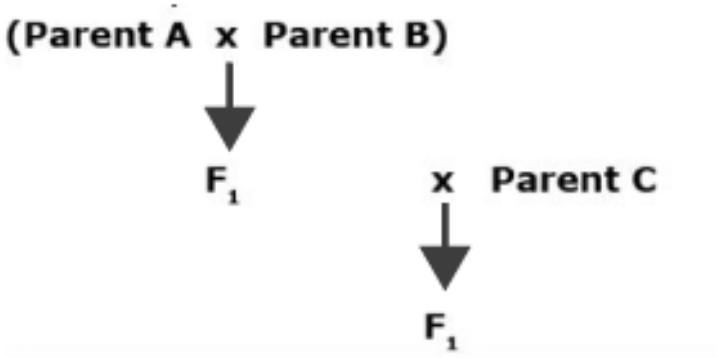

Fig. 2 Three way cross of F1 from Parent 'A' by Parent 'B' crossed to Parent 'C' (Parent 'A' and 'B' each contribute 25% (genetically), while parent 'C' contributes 50% genetically to the final F_1.

Double Cross

A double cross or four-parent cross population can be produced by crossing two single cross F_1 hybrids, each formed from two inbred lines (Fig. 3). Each of the resulting individuals in the double cross generation will be genetically distinct.

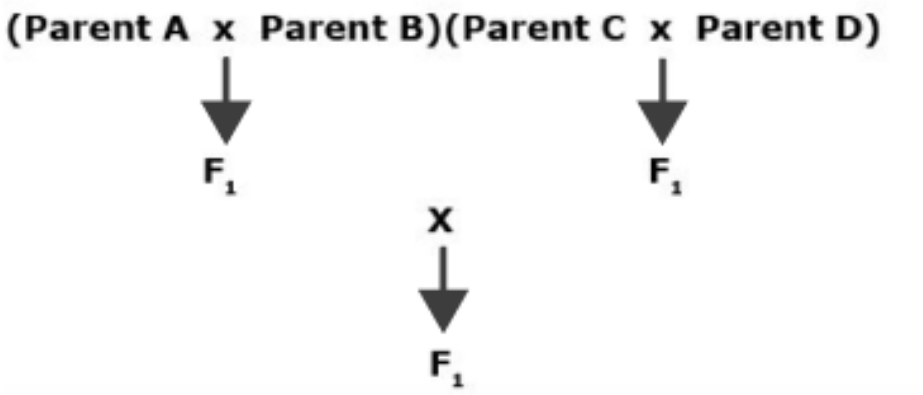

Fig. 3 Double cross of Parent 'A' by 'B', and Parent 'C' by 'D', and their F1s crossed to each other. Each parent contributes 25% genetically to the final F_1.

Complex Populations

Some of the reasons to use complex populations include: developing good information for parents and full-sib families, identification of heterotic groups, estimation of general and/or specific combining ability, develop estimates of additive, dominant, and epistatic genetic effects and genetic correlations. Complex populations deriving from more than four parents can be constructed in several ways:

- Nested designs
- Factorial designs
- Diallel cross
- Half-diallel
- Partial diallel
- Polycrosses

Nested Designs

A **North Carolina Design I** (**nested design**) involves mating of each of the male parents to a different subset of female parents as shown in Table 1.

Table 1 Mating of four male parents and eight female parents, each male mated to two different female.

n/a		**Male Parent** (♂)			
		A	**B**	**C**	**D**
Female Parent (♀)	**1**	X	n/a	n/a	n/a
	2	X	n/a	n/a	n/a
	3	n/a	X	n/a	n/a
	4	n/a	X	n/a	n/a
	5	n/a	n/a	X	n/a
	6	n/a	n/a	X	n/a
	7	n/a	n/a	n/a	X
	8	n/a	n/a	n/a	X
Note: X means “cross”. n/a means cell is blank.					

A **North Carolina Design II** (**factorial design**), Table 2, involves mating each member of a group of males (A, B, C, D) to each member of the group of females (1, 2, 3, 4, 5, 6, 7, 8).

Table 2 Mating of four male parents each to eight female parents in a factorial design.

n/a		Male Parent (♂)			
		A	**B**	**C**	**D**
Female Parent (♀)	**1**	X	X	X	X
	2	X	X	X	X
	3	X	X	X	X
	4	X	X	X	X
	5	X	X	X	X
	6	X	X	X	X
	7	X	X	X	X
	8	X	X	X	X
Note: X means "cross"					

Table 2 Mating of four male parents each to eight female parents in a factorial design.

n/a		Male Parent (♂)			
		A	**B**	**C**	**D**
Female Parent (♀)	**1**	X	X	X	X
	2	X	X	X	X
	3	X	X	X	X
	4	X	X	X	X
	5	X	X	X	X
	6	X	X	X	X
	7	X	X	X	X
	8	X	X	X	X
Note: X means "cross". n/a means cell is blank.					

Diallel Cross

A **diallel** refers to a crossing scheme in which all pairwise crosses among the parents are made as a series of single crosses (Table 3). Diallels can be "complete," in which crosses are made

in both directions i.e., including reciprocal crosses, as well as self-pollinations of parents. In other words, each parent is mated with every parent in the population (including selfs and reciprocals).

Table 3 Mating of a group of eight individuals each crossed to every other individual both as male parent and female parent, and also self-pollinated.

n/a		**Male Parent** (♂)							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Female Parent (♀)	**1**	X	X	X	X	X	X	X	X
	2	X	X	X	X	X	X	X	X
	3	X	X	X	X	X	X	X	X
	4	X	X	X	X	X	X	X	X
	5	X	X	X	X	X	X	X	X
	6	X	X	X	X	X	X	X	X
	7	X	X	X	X	X	X	X	X
	8	X	X	X	X	X	X	X	X

Note: X means “cross”. n/a means cell is blank.

From a breeding standpoint, selfs do not contribute any interesting recombination if the parents are inbred, and because crosses in different directions are functionally the same in terms of recombination in later generations (unless there are maternal and paternal effects), the complete diallel is usually used only as a research tool. In the example given above, Parent 1 will be selfed, as well as used as male and as female in crosses with the other parents, e.g. parent 2, thus doubling the number of crosses with parent 2, i.e. 1 x 2 and 2 x 1.

Half-Diallel Cross

In a **half-diallel cross**, each parent is mated with every other parent in the population excluding selfs and reciprocals (Table 4).

Table 4 Mating of a group of eight individuals each crossed to every other individual either as male parent or female parent only, and no self-pollination.

		Male Parent (♂)							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Female Parent (♀)	**1**								
	2	X							
	3	X	X						
	4	X	X	X					
	5	X	X	X	X				
	6	X	X	X	X	X			
	7	X	X	X	X	X	X		
	8	X	X	X	X	X	X	X	

Note: X means "cross". n/a means cell is blank.

Partial Diallel Cross

Partial diallel refers to a crossing scheme in which only selected subsets of full diallel crosses are made (Table 5). A partial diallel could be made among a large number of parents, followed by a diallel among the single crosses, allowing for sampling more recombination events among favorable parents. This would allow incorporation of a diversity of germplasm without having to make a massive number of crosses.

Table 5 Mating of a group of eight individuals using selected individuals as male parent particular female parent only, and no self-pollination.

		Male Parent (♂)							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Female Parent (♀)	**1**		X	X	X				
	2			X	X	X			
	3				X	X	X		
	4					X	X	X	
	5	X					X	X	X
	6	X	X					X	X
	7	X	X	X					X
	8	X	X	X	X				

Note: X means "cross". n/a means cell is blank.

Practically making crosses for a diallel (or other methods of population formation) in the field requires careful planting arrangements. There are several things to consider: such as flowering time, length of time pollen and stigma will be viable, and susceptibility to drought or pest. From a planting perspective in the field, the considerations are: (1) physical distance between parents, i.e., distance between rows or plants (how close do you want to have each of the two parents you are crossing), such as side-by-side or not; and (2) how much land area is available for the population development. Planting arrangements vary from unpaired parents, in which parents are not very close to each other but less land is required for example circular crossing (aka, chain crossing), in which parents are crossed in pairs sequentially, A x B, B x C, etc., with the final parent crossed back to A.; or paired parents, in which all parents to be crossed are grown in adjacent rows, however, a large area is required for crossing nursery.

Polycrosses

Polycrosses are used to intercross a number of selected plants. Polycrosses are primarily used for cross-pollinating crop species allowing natural conditions (e.g., wind or insects) to make the crosses. Thus, the pollen for a polycross comes from the population of selected (or unselected) individuals as pollen parent source, and no control on the success of any particular parental

pairing is known. Some plants will undoubtedly produce more pollen than others, thereby resulting in a higher percentage of pollinations than others. In a clonal crop, parent genotypes may be replicated in a manner to ensure each genotype is adjacent or surrounded by all other genotypes to provide equal frequencies of crossing among all genotypes. If sufficient land is available and not too many entries are included in the crossing, a Latin Square arrangement (each parent is present in each row and each column of the design) is a good way to enhance the equal chance of pollination among the genotypes. Another option will be a randomized complete block design if the number of parents is large. A higher number of replications are planted (two or more); however, each parental genotype will not be surrounded by all other genotypes in equal frequency therefore non-random and unequal mating occurs. A number of other aspects need to be considered for successful intercrossing which include flowering time, wind effects, and insect pollinator activity. Flowering time needs to be similar among the parents to prevent certain parents intercross more frequently (due to overlapping flowering time) than they should by random chance. For polycrosses done in the greenhouse by hand (e.g., in alfalfa), flowering can be controlled more easily than in a field planting and this is something breeders can consider using, resource permitting. If a breeder is relying on wind pollination, the dominant direction of the prevailing wind will affect pollination and lead to a non-random pollination. For insect-pollinated crops, placing bee hives near the field is often done to ensure successful pollination.

Polycross Nursery Harvest Procedures

To ensure harvested seed of the polycross is representative of seed from random and equal pollinations (which is what is intended) three major procedures exist for harvest:

1. **Bulk harvest the entire plot.** This is the easiest method but will result in unequal contributions by both paternal and maternal parents to the population because maternal or paternal parent that produces more seed will represent a higher proportion of seed in the lot.
2. **If replications were used in the crossing design, bulk each parental genotype's seed across all the replications.** Composite equal amounts of seed from each parent to make the population. This is the most commonly used method but there is an element of unequal contribution. Different clones (or inbred or doubled haploid) of the same parental genotype may not produce the same amount of seed, so this method will skew the population toward the pollen parents surrounding the highest yielding maternal clone.

3. **One method that will overcome the problem listed in #2 above is to composite an equal amount of seed from each clone in the polycross – that is, equal amounts from each parental genotype in each replication.** This method provides the most balanced contribution to the population possible. If there is a replication that didn't perform to provide the minimum seed, some adjustments (in either among sample per replication or pulling more from other reps for that genotype) will be needed, leading to some un-equal contribution.

Qualitative and Quantitative Traits

Qualitative traits are traits that are generally controlled by a single or few genes, the expression of which have phenotype that can be classified into distinct categories. These traits are generally not influenced by environment and are recorded/scored as presence versus absence, or yes versus no, different color, seed shape type, etc. Examples include the presence of awns in wheat (awned versus awnless), flower color (purple versus white), round versus wrinkled seed (Mendel's garden pea experiment). These traits will be in the 'yes versus no' classification and their expression will be the same irrespective of the environment the plants are grown, that is, genotypes with round seed will produce round seed in all environments.

Quantitative traits are controlled by several genes, whose expression produce a phenotype that cannot be classified into distinct categories, i.e., there will be a continuum of phenotypes. These traits are influenced by the environment, such that the same genotype will produce different phenotypes in different environments. Examples of such traits are yield, protein %, oil %, and seed weight.

Traits such as plant height are described as qualitative because they can be classified as short versus tall. However, it is important to note that plant height can occur across a range of values (cm) meaning that these are not innate categories and most appropriate measurement is on a numerical scale, which makes plant height a quantitative trait from a trait measurement perspective. In most crops, several plant height genes have been identified, again validating that plant height is not a truly qualitative trait.

Disease resistance can be qualitative or quantitative, and this distinction between them will be driven by genetic control, influence of environment, and phenotypic expression. Most traits that a plant breeder works to improve are quantitative.

Types of Gene Action

Expression of genes can be described as additive or non-additive (dominance or epistatic).

Additive gene action

A gene acts in an additive manner when the substitution of one allele for another allele at a particular gene locus always causes the same effect. For example,

A1A1 – A1A2 = A1A2 – A2A2

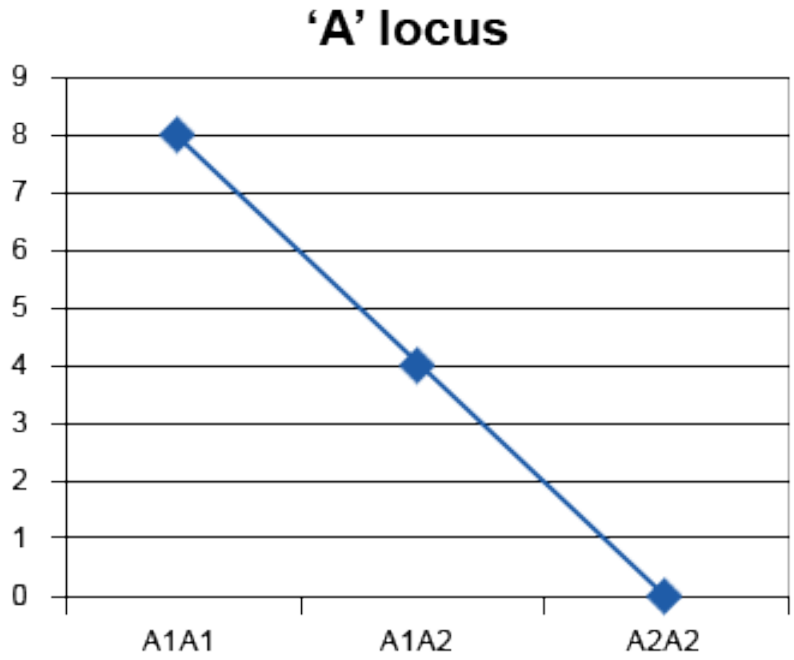

Fig. 4 Linear regression with common slope between genotypes to describe the additive effects.

That is, for this case, the effect of substituting A1 for A2 is the same whether the substitution occurs in genotype A2A2 or in genotype A1A2 (Fig. 4, Table 1).

Table 6 Genotypic values of three genotypes at 'A' locus.

A1A1	8
A1A2	4
A2A2	0

Thus the effect of substituting an A1 allele with an A2 allele is +4.

Genotypic Values at 'B' Locus

As shown in Table 7, if we assume genotypic values at 'B' locus as:

Table 7 Genotypic value of three genotypes at 'B' locus.

B1B1	4
B1B2	2
B2B2	0

then, as shown in Table 8, the effect of substituting allele B1 for B2 is +1, indicating an additive effect.

Table 8 Hypothetical example to demonstrate additive effects.

Genotype	**Genotypic value**
A1A1B1B1	12
A1A1B1B2	10
A1A1B2B2	8
A1A2B1B1	8
A1A2B1B2	6
A1A2B2B2	4
A2A2B1B1	4
A2A2B1B2	3
A2A2B2B2	0

When a gene acts additively, the maximum trait expression will occur in the genotype which possesses all the "favorable" alleles.

Non-additive Gene Action

Non-additive gene action results from the effects of dominance (intra-locus interactions, i.e. A1/A2) and/or the effects of epistasis (inter-locus interactions, i.e. A1/B1 or A2/B2).

Dominance effects are deviations from additivity, therefore A1A1 – A1A2 ≠ A1A2 – A2A2. This deviation results in the heterozygote being similar to one of the parents rather than the mean of the homozygotes (Table 9).

Table 9 Genotypic value of three genotypes at 'A' locus.

A1A1	6
A1A2	6
A2A2	1

Note that A1A2 can take different values, and that genotypic values are hypothetical for the purpose of explaining the concept here.

Non-Linear Regression

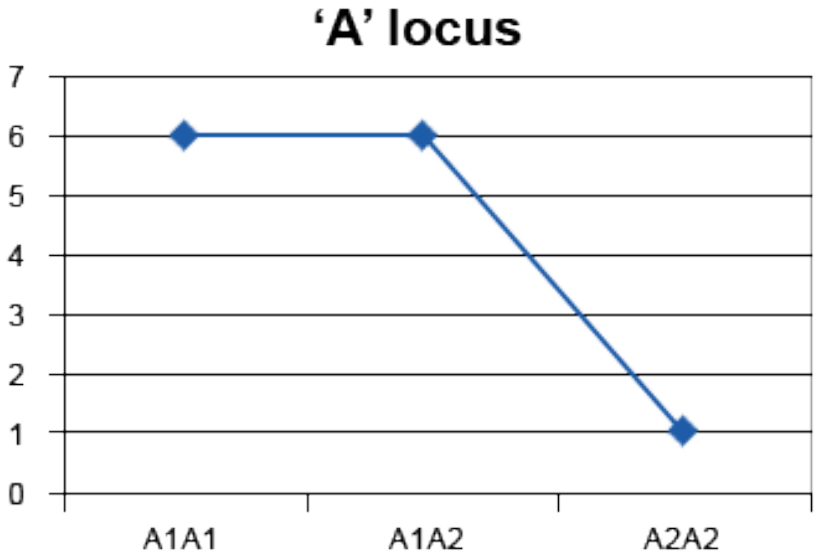

Fig. 5 Non-linear regression with non-common slope between genotypes (A1A1-A1A2; A2A2-A1A2) to describe the non-additive (dominance) effects.

Figure 5 describes dominance effects by showing the non-linear regression (with non-common slope) between genotypes to describe non-additive effects (in this case dominance effect).

Will selection for maximum expression always lead to selection of true breeding individuals? [Hint: consider individuals in early generations compared to purelines in a self-pollinating crop]

Hypothetical Example

If we consider a hypothetical example of two gene loci (no linkage), the proportions of F_2 genotypes are as shown in Table 10.

Table 10 Hypothetical example to demonstrate two gene loci (no linkage).

Genotype	Ratio
A1A1B1B1	1/16
A1A1B1B2	2/16
A1A1B2B2	1/16
A1A2B1B1	2/16
A1A2B1B2	4/16
A1A2B2B2	2/16
A2A2B1B1	1/16
A2A2B1B2	2/16
A2A2B2B2	1/16

Let's assume genotypic value as:

A1A1 = A1A2 = 4, A2A2 = 0

B1B1 = B1B2 = 3, B2B2 = 0.

Also assume complete dominance at A and B loci. Then, the genotypic values are as shown in Table 11.

Table 11 Hypothetical example to demonstrate complete dominance at A and B loci.

Genotype	Genotypic value
A1A1B1B1	7
A1A1B1B2	7
A1A1B2B2	4
A1A2B1B1	7
A1A2B1B2	7
A1A2B2B2	4
A2A2B1B1	3
A2A2B1B2	3
A2A2B2B2	0

Proportions

If a breeder makes selections based only on phenotype, she/he will select plants that have the following genotypes (A1A1B1B1, A1A1B1B2, A1A2B1B1, and A1A2B1B2) in the proportions listed below (with the assumption of independent assortment at the A and B loci).

A1A1B1B1 = 1/16 of the total population,

A1A1B1B2 = 2/16 of the total population,

A1A2B1B1 = 2/16 of the total population,

A1A2B1B2 = 4/16 of the total population,

In this case, the breeder is not able to distinguish between homozygous and heterozygous individuals of the four genotypes above as they have similar phenotypes.

Therefore, if a breeder only wanted homozygous dominant (A1A1B1B1) plants and they only used phenotype to make their selection, they will end up selecting 9 out of 16 plants; however, only 1 out of 16 plants should have been selected.

In a **self-pollinated** species where a cultivar is an inbred line, non-additive gene effects can rarely be fixed, and therefore, selection response is unpredictable when a trait is controlled by genes acting in a non-additive manner. In a **cross-pollinated** species where hybrid cultivars are used, non-additive gene effects, especially dominance effects, are important.

Types of Interaction

In the example below (Table 7; Fig. 6), we will look at a hypothetical case of two genes controlling plant height to demonstrate epistatic effects (i.e., the interaction of genes at different loci).

Assume that 'A' and 'B' loci both affect plant height (shown in cm in Table 12).

Table 12 Genotypic values for two loci 'A' and 'B' controlling plant height.

	B1B1	B1B2	B2B2
A1A1	90	105	110
A1A2	90	95	102
A2A2	90	94	95

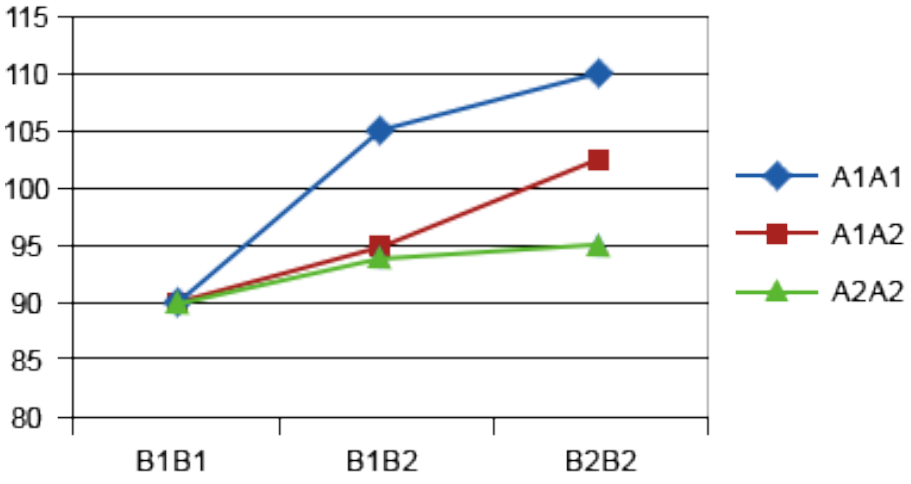

Fig. 6 Plot of the genotypic values for two loci 'A' and 'B' controlling plant height. Non-parallel lines indicate epistatic interaction.

Epistatic interactions can be additive*additive, dominance*dominance or additive*dominance, or a higher order for three loci or more. These interactions are important for most traits as these interactions are common.

Concept of Variability

Johannsen Experiment

If you received a seed lot that had variable seed size, you can select for small and large seed types and grow these seed as individual plants. Each individual plant can be harvested separately and a unique ID given to each row of plants of these individual plants. If you grew each row and measured seed size, what will you expect to find among and within lines in the progeny generation if the original seed lot consisted of a homogenous mixture of purelines (true breeding = homozygous)?

Johanssen, in 1903, conducted an experiment in beans (*Phaseolus vulgaris*), a highly self-pollinated species, to study the effect of selection for seed weight using a seed lot from the cultivar 'Princess'. His experiments showed that: a) selection for seed weight was effective in the original unselected population (i.e., lines selected for differences in seed weight showed consistent differences in seed weight in subsequent generations; large seeded parents produced large seeded progeny and small seeded parents produced small seeded progeny), and b) selection within a line was not effective (i.e., irrespective of whether the parent was small or large seeded, all of the progeny of the selected seed always showed the average seed weight typical of the parent line).

Johanssen concluded from this experiment that the original seed lot was composed of a mixture of different genotypes/purelines (that were each homozygous for genes controlling seed weight), and even when the progeny of a seed lot differed phenotypically, each seed of that line possessed the same genotype for seed weight.

Mathematical Representations

Mathematically, the phenotypic value for an individual (i.e., a single seed in Johanssen's experiment) in a population is equal to its genotypic value plus an environmental (non-genetic) deviation:

$$P = G + E$$

where:

P = phenotype (observed seed weight)

G = genotype (genetic potential for seed weight)

E = environment (environmental effects, i.e., factors determining the extent to which genetic potential is reached)

For a population of seed, the phenotypic variability is represented mathematically by the equation below:

$$V_P = V_G + V_E$$

where:

V_P = phenotypic variability (total variability observed)

V_G = genotypic variability (variability due to genetic cause)

V_E = environmental variability (variability due to environmental causes)

Genetic variability is heritable, i.e., variability that can be manipulated by plant breeders and transmitted to progeny. The presence of genetic variability (as we saw earlier) is ESSENTIAL for selection to be effective.

Environmental variability is not heritable and it can mask the true expression of a trait.

Phenotype Interactions

If we assume that the mean value of "E" for all individuals across the population is zero, then the mean phenotypic value equals the mean genotypic value. Thus, the population mean is both the phenotypic and genotypic value. To prove this, consider a theoretical experiment using replicated genotypes – either as clones or as inbred lines – and measure them under "normal" environmental conditions. The mean "E" will be zero across the population, so that the mean phenotypic value would equal the mean genotypic value.

However, in reality, plant breeders deal with segregating populations that are not genetically uniform when they are selecting. So let's explore the types of gene actions and their importance to breeding.

Phenotype, Genotype, Environment, and Genotype X Environment Interactions

Phenotype is governed by Genotype (σ^2_G), and Genotype × Environment interactions (σ^2_{GE}). Not all variation for a phenotype is accounted for by Genotype and GxE interaction with the remaining variation attributed to error. Any trait that you observe for a plant, a plant family, or population is a phenotype. Genotype is the genetic basis of a trait (e.g., gene or gene × gene interactions). GxE interaction is the interaction of genotype with environment, where each genotype may perform or look different in different environments. The environment of a single plant consists of all things other than the genotype of the individual.

The environment includes differences in soil, temperature, humidity, rainfall, day length, solar radiation, wind, salinity, pathogens, pests, etc.

Environment

Environment can be micro-environment or macro-environment. A micro-environment refers to a unique set of factors that alter the development of a single plant. Groups of plants growing at the same time in the same space each encountering similar micro-environment are classed under experiencing a macro-environment (i.e. a class of micro-environments). For example, if a field of beans is exposed to excessive moisture stress (i.e., water logging), individual plants may suffer slightly different levels of water logging (micro-envirionment), but all plants would suffer some degree of water logging (macro-environment). This beans field's macro-environment will be described as water logged. In breeding, we are more interested in the macro-environments and these are classified as location or year, or a combination of location x year, or simply environment.

To describe the phenotypic value of a genotype in terms of microenvironment and macro-environment, let's consider the equation below:

$$P_{ijk} = G_i + E_k + (G * E)_{ik} + e_{ijk}$$

where:
G_i = effect of the i^{th} genotype
E_k = effect of the k^{th} macro-environment
$(G * E)_{ik}$ = effect of interaction between i^{th} genotype and k^{th} macro-environment

e_{ijk}= residual composed of deviation of the j^{th} micro-environment from the mean of such effects in the macro-environment k, and deviation of the interaction from the mean of interactions.

Graphical Representations of GxE Interaction

Assume two genotypes are tested at two locations. On the y-axis, we present yield/ha, and the x-axis is environment (i.e., locations). Figs. 7, 8, and 9 show different types of GxE interaction:

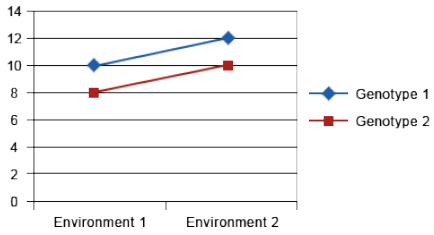

Fig. 7 Example of no Genotype x Environment interaction.

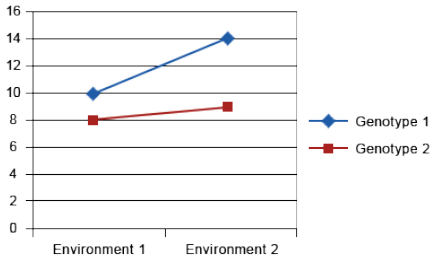

Fig. 8 Example of Genotype x Environment interaction (non-crossover) illustrating a change in magnitude but not in rank.

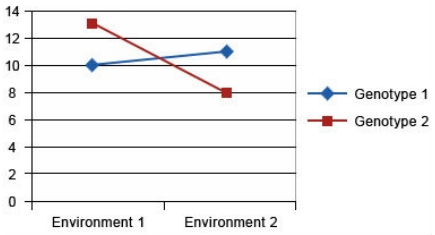

Fig. 9 Example of Genotype x Environment interaction (crossover) illustrating a change in rank.

Crossover Interaction

Crossover interaction is the most important type of genotype x environment interaction because different genotypes will be selected in different environments. Crossover interactions are often due to differences in how genotypes respond to different environments. For example in Figure 6, let's consider that environment 1 is disease free, while pathogen 'A' is present in environment 2. Genotype 2 is a high yielding, disease susceptible genotype and genotype 1 is a lower yielding, disease resistant genotype. Genotype 2 will yield higher in environment 1, while genotype 1 will yield higher in disease prevalent environment 2. The goal of a breeder should be to combine the better response to both environments into a single genotype. However, a breeder needs to first determine if yield and disease resistance are mutually exclusive. For example, it's possible that the gene for disease resistance could be linked to gene(s) that reduce yield. In this case, a breeder would need to grow a large segregating population to identify progeny with useful recombination that combines high yield and disease resistance. If however, a single pleiotropic gene controls both disease resistance and yield, a breeder can only improve both traits by

complementation (i.e., the building or bringing together of other useful genes to improve the responses).

Practical Considerations of GxE Interaction for Plant Breeders

1. Breeders working at international institutions (CGIAR institutes such as CIMMYT, ICARDA, IRRI, and CIAT) have a mandate of a wider adaptation, while a provincial or state breeding institute's mandate will be more localized (specific area, perhaps one macro-environment). CGIAR breeders often utilize many (more than 20) diverse locations to identify cultivars with wider adaptation, while breeders at a state breeding institute use fewer environments that are representative of one or two macro-environments.
2. If the mandate of the program is to develop cultivars for specific purposes (i.e., disease resistance, stress tolerance, or quality traits), then the testing sites need to be selected by breeders for this objective. For example, malt barley has a very specific crop quality requirement. Stable performance on quality (malt quality for consistent and high quality and better taste for beer-making) is a must and breeders will discard cultivars if they show specific adaptation for malt quality, i.e., only very specific sites produce good malt.
3. Resource allocation: A breeder should be aware of the relative importance (i.e., magnitude) of G × Location, G × Year, and G × Location × Year interactions in order to appropriately allocate resources for cultivar testing. This information will help a breeder decide on how many locations and years should be used for testing materials.
4. While we have not discussed different stability analysis methods, these methods (such as AMMI type analysis) will help to determine which environments are more similar to each other. Each mega-environment will consist of several individual locations or sites. Within a mega-environment, the genotypes perform more similarly compared to genotypes in different mega-environments. In other words there is little or no G×E interaction among environments within a mega-environment. In such a scenario, breeders will gain little by testing in more similar environments, and should aim to test across dissimilar environments to test for stable performance of genotypes in a range of environments. Breeders should therefore aim to sample one or more locations from each mega-environment (or testing zone). Environmental parameters such as rainfall, soil type, pH, etc., may also be a good way to cluster environments. If there are larger agro-ecological regions that grow predominantly a single cultivar and you as a breeder are targeting for that region, the cultivar acreage map may also serve as a good source to identify mega-

environments to develop a better yielding alternative to that large acreage cultivar. Another useful exercise is to perform genetic correlations (genotype means analysis) to see if the correlations are high or low. High correlation will mean that predictive ability of those environments is similar and a breeder does not gain as much information on stability as he/she would gain by testing in environments with lower correlation among genotype means.

5. If G×E cannot be measured (due to lack of resources to have more than one site), a breeder should still consider putting that test in two dissimilar conditions, for example dryland versus irrigated nurseries.

Selecting a Testing Site

The site where you grow your trials may have seasonal patterns, such as cycles of drought at seedling stage, heat stress at flowering stage, and years with no apparent stress. It is advisable to keep track of this information (through the use of long term checks that have known and consistent response to these stresses and weather parameters) and use this site for selection to improve tolerance for these factors. If you are breeding for another trait, say salinity tolerance, a new site more appropriate for screening of this trait will be required.

Factors to consider in the selection of testing site include the following:

1. Good correlation with the performance in farmer growing conditions.
2. Ability to handle different tests (infrastructure) and ability to respond to mitigate threats (such as an ability to irrigate if needed to avoid impact of water deficit on response to the selected treatment).
3. Low environmental error, i.e., higher heritability to differentiate 'keeps' (i.e., desirables) from discards.
4. Infrastructure to implement breeding decisions of timely planting, maintenance, harvest, processing etc.

Adaptation Factors

As we previously covered, the debate on wide versus specific adaptation is still ongoing among breeders and really boils down to:

- the mandate of the program
- target region, and
- farmers.

Ultimately, breeders need to remember that their job is to ensure that the product (cultivar) that reaches farmers can help them to make a profit, and that it meets the requirement of end-user (e.g. the processing industry) who buys from farmers.

If you, a breeder, produce the highest yielding cultivar but it lacks the necessary quality or protection against biotic or abiotic stress, farmers will not grow this variety. Therefore, always think of the cultivar you develop as a "package". A package needs to have all the ingredients that will make it ready to be adopted by farmers as well as the processing industry.

Heritability

Concept

Definition: Heritability can be defined as the degree to which the characteristics of a plant are repeated in its progeny. Mathematically, we have seen already that it is the proportion of total variability for a character due to genetic causes.

Further theoretical information can be obtained here.

$$\text{Broad sense heritability: } \left(h_b^2\right) = \sigma_G^2 / \sigma_P^2$$

$$\text{Narrow sense heritability: } \left(h^2\right) = \sigma_a^2 / \sigma_P^2$$

where:
σ_G^2 is total genotypic variance
and
σ_a^2 is the additive component of the genotypic variance.

Narrow sense heritability is more valuable since it indicates how much of the total observed variability is due to additive gene action (which can be selected for effectively and fixed in homozygous condition).

Broad sense heritability is less valuable since it also includes dominance and epistasis (these gene actions cannot be fixed and occur only in specific gene combinations).

Importance

It is important for breeders to have a sense of the heritability of traits that they are selecting for in their programs. This can be obtained by using data from their own experiments, or for a new program, using information available in literature or from previous experiments. The reason heritability is important is that selection response is related to heritability. The higher the heritability, the more the phenotype reflects the genotype and the more effective selection will be. More extensive testing (more environments, more replications) reduces the phenotypic variance and increases heritability. Heritability can be increased more by using higher number of locations rather than by increasing the number of years [this is due to smaller variance component of Genotype × Year, relative to other component such as Genotype × Location]. This suggests that in most cases, a breeder does not need to do selection based on more than one year of data [except in cases where selections are made in each generation as plants are achieving 'true breeding' status].

Methods for Estimating Heritability

1. *Variance Component method*: Comparison of segregating and homogenous populations is applicable to only self-pollinated or clonally propagated species. This method estimates broad sense heritability. This method involves estimating the magnitude of various types of genetic and environmental variability.
   In such experiments, $\sigma^2_{P1} = \sigma^2_{P2} = \sigma^2_{F1} = \sigma^2_E$
   In a self-pollinated species, parent 1 and parent 2 being inbred, their estimates of genetic variability will be similar to each other ($\sigma^2_{P1} = \sigma^2_{P2}$) as well as to F_1 ($= \sigma^2_{F1}$), and these will be equal to environmental variability (as these three are genetically uniform and therefore any variability observed will be due to environment). Variance of F_2 or any other segregating generation can then be used to obtain $\sigma^2_P - \sigma^2_E$), where σ^2_P is variance estimated from the segregating generations.
2. *Covariance between relatives* (or resemblance among relatives as measured by regression analysis): Examples are, parent-offspring regression, covariance between half-sibs and full-sibs, covariance between inbred or partially inbred families. Variability estimates can be

obtained in other ways such as special mating designs (half-sib, full-sib, North Carolina designs, etc.), or analysis of trials conducted in a range of environments.

3. Realized heritability [see Response to Selection section, below]

Example 1

Table 13 is an example of analysis of variance (ANOVA) where a random set of genotypes were evaluated over '*l*' locations for '*y*' years, and '*r*' replications used in each test. Multiple locations, years and reps.

Table 13 ANOVA formulas for determining degrees of freedom, mean squares, and expected mean squares for a multi-location, multi-year trial.

Source of variation	Degrees of freedom	Mean square	Expected mean square
Location (L)	$(l-1)$	n/a	\sigma ^2_{e} + r\sigma ^2_{gly} + ry\sigma ^2_{gl} + g\sigma ^2_r + gr\sigma ^2_{ly} + gry\sigma ^2_l
Year (Y)	$(y-1)$	n/a	$\sigma_e^2 + r\sigma_{gly}^2 + ry\sigma_{gl}^2 + g\sigma_r^2 + gr\sigma_{ly}^2 + grl\sigma_y^2$
$L * Y$	$(l-1)(y-1)$	n/a	$\sigma_e^2 + r\sigma_{gly}^2 + ry\sigma_{gl}^2 + g\sigma_r^2 + gr\sigma_{ly}^2$
Rep ($L * Y$)	$ly(r-1)$	n/a	$\sigma_e^2 + g\sigma_r^2$
Genotype (G)	$(g-1)$	MS_1	$\sigma_e^2 + r\sigma_{gly}^2 + ry\sigma_{gl}^2 + rl\sigma_{gy}^2 + rly\sigma_g^2$
$G * L$	$(g-1)(l-1)$	MS_2	$\sigma_e^2 + r\sigma_{gly}^2 + ry\sigma_{gl}^2$
$G * Y$	$(g-1)(y-1)$	MS_3	$\sigma_e^2 + r\sigma_{gly}^2 + rl\sigma_{gy}^2$
$G * L * Y$	$(g-1)(l-1)(y-1)$	MS_4	$\sigma_e^2 + r\sigma_{gly}^2$
Pooler Error	$ly(g-1)(r-1)$	MS_5	σ_e^2

where:

$\sigma_e^2 = MS_5$

$\sigma^2_{gly} = (MS_4 = MS_5)/r$
$\sigma^2_{gy} = (MS_3 - MS_4)/rl$
$\sigma^2_{gl} = (MS_2 - MS_4)/ry$
$\sigma^2_g = (MS_1 - MS_2 - MS_3 + MS_4)/rly$

σ^2_P (phenotypic variance of genotypic means) = $\sigma^2_g + \frac{\sigma^2_{gl}}{l} + \frac{\sigma^2_{gy}}{y} + \frac{\sigma^2_{gly}}{ly} + \frac{\sigma^2_e}{rly}$

and if we substitute for Mean squares we will obtain,

$$\sigma^2_p = \frac{MS_1}{rly}$$

Broad Sense heritability can be calculated using the equations above.

Example 2

Table 14 is an example of analysis of variance (ANOVA) where a random set of genotypes were evaluated over 'e'
environments (can be locations, years or combination of years and locations), and 'r' replications used in each test. Multiple environments and reps within a year.

Table 14 ANOVA formulas for determining degrees of freedom, mean squares, and expected mean squares for a multi-environment trial.

Source of variation	Degrees of freedom	Mean square	Expected mean square
Environment (E)	$(e-1)$	n/a	$\sigma^2_\varepsilon + g\sigma^2_{r(e)} + r\sigma^2_{ge} + rg\sigma^2_\varepsilon$
Rep (E)	$e(r-1)$	n/a	$\sigma^2_e + g\sigma^2_{r(e)}$
Genotype (G)	$(g-1)$	MS_1	$\sigma^2_e + r\sigma^2_{ge} + re\sigma^2_g$
$G * E$	$(g-1)(e-1)$	MS_2	$\sigma^2_e + r\sigma^2_{ge}$
Pooler Error	$e(g-1)(r-1)$	MS_3	σ^2_e

Note: all factors considered random in ANOVA.

$\sigma_e^2 = MS_3$

$\sigma_{ge}^2 = (MS_2 - MS_3)/r$

$\sigma_g^2 = (MS_1 - MS_2)/re$

Variables

σ_p^2 (phenotypic variance of genotypic means) = $\sigma_g^2 + (\sigma_{ge}^2)/e + (\sigma_e^2)/re$,

If we substitute for Mean squares we will obtain, $\sigma_p^2 = (MS_1)/re$

Total phenotypic variance $\sigma_p^2 = \sigma_g^2 + \sigma_{ge}^2 + \sigma_e^2$

Heritability on individual *experimental unit basis* is: $\dfrac{\sigma_g^2}{(\sigma_g^2 + \sigma_{ge}^2 + \sigma_e^2)}$

Heritability on genotypic *mean basis* is: $\dfrac{\sigma_g^2}{(\sigma_g^2 + (\sigma_{ge}^2)/e + (\sigma_e^2)/re)}$

This estimate of heritability is obtained if the genotypes represent the population and are chosen randomly. If the genotypes are not chosen randomly (e.g. selected genotypes), the ratio between genetic and phenotypic variation is called *repeatability* and this estimate is a measure of the precision of data and a measure of the proportion of genetic variation, which helps breeders to detect significant difference among genotypes.

Summary

Each heritability estimate is unique and reflective of the method of calculation, testing environment, generation used in estimation, and genotypes studied. While the heritability estimates are going to somewhat differ based on different conditions described above, a plant breeder can get a good handle on heritability based on published literature, and their or their predecessors' experiences working on the crop and for various traits.

Heritability is used to estimate the expected response to selection and to choose the best

breeding approach to improve the target trait(s). Traits with high heritability can be selected on a single-plant basis in an early generation and in fewer (even single) environments.

A breeder should consider a range of heritability (rather than absolute value) as well as have some precision around their estimate (confidence interval). Higher heritability, say, 0.7, or higher narrow sense heritability means a breeder can expect that selection in early generation can be effective for that trait. High broad-sense heritability only indicates that effect of environment is smaller but does not provide insight into the relative importance of additive (which can be fixed) or non-additive (which cannot be fixed) gene effects.

Selection Theory

Truncation

When individuals are selected based on their individual phenotypic value, we call this artificial selection or individual selection. Truncation is a type of individual selection and very common in plant breeding programs. The curve in Figure 10a represents the normal distribution of a quantitative trait in a population, and the shaded part 'T' (i.e., truncation point) represents the individuals selected for the next generation of breeding – could be cross- or self-pollinated. μ is the mean of the unselected population (or mean of the population in generation 1) and μ_s is the mean of selected parents. If these selected parents are mated at random, their offspring will have the phenotypic distribution in Figure 10b and a mean equal to μ'. Generally, $\mu_s > \mu' > \mu$.

- μ' is greater than μ because some of the selected parents have favorable genotypes and therefore pass favorable genes on to their offspring.
- μ_s is greater than μ' because some of the selected parents did not have favorable genotypes, but instead had superior phenotype due to the favorable environment where they were tested (chance exposure to favorable environment, e.g., low spot in the field that received more water, a spot in the field that received more fertilizer, or a spot in the field that was not exposed to high winds). Secondly, alleles, not genotypes, are transmitted to the offspring and favorable genotypes may segregate or recombination may cause breakage of favorable linkages.

Equations

The difference in mean phenotype between the selected parents (μ_s)
and generation 0 (μ)
is called **selection differential** (S).

$$S = \mu_s - \mu$$
Equation 1

The difference in mean phenotype between the progeny generation (generation 1) (μ')
and generation 0 (μ)
is called the **response to selection** (R).

$$R = \mu' - \mu$$
Equation 2

The prediction equation defines the relationship between S
and R. For truncation selection, the prediction equation is:

$$R = h^2 * S$$
Equation 3

where, h^2 is heritability of the trait.

Graphical Representation

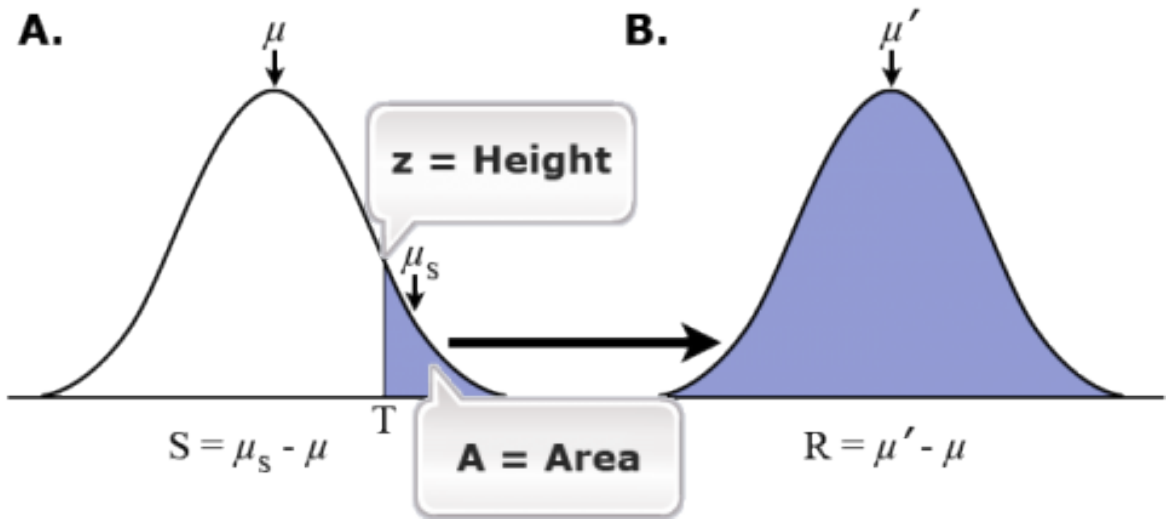

Fig. 10 Example of truncation selection.
A. phenotype distribution in parental population with mean [latex]\mu[/latex]. Shaded area shows the individuals that were advanced to the next generation (individuals with phenotypes above the truncation point ([latex]T[/latex]). Selected individuals (shaded area) have a meaning phenotype ([latex]\mu_S[/latex]).
B. distribution of phenotypes in offspring generation derived from the selected parents. the mean phenotype is donated with ([latex]\mu'[/latex]). [latex]S[/latex] = selection differential, [latex]R[/latex] = response to selection. Source: Harti, D. (1988). Primer of Population Genetics, 2nd edition. Sinauer Association.

z/A = frequency at the truncation point (in a normal distribution)/Area under the selected portion of the curve. This is equal to selection differential/phenotypic variance, i.e.,

$$z/A = (\mu - \mu')/\sigma^2$$

Equation 4

assuming that the effects of each allele are small relative to phenotypic variation, and phenotypic values are normally distributed.

Response to Selection

Calculations

We cannot get into detailed calculation, but it can be shown that:

$$\mu' = \mu + 2\Big[a + (q - p)d\Big]\Delta p$$

Equation 5

where:

Δp = change in allele frequency of A1 $= (z/A) * pq\Big[a + (q - p)d\Big]$

a = genotypic effect
d = measure of dominance
p = allele frequency of A1
q = allele frequency of A2
μ' = mean phenotype of progeny generation
μ = progenitor generation

Substituting z/A = (μ_s – μ)/ σ^2_p and Δp, equation can be written as:\

$$\mu - \mu' = \Big\{(\mu_s - \mu) \times 2\text{pq} \times [\text{a} + (\text{q-p})\text{d}]^2\Big\}/\sigma^2_\text{p}$$

Equation 6

Since, S = μ_s – μ and R = μ' – μ,

$$\text{R} = \Big\{\text{S} \times 2\text{pq} \times [\text{a} + (\text{q-p})\text{d}]^2\Big\}/\sigma^2_\text{p}$$

Equation 7

Additive Genetic Variation

We already have seen that **R** = $\mathbf{h^2S}$ (Equation 3), therefore, we can now define heritability in the genetic terms of: a, p, q, d, σ^2_p as:

$$h^2 = \left\{ 2pq \times [a + (q\text{-}p)d]^2 \right\} / \sigma_p^2$$

Equation 8

This heritability definition is valid when the trait is under single gene control. This is hardly the case for most of the traits, therefore heritability (narrow sense) can be defined as:

$$h^2 = \left\{ \sum 2pq \times [a + (q\text{-}p)d]^2 \right\} / \sigma_p^2$$

Equation 9

$\mathbf{\Sigma 2pq[a + (q - p)d]^2}$ = additive genetic variation of the trait = σ^2_a

Selection Intensity

Selection Intensity and Response to Selection Equation

$\mathbf{R = h^2 S}$ can also be written as:

$$R = i\sigma h^2$$

Equation 10

$$S = i\sigma = \sigma^2 z / A$$

Equation 11

where:

i = standardized selection differential.

Intensity of selection depends on the proportion of the total population selected. For example, Table 15 shows some selection intensities based on % selected (assuming a completely normal trait distribution). The full table can be seen in most plant breeding books.

Table 15 Percent of individuals selected and the corresponding standardized selection differential.

% selected	Standardized selection differential (i)
0.01	3.959
0.1	3.367
0.5	2.892
1	2.665
5	2.063
10	1.755
15	1.554
20	1.400
25	1.271
30	1.159
35	1.058
40	0.966
45	0.880
50	0.798

h^2 is narrow sense heritability

σ_p = phenotypic standard deviation (=square root of phenotypic variance)

Breeder's Equation

This equation $R = i\sigma h^2$ (Equation 10) is fundamental in plant breeding. Plant breeders generally do not use it to calculate an actual numerical value for selection response. However, this equation is important as it shows that selection response depends on:

1. Selection intensity
2. Heritability
3. Phenotypic variability present in the population, say from a cross.

The equation R = μ' – μ can be written as

$$\mu' = \mu + i\sigma h^2$$

Equation 12

which clearly indicates that to maximize the expression of a trait in the offspring generation, a breeder needs to start with high expression of the trait, maximum heritability, and high selection intensity (although diminishing returns apply beyond a certain level).

Square root of heritability (h) is a measure of the correlation between the observed phenotypic value and the underlying genotypic value. In a breeding program, a breeder will try to maximize these factors (higher standardized selection differential, genetic variation, heritability). One has to keep in mind that optimum balance needs to be obtained between increased expected response (which is a good thing and what a breeder is after) and increased variability of that response (undesirable characteristics of selection). We will look at a few examples below to understand the concept of variability of response.

$$(C.V.\ of\ R) = \sqrt{\frac{1 + (p \times h^2) - (h - h^2)}{p \times n \times i \times i \times h^2 \times h^2}}$$

Equation 13 (From Baker, 1971.)

where:

n = total number of lines evaluated

p = proportion of lines selected

h^2 = heritability

i = standardized selection differential

C.V. = coefficient of variation

For example, assume a breeder started with n=1000, % selected (p) = 0.01, heritability (h^2) = 0.2, and i = 3.959, then CV (Response to selection) = 39% by substituting values in Equation 10.

n = total number of lines evaluated = 1000

p = proportion of lines selected = 0.01

h^2 = heritability = 0.2

i = standardized selection differential = 3.959

C.V. (R) = 30%

Compared to starting with a smaller number of lines, if a breeder started with n=100, % selected = 0.01, heritability = 0.2, and i = 3.959; CV (Response to selection) = 124%.

n = total number of lines evaluated = 100
p = proportion of lines selected = 0.01
h^2 = heritability = 0.2
i = standardized selection differential = 3.959
C.V. (R) = 124%

Importance of h-squared

However, h^2 importance can be seen with the same calculations: if a breeder started with n=1000, % selected = 0.01, heritability = 0.7, and i = 3.959; CV (Response to selection) = 8%.

n = total number of lines evaluated = 1000
p = proportion of lines selected = 0.01
h^2 = heritability = 0.7
i = standardized selection differential = 3.959
C.V. (R) = 8%

If breeder had started with n=1000, % selected = 5, heritability = 0.7, and i = 2.063; CV (Response to selection) = 2%.

n = total number of lines evaluated = 100
p = proportion of lines selected = 5
h^2 = heritability = 7
i = standardized selection differential = 2.063
C.V. (R) = 2%

These calculations show that while the response to selection equation is an essential equation for any breeder to consider for trait improvement, it is also worthwhile to consider the extent of variability in relation to the mean of the population (CV).

Selection Explanation

One of the ways to maximize genetic standard deviation is to cross diverse parents. However,

if crosses between diverse parents have lower unselected means than crosses between adapted (elite) parents (which will have lower genetic variability between them, generally), then a breeder may be reducing the mean genotypic value of the subsequent population by crossing diverse parents. Therefore, best x best (or elite x elite) crosses is one way to maximize genotypic mean of the starting population although it may reduce the genotypic variance and even response to selection, R. Most cultivar development programs will work with best x best configuration, or have at least 75% elite, for example, (best x exotic) x best.

Standardized selection differential can be increased by selecting fewer lines (but we saw earlier that this can cause increased variability of response, which is undesirable) or testing more units but selecting fewer units (this will require more resources). If a breeder makes compromises between testing more lines to advance a few, it will likely be done at a compromise of not doing a thorough evaluation of units. Less thorough evaluation will result in lower correlation between phenotypic and genotypic values (lower h), therefore a breeder should not compromise on proper trait measurement protocols. Optimum balance needs to be achieved for each trait for more thorough testing to increase the correlation between phenotypic and genotypic values (higher h) as well as increase the standardized selection differential.

Expected Genetic Gain

Expected genetic gain **formula** is shown below.

$$\Delta G = \frac{ic}{y}\frac{\sigma_G^2}{\sqrt{\sigma_p^2}} = \frac{ic}{y}\frac{\sigma_G^2}{\sqrt{\frac{\sigma_e^2}{re} + \frac{\sigma_{GE}^2}{e} + \sigma_G^2}}$$

Equation 14

This formula is an extension of response to selection:

$$R = h^2 S = ih^2\sigma_P = \frac{i\sigma_g^2}{\sqrt{\sigma_P^2}} = \frac{c}{y} = \frac{i\sigma_g^2}{\sqrt{\sigma_P^2}}$$

Equation 15

and includes two additional variables: number of years (y) and parental control (c) (Eberhart, 1970) compared to what we have seen so far.

Heritability equation is

$$\frac{\sigma_g^2}{(\sigma_g^2 + (\sigma_{ge}^2)/e + \sigma_e^2)/re)}$$

Equation 16

where:
r =number of replications
e =number of environments

We have already looked at i, which is standardized selection differential, c = parental control, and y = seasons per cycle.

Practical Considerations

1. Increase the numerator of this equation by increasing genetic variance (larger population sizes, diverse parents (but keep the proportion of elite parents high), increasing selection intensity (without getting genetic drift problem).
2. Parental control will allow for increased response to selection. Parental control, c, can be increased by recombining genotypes where both sources of gametes originated in selected genotypes (c = 1), which will be generally true in self-pollinated crops. In cross-pollinated species, c = 0.5 if the male gametes are coming from unselected genotypes. Therefore, it is recommended that if possible, conduct selection before pollination so that only selected genotypes contribute to the next generation. C = 2.0 if the selected seed of selected genotypes is used for establishing the next generation.
3. Another way to increase genetic gain is to decrease the value of the denominator. This can be achieved by decreasing the number of seasons per cycle or the phenotypic variance (which can be decreased by reducing g x e and e variances. The phenotypic variance can be decreased by increasing the number of locations (or environments) and by increasing replication. Increasing locations is generally considered to play a more important role in reducing phenotypic variance rather than replications.
4. Usage of proper experimental methods and field design and analysis will reduce error

variance and improve confidence in estimate of progeny performance. These methods may include augmented designs, or moving means in earlier generations where no replication is used per environment and using incomplete lattice (for example, alpha-lattice) or RCBD in replicated tests.

5. Different generations and type of progenies have different genetic variance components and therefore affect the equation. The theoretical proportion of additive variance to total genotypic variance of half sib is 0.25, full-sib is 0.5 and 1 for S_1 progenies.
6. The number of seasons required to complete a cycle can be reduced by using off-season nurseries, or by using an off-season nursery with high correlation to the home environment to facilitate selection for high to moderate heritability traits and to reduce the 'y' in equation above. If resources permit, greenhouse or growth cabinet can be used instead of off-season nursery and complemented with marker assisted selection to increase 'i' as well as reduce 'y'.

Reducing Effect of Environment

One way to obtain higher heritability is to reduce environmental effects (remember, higher heritability implies that selection will be more effective as 'what you see is what you will get').

Here are some recommendations to reduce the effect of environment:

1. Use best quality land (uniform area – less gradients in field, highly productive) if selection will be performed (on single plant, rows, or yield trials; in early or later generations).
2. Use best management practices (reflective of the recommended fertilizer, irrigation, crop rotation, time of planting, weeding, harvesting). An advice is to avoid pest or pathogen control as this will provide another trait to select for if naturally present].
3. Use check cultivars frequently (this will allow breeders to have a better handle on variability).
4. Use appropriate statistical designs (lattice, RCBD, augmented designs as needed).
5. Use replication (improves precision, and provides better handle to measure variation) and randomization (improves accuracy).

Multiple Trait Selection

1. **Tandem selection**: A breeder selects sequentially for each trait in successive generations.

In this scenario, population is improved first for one trait, then for the next trait and so on. This will lead to improvement over generations. One disadvantage of this strategy is long selection cycle, and is generally not followed in commercial plant breeding. Another disadvantage of this strategy is the potential reduction in the level of performance of the first trait selected.

2. **Independent culling** (**or truncation selection**): Selection is practiced successively in the same generation. This is probably the most common selection strategy deployed by breeders worldwide. In this scheme, a breeder will discard all individuals that fail to meet the desired level for one trait, irrespective of the value for any of the other traits. This will be followed by selecting among the surviving lines for the second trait and the process is repeated until all selections are made. Experienced breeders will know the culling point keeping in mind the trait value of the most important trait, and may allow some relaxation for major traits when culling for traits that have less significance or importance. One issue with independent culling is that with each successive trait cull, the population size and genetic variability is reduced.
3. **Index selection**: An index is developed based on the combination of the heritability and economic value of each of several traits under selection, simultaneous selection is happening for every trait in the same generation. Each line is given an index score based on the trait expression and weight given to the trait. Most breeders use a "mental" index selection. For example, visual selection may be done for a number of traits, an overall mental assessment done, and selection is made. For example, in a space planted nursery where single plants are growing, a breeder may make a mental assessment on the criteria for different traits such as height, seed fill, plant health, lodging, inflorescence and either keep or discard. Since there are likely several thousand plants in a nursery, "mental" index approach needs to be used, as the most feasible. There are more sophisticated methods described such as Pesek baker index (Pesek and Baker, 1969) but these require estimation of variance and co-variances. Using economic weights is a good compromise to remove the need to know variance and co-variances. However, it is still not an easy task to develop an index.

Combining Ability

GCA and SCA

Combining ability of inbred lines is of paramount importance in determining future usefulness and commercial potential of the inbred lines for hybrid production. Combining ability can be divided into general combining ability (GCA) and specific combining ability (SCA) (Sprague and Tatum, 1942). This concept has been very important in the commercial success of maize breeding and hybrid development.

GCA is defined in terms of the average performance of a line in hybrid combinations. The GCA is calculated as the average of all F_1s having this particular line as one parent, the value being expressed as a deviation from the overall mean of crosses.

SCA is defined in terms of instances in which performance of certain hybrid combinations (between two inbred lines in a single cross) is either better or poorer than would be expected based on the average performance of the parent inbred lines. That is, each cross has an expected value that is the sum of GCAs of its two parental lines. However, each cross may deviate from the expected value to a greater or lesser extent, and the deviation is referred to as the specific combining ability (SCA) of the two lines in combination. Estimates of GCA and SCA are applicable to the particular set that a breeder has used in the crossing. These crossings are generally in a diallel design (full or partial, or other designs such as NC designs). Sprague and Tatum (1942) reported that for unselected inbred lines, GCA was relatively more important than SCA, whereas for previously selected lines SCA was more important than GCA. GCA is an indication of genes having largely additive effects (differences of GCA are due to the additive and additive × additive interactions in the base population) and therefore more important in a population such as synthetics, while SCA is indicative of genes having dominance and epistatic effects (differences in SCA are attributable to non-additive genetic variance) therefore more important in a hybrid combination.

Calculations

NOTE: GCA is the average performance of a plant in a cross with different tester lines, while the SCA measures the performance of a plant in a specific combination in comparison with other cross combinations.

Let us look at some calculations:

As we previously described, deviation of the parent mean (X) from the mean of all crosses or population mean (μ) is the general combining ability, therefore we can calculate GCA as:

$$GCA = X - \mu$$

GCA of a parent A can be defined as

$$Y_i = \mu + GCA_A + e_i$$

Example

Below is an example to show GCA calculation using an experiment where eight inbreds were mated to produce 16 crosses. Response variable was grain yield.

Table 16 Grain yield data from a 4 x 4 cross.

Cross mean	**E**	**F**	**G**	**H**	**Half-sib mean**
A	91	92	77	84	86
B	83	86	75	120	91
C	82	98	85	103	92
D	104	112	87	101	101
Half-sib mean	90	97	81	102	92.5

GCA_A -6.5 (=86 – 92.5)
GCA_B -1.5
GCA_C -0.5
GCA_D 8.5
GCA_E -2.5
GCA_F 4.5
GCA_G -11.5
GCA_H 9.5

SCA_{AE} = 3 (i.e., full-sib mean – (mid-parent value)) or [91 – (86+90)/2]
SCA_{AF} = 0.5
Etc.

Diallel Example

In the case of a diallel, the calculations of GCA are shown in Table 17 below.

Table 17 Example calculation of GCA for a diallel.

Cross mean	B	C	D	E	Total	GCA
A	91	92	77	84	344 (=91+92+77+84)	-7.2
B	n/a	86	75	120	372 (=91 + 86 +75 + 120)	2.1
C	n/a	n/a	85	103	366 (=92 + 86 + 85 +103)	0.1
D	n/a	n/a	n/a	101	338 (=77 + 75 + 85 + 101)	-9.2
E	n/a	n/a	n/a	n/a	408 (=84 + 120 + 103 + 101)	14.1
n/a	n/a	n/a	n/a	n/a	1828 (=344+372+366+408)	n/a
Note: "n/a" means cell is blank.						

Expected value of a cross between inbred lines 'A' and 'B' is

$$X_{AB} = \mu + GCA_A + GCA_B + SCA_{AB}$$

where:
μ = general mean

and

$$GCA_A = \left[T_A/(n-2)\right] - \left[\sum T/n(n-2)\right]$$

where:
A represents a specific inbred
T = Mean of hybrid performance across each parent for a trait
n = number of parents used in crosses, which is **5** in this case

(From Acquaah, 2007.)

GCA_A = [344/(5-2)] – [1828/5(5-2)] = -7.2
GCA for other inbreds can be calculated similarly.

Expected value of the cross between A and B = -7.2 + 2.1 + 91.4 = 86.3
[91.4 = average of all SCA's]

The SCA is calculated as follows: SCA_{AB} = 91 – 86.3 = 4.7

Heterosis

Heterosis is the superior performance of crosses relative to their parents (Shull 1910; Falconer and Mackay, 1996). Mid-parent heterosis is the difference between the hybrid and the mean of the two parents used in developing the hybrid and can be calculated as

$$\text{Heterosis}_{(\text{Mid-Parent})} = ((\mu_{F1} - \mu_{MP})/\mu_{MP}) \times 100$$

where:
$\boldsymbol{\mu}$ = trait mean of the hybrid
$\boldsymbol{\mu}_{\mathbf{MP}}$ = trait average of the two parents

High-parent heterosis is the superiority of a hybrid over the better parent.

Heterosis is dependent on the presence of dominance and summation of allele frequency differences across loci. In maize and other cross-pollinated crops, heterotic groups have been created such that they maximize the difference in allele frequencies in genes affecting target trait(s) thereby maximizing heterosis.

Examples of hybrid cultivars include: commercial single-cross maize hybrids, commercial three-way cross maize hybrids, and sunflower hybrids. Hybrid cultivars are usually utilized for

allogamous species but some hybrids are produced for some autogamous species (i.e., sorghum, tomato, rice). Single-cross hybrid cultivars are homogeneous and heterozygous. Three-way hybrids are both heterogeneous and heterozygous.

References

Baker, R J. 1971. Theoretical variance of response to modified pedigree selection. Can J Plant Sci, 51, 463-468.

Eberhart, S.A. 1970. Factors affecting efficiencies of breeding methods. Afr. Soils. 15:669-680.

Pesek, J., and R.J. Baker. 1969. Desired improvement in relation to selection indices. Can. J. Plant. Sci. 9:803-804.

Shull, G.H. 1910. Hybridization methods in corn breeding. Am. Breed. Mag. 1:98-107.

Sprague, G.F., and L.A. Tatum. 1942. General vs specific combining ability in single crosses of corn. J. American Soc. Agron., 34: 923-932.

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Chapter 5: Steps in Cultivar Development

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Cultivar development is one of the four plant breeding projects (other three are genetic improvement, trait integrations and product placement) of many breeding programs. The end product depends on the specific objectives, the mode of propagation and commercial production, and like most other processes, specific steps need to be followed in succession (in parallel in certain cases) to ensure the set objectives are met.

Learning Objectives

- Describe the basic steps in the development of clonal, inbred, synthetic, hybrid, multilines, and blended cultivars
- Distinguish among the different clonal types and source of variation
- Know the application of male sterility in hybrid crop development

Asexually Propagated or Clonal Cultivars

In Chapter 1, we looked at different types of cultivars that are grown by farmers. These cultivars may be sexually or asexually propagated. Clones are types of cultivars that are asexually propagated. We also learned in this module the concept of heterogeneous versus homogenous and heterozygous versus homozygous. Clonal cultivars are heterozygous and homogenous and can be maintained through vegetative plant parts. Figure 1 depicts the gradation of heterogeneity and heterozygosity in different types of varieties.

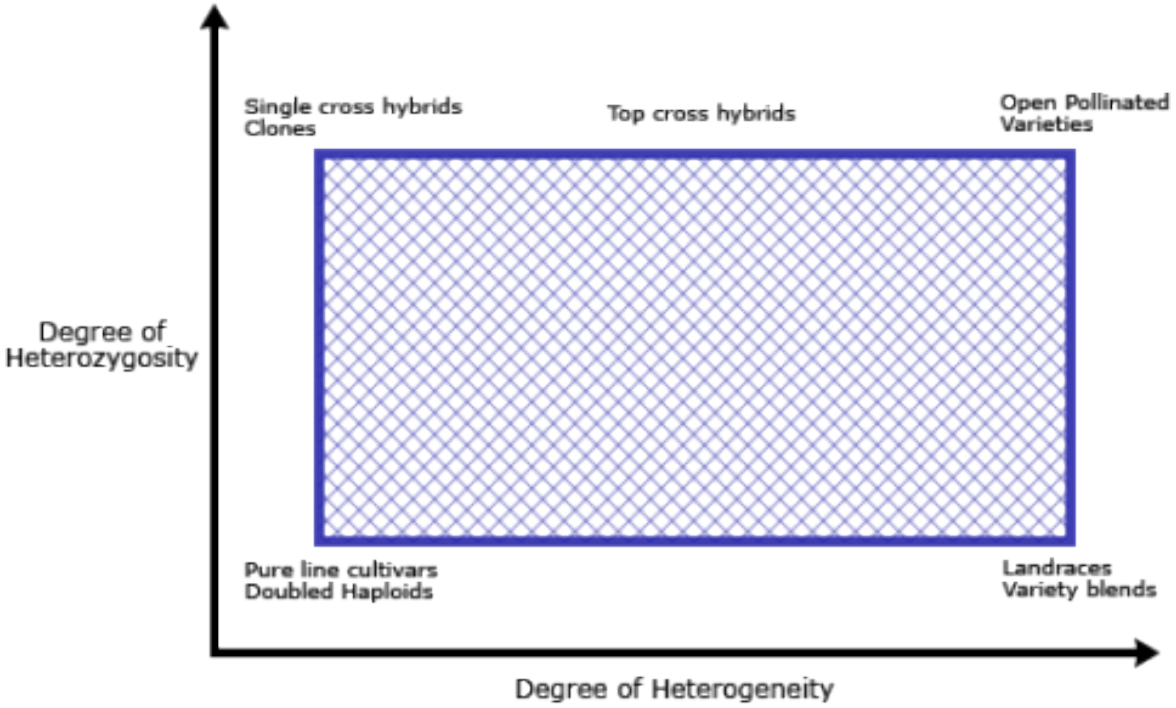

Fig. 1 Degree of heterozygosity and heterogeneity of different types of varieties.

Clones

Clones, as the name implies, are identical copies of a genotype. A population of clones of the same genotype is homogeneous (since they are identical). However, individually, they are highly heterozygous. Asexually or clonally propagated plants produce genetically identical progeny. Since the cultivar can be asexually propagated, heterosis can be fixed as long as the propagation continues. Clones can be the product of inter-generic or inter-specific crosses because even sterile hybrids can be maintained in clonally propagated crops.

One of the biggest challenges in clonal crops production is to keep parental lines and breeding stocks free of virus and other disease that can be transmitted through vegetative propagation.

In-vitro methods (such as, tissue culture) are often used to rapidly increase clonal stocks which can be kept disease free. The tissue culture methods of plant propagation, known as micropropagation, utilizes the culturing of apical shoots, axillary buds and meristems on sterile suitable nutrient medium to grow new clones. Micropropagation offers an ability to continually produce clones (all year round), produce and propagate hybrids, and produce disease free plants. It is a cost effective technique and requires small space.

Somaclonal Variation

Clones are products of mitosis. Any variation occurring among them is environmental in origin. Micropropagation or tissue culturing can lead to somaclonal variation (SV). SV can be defined as genetically stable variation generated through plant tissue culture (Larkin and Scowcroft, 1981). It has been used by breeders as an approach to create and exploit greater genetic diversity and provides a mechanism to expand germplasm pool for plant improvement and cultivar development.

While the resultant success of SV has not been as great as initially promised, it has led to identification of valuable genotypes, for example, Aluminum tolerance in rice (Jan et al 1997). SV can also cause negative effects, therefore mechanism of genotype purity is desirable in clonal crops. Whether natural (spontaneous) or artificial (induced), somatic mutations are characterized by tissue mosaicism, called chimerism. In a chimera, an individual consists of two or more genetically different types of cells, i.e. mosaics, which can only be maintained by vegetative propagation (not transferable to progenies by sexual means as clones are products of mitosis and change did not happen in sex cells).

Examples of Clonal Cultivars

Many clonal cultivars are in use. Fig. 2 and Fig.3 show some examples.

Fig. 2 Examples of vegetative tissue used for the propagation of clonal cultivars. Photos A and B from htttp://www.freeimages.com. Photos C and D by Iowa State University.

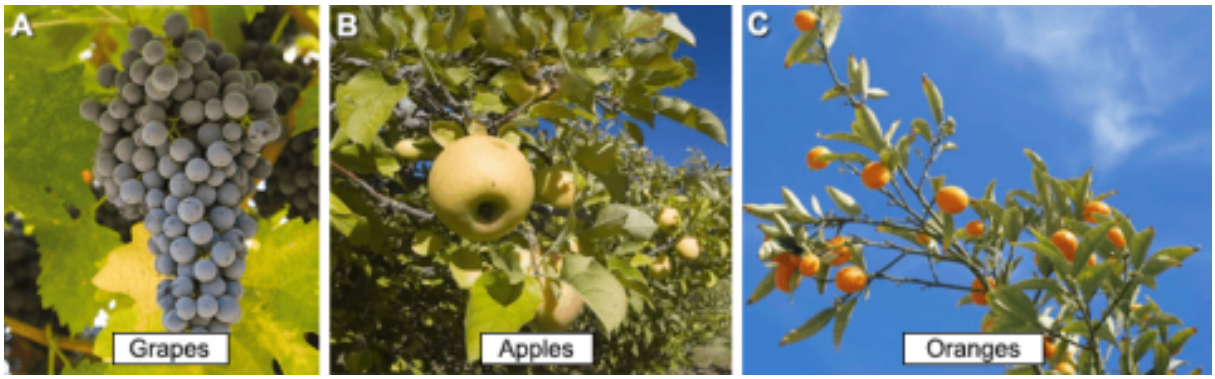

Fig. 3 Examples of plant species for which clonal cultivars are used for commercial production. Photos A and B by USDA NRCS.

Steps in Development of Clonal Cultivars

Clonal cultivars are developed using the micropropagation method, which involves the following steps.

1. Selection and maintenance of stock plants for culture initiation,
2. Initiation and establishment of culture – from an explant like shoot tip, on a suitable nutrient medium,
3. Multiple shoots formation from the cultured explant,
4. Rooting of in vitro developed shoots, and
5. Transplanting and hardening, i.e., acclimatization before transplanting to the field.

Breeding Approaches Used In Asexual Crops

Clean (disease-free) clonal material is essential starting material for multiplication for propagation. It is very important in clonal crops to maintain disease-free and/or purify an infected cultivar (Fig. 4). Infection can occur due to bacteria or viruses. Viruses are more damaging due their systemic nature. In order to screen for disease-free material plant material is visually inspected for the presence of pathogens, however this is not the most effective method for viruses as there may be no obvious symptoms.

Detection of Specific Pathogens

Two main methods are used to detect the presence of specific pathogens:

- Serological, such as enzyme-linked immunosorbent assay (ELISA)
- Nucleic acid based, such as Real Time-PCR.

These techniques can detect latent viruses as well as other pathogens. Note that a negative test may not always be proof of the absence of pathogens, and could just be due to ineffective assay.

If diseases or viruses are detected, it is important to eliminate them. Methods used include:

- *Tissue culture*: Even when the pathogen is systemic, tissue from the terminal growing points can be used for further propagation as it is often pathogen-free.
- *Heat treatment*: works well for fungal, bacterial, and nematode infections. For viruses, a longer treatment is required relative to other pathogens.
- *Chemical treatment*: surface sterilization with chemicals can be used to eliminate pathogens.
- Use of apomictic seed

Apomixis

Fig. 4 Virus-indexing of potato. Meristem tissue is isolated and small explants (circled) are collected and placed in sterile test-tubes containing the appropriate growth medium. The tissue culture-derived plantlets are later transferred to the greenhouse and monitored for disease symptoms (middle panel). The tubers produced in the green house (right panel) will be grown in the field for tuber increase. Photos by Shui-zhang Fei, Iowa State University.

Apomixis is the formation of seeds without meiosis, and two forms are present.

1. Gametophytic apomixis in which the asexual embryo is formed from an unfertilized egg; and
2. Adventitious embryony, in which the asexual embryo is formed from nucellus tissue.

Steps in Development of Apomictic Cultivars

Apomictically produced seeds are genetically identical to the parent plant. Breeding of apomictic species requires developing population improvement by sexual reproduction and subsequent variety development by apomixis.

Example of plant species in which apomictic cultivar has been produced is Kentucky bluegrass.

Summary of Steps

Step 1: Defining objectives
Step 2: Develop a segregating population
Step 3: Select superior plants (clones)
Step 4: Preparation of seedstock for commercial planting

After objectives are clearly defined and are considered biologically feasible, genetic improvement in clonal crops starts with the assembly and evaluation of a broad germplasm base, followed by production of new recombinant genotypes derived from selected elite clones and careful evaluation in a set of representative environments.

In a crop with few years of breeding efforts, the divergence between landraces and improved germplasm is not as wide as in crops with a more extensive breeding history. As a result, landrace accessions play a more relevant role in clonal crops such as cassava (with fewer years of directed breeding efforts compared to other crops such as potato).

Longer-Term Breeding Experience

With longer term breeding experience, a breeder will have a better understanding and handle on combining ability of different clones as well as more information on other traits such as

quality and pest resistance. Thus it is essential to pick parents that will have a higher chance to produce offspring that will be superior and a commercial success. It is important to recognize the difference between botanical (or biological) seed and vegetative (clonal) parts (used for propagation).

The F_1 obtained from crossing is biological seed, while the plant parts used from that F_1 for further testing in subsequent generations is are clones (identical to the F_1 plant).

Several cuttings or plant parts taken for testing from a single F_1 will be identical to each other and to the F_1, while plant parts taken from different F_1 plants will be dissimilar to each other.

In the case of Cassava, the botanical seed obtained after crossing is either planted directly in the field or first germinated in greenhouse conditions and then transplanted to the field when growth is suitable to transplant. Root systems in plants derived from botanical seed and vegetative cuttings may differ considerably in their starch content and therefore selections should not be made for traits that will differ between biological seed and cuttings due to low correlation between trait data from plants developed from biological seed and vegetative cuttings.

Root Data

Since the commercial product is derived from vegetative cutting, root data from biological seed is not relevant for use in selection (unless the correlation is high). One way to overcome this issue is to germinate the biological seed and then transplant, which then develops a root system similar to what will be observed with vegetative cuttings. In clonal crops, the area of plant used to get cutting also influences the performance. Therefore plant breeders should be aware if these are issues in the crop they are working on. In Cassava, vegetative cuttings from the mid-section of the stems usually produce better performing plants than those at the top or the bottom. This variation in the performance of the plant, depending on the physiological status of the vegetative cutting, results in larger experimental errors and undesirable variation in the evaluation process. Consistency in vegetative cutting is important to remove this unwanted source of error. Breeders should also be aware of the number of cuttings that can be obtained per plant as one of the biggest constraints in getting to multi-location trial is the inability to produce enough cuttings per plant to put them into replicated evaluations across several locations.

Crossing Block

Crossing block can be in the field for over a year. In the case of Cassava, synchronization of flowering may require that crossing blocks are maintained for as many as 18 months and on average only one or two seeds per cross can be obtained (in directed crosses). The first selection can be conducted in the third year in the nurseries with plants derived from botanical seed. Due to low correlations between the performance of individual plants and yield plots, selections should be done only on traits with high heritability. Traits with higher heritability can be plant type, branching habits, reaction to diseases. Breeders will be able to reduce their population by up to 60-80%, making the numbers suitable for clonal evaluation trials (generally based on 6-10 vegetative cuttings in Cassava). At this stage, breeders may just use spray paint or tags or another method to identify the discards as some of the traits are visually assessed, and data collection may not be feasible in all cases. From about 100,000 F_1 plants a breeder will be able to use single plant selection to reduce the entries going to clonal evaluation trials to about 2,000 to 3,000 clones, where each clone is going to consist of 6-10 plants (coming from vegetative cuttings). In each trial, care is taken to ensure that the same number of plants are grown (for each entry) to avoid bias in selection.

Plot Considerations

As in row crops, plant breeders will use ways to ensure that plots are of the same size (length and width) so that no plot is given unnecessary advantage just because it had a larger area. Either a GPS planter is used so that plots are of the same size, or a trimmer is used to cut the plots to be equal size, or proper measurements are made prior to planting (i.e., if hand planting).

These clones are planted in an un-replicated single row trial generally. Since there is going to be a lot of variability in canopy coverage, height, branching (plant architecture traits), breeders will either try to group more similar entries into same test, or select for high heritability traits (as mentioned above, including harvest index, plant type, branching habit, leaf type, or some other higher heritability quality trait).

To reduce this problem of inter-plot competition, the distance between rows can be increased and plant-plant distance reduced, or leave an empty row between plots. This planting strategy increases the competition among plants from the same genotype and reduces the competition between plants from different genotypes.

It is also advisable to consider dividing fields into smaller blocks and conducting selection within a block (with commercial or elite checks present regularly across the field within each block). It is advisable to record all trait data as it helps a breeder to assess within (all clones from a cross) and among (between different crosses) family performance, and gives an indication of the parents used in crossing.

Trait Assessment

Traits with intermediate to lower heritability can be selected for in the evaluation trials including root or tuber dry matter (which are the economic part and most important breeding objective). The clones keep getting included in advanced trials (from clonal evaluation tests > Preliminary yield tests > advanced yield test > regional tests; number of entries reducing at each stage, vigor of testing increasing in terms of locations, replication, traits) to assess their yield and stability. Also processing and consumer preference (i.e. end-use quality) traits are assessed, and since such traits are most expensive or cumbersome to test, only the most advanced material is tested for these traits. As the most promising material is getting evaluated for processing and end use quality, the breeder needs to start the steps to multiple the stock for commercial planting.

It is generally accepted that at the PYT and AYT stages, testing at more locations will be more beneficial than using more replications, and 2-3 replications per entry should suffice in most cases.

Steps in Development of Self-Pollinated Cultivars

Pure Line Cultivars

Pure line cultivars are developed in self pollinating species. Pure line cultivars are homogeneous and homozygous and, once created, can be maintained indefinitely by selfing. Inbred lines are different from pure lines, although sometimes people use the terms interchangeably.

Inbred lines are developed in cross-pollinated species through inbreeding and these lines are used as parents in the production of hybrid cultivars and synthetic cultivars. Inbred lines are not meant for commercial release to farmers for commercial production because inbred lines suffer from inbreeding depression (yield will be lower than in hybrid and even an open-pollinated

variety (OPV). Inbred lines are homogenous and homozygous, similar to pure lines; however, artificial selfing needs to be done at each generation to maintain or increase seed. Sib-mating can be used to avoid severe inbreeding depression.

Summary of Steps in Development of Self-Pollinated Cultivars

Step 1: Define objectives
Step 2: Form the genetic base by creating segregating population(s)
Step 3: Perform selection to make pure lines
Step 4: Conduct Trials (testing of experimental lines) and Seed Multiplication

Clearly Defined Objectives

Step 1: It is critical that a plant breeder has clearly defined objectives before any other activity happens. Objectives need to be clearly defined and biologically possible. Also consider the following when defining objectives:

1. Will it meet the needs of the producer, processor and consumer? The best way to accomplish this is by having direct interactions with these three groups. Reading news print and other sources will also give a breeder an indication of the requirements by these groups. If possible, a breeder should attend farm shows, farm group meetings, meet and visit processing companies, colleagues in other disciplines, and marketing companies.
2. Available resources. Do you have the necessary resources to achieve the objectives? For example, if you would like to select for resistance to a disease using molecular markers very closely linked to the gene as you don't have disease nursery available. However, if you don't have access to either the disease nursery or marker screening lab, it will be near to impossible to meet the objective of developing cultivars with resistance to that disease.

Without clear and logical objectives, a breeder is working aimlessly. It is analogous to driving a car without knowing the destination.

Making Strategic Decisions

Setting objectives allows a breeder to make strategic decisions, such as:

1. Picking parents that have the necessary complementation of traits to develop progeny that possesses desirable traits from both parents.
2. Which breeding method to use
3. Determine selection strategy and plan for any specialized nursery or tools.
4. Breeder can also make decisions on which traits to select for and in which generations.

Step 2: Based on the objectives, a breeder can pick the parental material which can be:

1. Advanced lines from the breeding program
2. Advanced lines from another breeding program
3. Released cultivars
4. Germplasm line from gene bank or a pre-breeding program
5. Introductions (from other countries) from colleagues or genebank
6. Mutant lines, populations (unselected or selected)
7. Wild relative (need to be crossable or resources available to do embryo rescue if needed)

Crops that have a long history of breeding efforts will rely on cultivars and advanced breeding lines and in specialized cases, introductions as the choice of parent material. Crops with less breeding effort will rely on populations and introductions. Majority of parents in a breeding program will be best lines (advanced lines or newest cultivars) derived from the continuous breeding cycle of the program.

Parents Selection

All the traits desired in the cultivar you want to develop need to be present in the parents. Parents selection has to be based on reliable and complete data (yield testing, adaptation testing, specialized nurseries for stress assessment, end use quality, etc.). If a breeder does not have the specialized nurseries, she/he would collaborate with other breeders in the same or different organizations to send material for testing and characterization (material transfer across organizations may need a material transfer agreement (MTA).

The number of crosses made by a breeding program depends on various factors, such as objectives, resources available, breeding method (determines number of breeding lines that will be generated). In self pollinated crops with pure-line cultivars, it is assumed that the parent seed being used is a pure-line (homogenous and homozygous), but in case a breeder decided to accelerate incorporation of a trait and uses a line that is still segregating visually for a trait,

more number of plants will need to be used to increase the probability of recovering desired recombinants. Cross configuration will also dictate how many F_1's to create. If a cross is made between two pure-lines, all F_1's will be heterozygous but homogenous. If a three way cross is made using three different pure-lines, F_1's of the three way cross will be heterozygous and heterogeneous, necessitating large population size of F_1's to be created. There is considerable debate about the relative importance of number of crosses versus population size per cross. In most scenarios there will be an inverse relationship between number of crosses and population size, primarily due to resources available in a breeding program. In their review on this topic, Witcombe and Virk (2001) suggest that the strategy is to make fewer crosses (but very careful decisions need to be made based on prior information and scientific principles to pick parents) that are considered favorable and produce large sized populations from them to increase the probability of recovering superior genotypes.

Appropriate Breeding Method

Step 3: Once the crossing scheme is decided and crosses made, the next stage is to choose an appropriate breeding method to develop inbreeding populations which will be composed of an array of different inbred homozygous lines (pure-lines) where genetic variability exists among but not within lines. All breeding methods in pure-line breeding lead to an increase in homozygosity, a reduction in the genetic variance within families, and an increase in the genetic variance between families. Cultivar development is aimed at identifying the best homozygous lines.

Selection should commence in an early generation and preferably as early as F_2 because it is the generation of maximum variability, and the minimum population size required to observe desirable type is lowest in F_2 and progressively increases. This means a breeder will not have to evaluate larger populations as the generations advance in order to recover the desirable types.

It is also important that a breeder makes selections in each generation (if possible) so as to continue with the development of pure-lines and to eliminate undesirable types. This may be accomplished through phenotypic or genotypic selection with molecular markers. If a high value marker is, or set of markers are, available and linked to the trait of interest, it will be very beneficial for a breeder to use the marker(s) to enrich and advance the desirable types while eliminating the undesirable types.

Selection Considerations

Consider for example, a trait under recessive gene control, which, in fixed homozygous recessive state, will never segregate to give the desirable dominant allele. Evaluating lines with the undesirable homozygous recessive genotype in this case will be a drain on resources. Therefore, the use of molecular markers (which are not influenced by environment) lends a breeder confidence in the selections made, provided the molecular marker is robust, tightly linked to or is on the gene, and not background dependent.

For selections in early generations, a breeder will likely handle several thousand plants, hills, or rows. It is therefore very important to handle this material in a selection environment that is very similar to the target region or is representative of the target environments of different agro-ecological regions if breeding for a sub-region. In other words ensure that the environment is ideal for selecting for targeted traits. For example, choose dryland environment if breeding for drought tolerance, or irrigated field nurseries if breeding for high-input environments.

It is best to grow these early generation trials at a location where a breeder has easy and quick access to observe the material to facilitate making breeding decisions, and also from a logistical viewpoint, to better manage the trial location.

It is extremely important that a breeder eliminates controllable sources of variation such as weeds, non-uniform land, animal damage, non-uniform application of chemicals.

Generation Trials

In early generations (F_2 to F_4), selection is restricted to traits of high to moderate heritability, whereas in later generations (F_4 to F_6 or F_8 depending on the complexity of the crop genome), evaluation is more detailed and involves multi-location testing and replication.

In situations of relatively large number of entries but limited resources, in early generation trials, single replication yield plots may be used to identify material to advance. Statistical approaches such as running mean, partial rep or augmented designs can be used to identify promising lines. As generations advance, more seed is available and population sizes are sufficiently reduced to allow for increased replications and locations. Selection is then done for traits of lower heritability, such as yield (in larger or paired rows) and for end use quality. However, techniques that require small sample sizes such near-infrared spectroscopy (NIRS)

can be effectively utilized in earlier generation testing of end use quality traits to select and remove the undesirables based on cut-offs developed in comparison with checks or industry requirement. Note that cut-offs for traits are variable in every test as they are generally developed based on checks or industry requirement.

Final Stages

Step 4: Final stages in the breeding cycle will involve lines that are considered pure-lines (non-segregating). At this stage, more extensive testing of few best recombinants from a cross is done for agronomic performance and end-use quality. Multi-environment testing is done for adaptation and stability, and environments may be locations or a combination of locations and years. At this stage, trials will be grown using lattice design (incomplete block if the number of entries is large) or RCBD, and detailed observations made and data taken. Since fewer number of lines is tested, more detailed assessment is feasible for an increased number of traits.

For optimal use of resources and to ensure timely adoption of pure-lines, a breeder needs to pro-actively initiate seed multiplication alongside advanced yield testing for production of sufficient quantities of certified seed for the launch of pure-lines.

In the case of doubled haploid (DH), steps 3 and 4 are very closely aligned and can even be considered as one because once the doubled haploid is generated, lines can go into multiple location replicated testing where seed quantity permits.

Steps in Development of Synthetic Cultivars

Hybrids and Synthetics

Cross-pollinated species have two main types of cultivars: Hybrids and Synthetics.

Hybrids are generally a product of a single cross (or two way cross; A x B), and to a much smaller extent three-way crosses (A x B) x (C) or double-crosses (A x B) x (C x D).

Synthetic cultivars consist of a mixture of heterogenous and heterozygous individuals (parental lines are generally clones or inbred lines). Synthetics are more common in some developing countries. These parental lines are maintained so that synthetic cultivars can be re-constituted

when needed. These parental lines (clones of inbreds) are assessed for their general combining ability and lines exhibiting superior combining ability are crossed in a polycross configuration to produce S_0. The S_0 plants are allowed to intermate to produce S_1, which, in the case of asexual propagated crops such as alfalfa, can be sold as a synthetic cultivar. Sometimes S_2 may be sold as commercial cultivar but maximum heterosis is observed in S_1 and is therefore a more favorable generation for cultivar development. In annual crops such as maize, asexual propagation is not feasible therefore the progression from Breeder Seed to Foundation Seed to Certified Seed production is done from S_2 to S_3 to S_4, respectively.

A synthetic cultivar *differs from open-pollinated variety* (developed by mass selection). A cultivar developed by mass selection is made up of genotypes bulked together without having undergone preliminary testing to determine their combining ability. This makes an open-pollinated cultivar the same as a landrace cultivar.

Synthetic Cultivars

Hybrids are preferred over synthetic cultivars in crops where hybrids can be created economically and commercialized. However, in crops that show heterosis but hybrid production is difficult, synthetics are important and preferred. Synthetic varieties are known for their hybrid vigour and for their ability to produce usable seed for succeeding seasons. Because of these advantages, synthetic varieties have become increasingly favored in the cultivation of many species, for examples forage crops such as alfalfa.

Summary of Steps in Development of Synthetic Cultivars

- **Step 1:** Define objectives
- **Step 2:** Form the genetic base by creating segregating population(s)
- **Step 3:** Perform selection to make pure lines
- **Step 4:** Conduct Trials (testing of experimental lines) and Seed Multiplication

We have already covered in details how to set up objectives. Similar principle can be applied to synthetic cultivars to set reasonable synthetics.

Assembly of Parental Lines

Assembly of parental lines can be from a previous synthetic cultivar or from other experimental populations. Parental lines can consist of different clones (forages) or inbred lines (maize). Clones will be highly heretogenous and heterozygous and each clone will be unique. These clonal lines are used to establish a *source nursery* with several thousand individual plants generally grown in a space planted grid system to reduce environmental variance and enable meaningful comparisons of experimental clones amongst each other or to a check within smaller grids. A breeder will select for higher heritability traits such as disease reaction, and morphological traits. Once the superior clones are identified, they are grown in a polycross nursery to either facilitate random pollination among clones or carefully set up clones to facilitate equal chance per clone to contribute pollen to other clones. Clones may be replicated to ensure uniform pollination. Further evaluation may be done, and seed is harvested (in equal amount per clone per replication). In a perennial species, clones may be grown for more than one year and seed harvest each year. This polycross nursery is used to produce seed for progeny testing in performance tests (in corn, one year but several locations and can be replicated; in forages and perennials, one or more years in several locations and can be replicated). Based on the progeny testing, superior clones from the polycross nursery are identified and crossed to each other to produce the synthetic. Syn1 or Syn2 may be released as a synthetic cultivar in a forage species (with clonal propagation). While in maize, two or three more rounds of pollination may be needed to have sufficient seed for commercial launch of a synthetic cultivar.

Half-sib Selection

Half-sib selection is widely used for breeding perennial forage grasses and legumes. A polycross mating system is used to generate the half-sib families from selected clones maintained vegetatively. The families are evaluated in replicated rows for 2-3 years. Selecting of traits with high heritability, e.g. oil and protein content in maize is effective.

Full-sib mating involves the crossing of pairs of plants from a population in which case control is exerted on both male and female parents.

A half sib is a plant (or family of plants) with a common but unknown pollen parent (i.e. pollen source). Therefore, in half-sib mating, the pollen source is random from the population, but the female plants are identifiable. Half-sib selection is based on maternal plant selection without pollen control, therefore half-sib selection is less effective for changing traits with low

heritability. The methods used by plant breeders in population improvement may be categorized into two groups: one group is based on phenotypic selection alone (no progeny testing), and the second group is based on genotypic selection (with progeny testing).

The specific methods include:

2. *Simple recurrent selection or mass selection*. The procedure does not involve the use of a tester so there is no estimation of general or specific combining ability. Selection is based on phenotypic observations and therefore this method is also known as phenotypic recurrent selection.
3. *Recurrent selection for general combining ability*. In this method a wide genetic base cultivar (i.e., a population) is used as a tester to cross with identified females, therefore deploying a half-sib progeny test procedure. Based on the test cross progeny performance in replicated or multi-environment trials, selections are made. Selected lines are advanced into the next round of testing. Generations may be advanced by sib-mating while the progeny test is on-going.
4. *Recurrent selection for specific combining ability*. In this method a narrow genetic base line (i.e., inbred line) is used as a tester to cross with females, also deploying a half-sib progeny test procedure. Similarly, based on the test cross progeny performance in replicated or multi-environment trials, selections are made. Selected lines are advanced into the next round of testing and here also, generation may be advanced by sib-mating while the progeny test is on-going.
5. *Reciprocal recurrent selection*. This scheme is capable of exploiting both general and specific combining ability. This is achieved by using two heterozygous populations, where each population serves as a tester for the other.

[Note: The difference between a synthetic and an open pollinated cultivar is the ability to re-constitute the seed in a synthetic because the parents (inbred, clones, hybrids) are used in a pre-determined manner and configuration, while in an OPV the original population cannot be created due to no control on parent configuration. After a limited number of generations, seed needs to be reconstituted for a synthetic using the breeder selected parental stock, while in OPV the random mating happens in each generation and population can be propogated indefinitely]

Steps in Development of Hybrid Cultivars

Hybrid Cultivars

Hybrid maize (*Zea mays* L.) in USA is an example of a success story in cross-pollinated crops. Up until the 1930s, open pollinated varieties were the more common type of cultivars in cross-pollinated crops. However since then in USA almost all commercial maize cultivars are hybrids.

A hybrid cultivar is the F_1 offspring of a planned cross between inbred lines, cultivars, clones, or populations. The hybrids may be the product of a single cross, a three-way cross, or a double cross hybrid. One absolute requirement for a hybrid cultivar is its superior performance over the parents (heterosis) and an ability to economically generate the seed for commercial seed sale. In the case of maize, farmers moved from OPV to hybrids due to several advantages offered by hybrids which include higher yield, improved tolerance to stalk lodging (i.e. better standability), and improved response to drought. Development of hybrids in maize was easier due to the ability to follow a configuration of males and females for seed production – female rows were detasseled and seed was only collected from female rows (in later years, male rows destroyed post-pollination to ensure no contamination). Therefore, the role of male rows is to serve as pollen source, with requirements that the male needs to be a good combiner to the female (higher SCA) and must possess good pollen shed, nicking well with the female, i.e, must have close flowering time to that of the female.

Cytoplasmic Male Sterility

Discovery of cytoplasmic male sterility (Fig. 5) has also helped in the development of hybrid crops in those species. Plants with sterile cytoplasm plus nuclear non-restorer genes are male sterile; plants with sterile cytoplasm plus nuclear restorer genes produce fertile pollen (fertility restoration). In contrast to plants with sterile cytoplasm, plants with normal cytoplasm are male fertile when they carry either of the nuclear genes: restorer or non-restorer. Fertility restoration may not be needed for crop species in which the vegetative part is of economic value. In addition to straight crossing (male on female) and male sterility, chemical agents have been used to create hybrid cultivars. An important consideration of hybrid cultivars is the expression of heterosis and efficiency (i.e., cost, labor, time) of seed production.

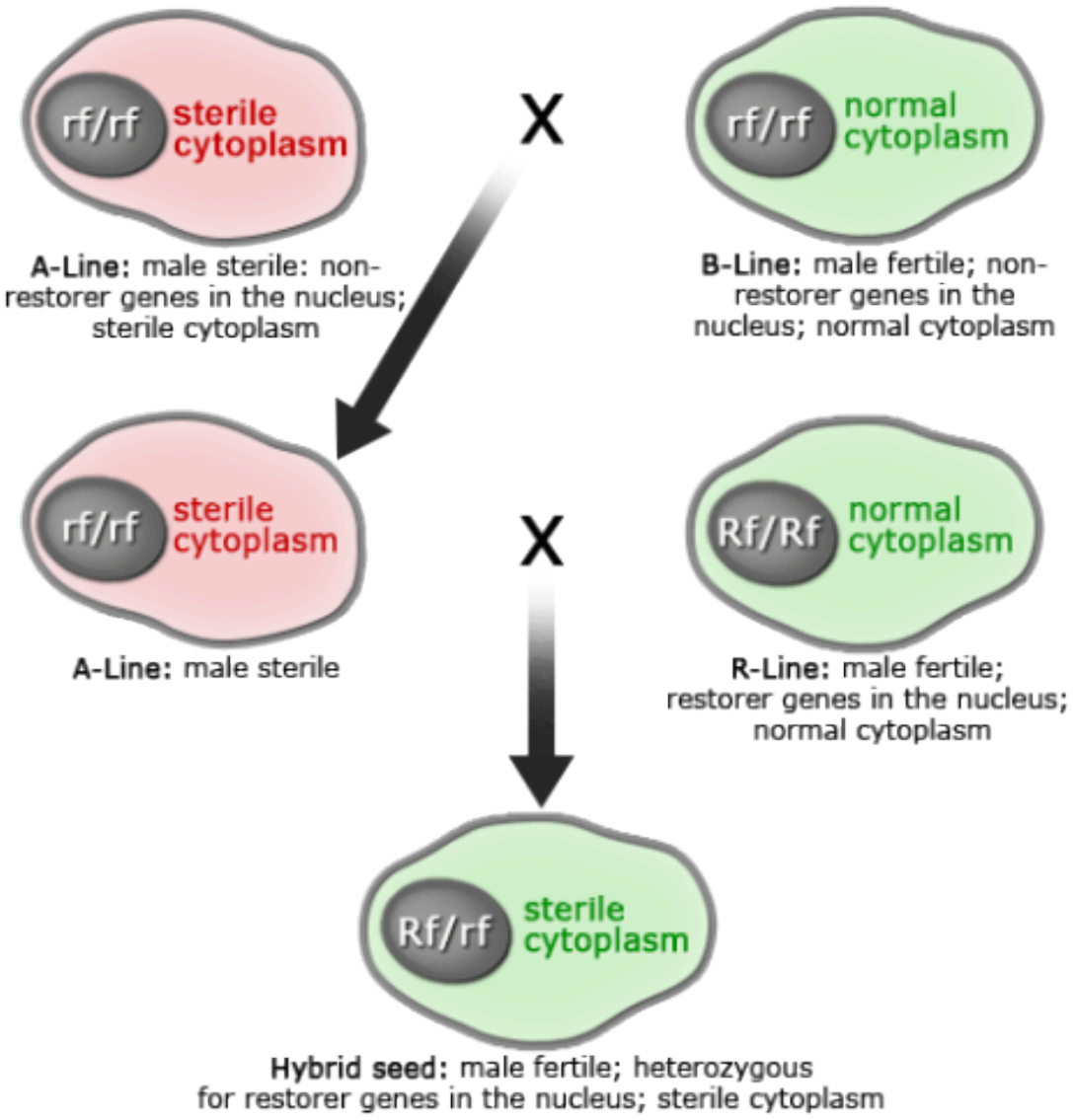

Fig. 5 The CMS system used for hybrid seed production. **Rf** refers to fertility restorer genes in the nucleus and **rf** refers to nuclear genes that cannot restore the fertility of a plant with sterile cytoplasm.

Practical Considerations

Some practical considerations for hybrid systems:

1. Farm production of field crops such as wheat, barley, rye, sunflower, grain sorghum requires large amounts of seed for planting the crop and return per unit area is relatively low compared to most horticultural crops or oilseed crops (such as canola), so expensive labor intensive methods of producing hybrid seed (such as hand emasculation) are not

preferred. Cytoplasmic male sterility is one approach that is useful in field crops to develop hybridization techniques in which seed parents and pollinators could be grown on a field scale.

2. Pollination also needs to be on a field scale, such as with wind or with bees. Successful hybridization via wind pollination requires that males shed abundant amounts of pollen and female lines be receptive (e.g. florets should open at appropriate times). There is a need to synchronous flowering times between females and males.
3. Female inbred line should generally be more productive and with a higher capacity to produce more seed. Male and female need to be chosen for their superior specific combining ability, and are generally distinct i.e. belonging to different heterotic groups as for example stiff stalk and non-stiff stalk in maize in North America, or origins as for example *indica* and *japonica* in rice.
4. Expression of heterosis needs to be sufficient to overcome the cost of development and hybrid seed production. For example, in wheat heterosis is not sufficient to warrant development of a hybrid. Floral morphology also prevents easy pollinations.

Hybrid Rice Breeding

A good example in hybrid rice breeding in China was produced by the International Food Policy Research Institute (Li, Xin, & Yuan, 2009).

History of hybrid rice technological development in China

1964 – Research on three-line hybrid rice initiated

1970 – Wild abortive (WA) rice identified on Hainan Island in China

1973 – Photo-thermosensitive genic male sterile (PTGMS) material identified

1974 – First sets of three lines (A, B and R lines) developed for three-line system hybrid rice

1976 – Hybrid rice commercialization started

1977 – Systematic hybrid rice seed production technique developed

1983 – Hybrid rice seed yield more than 1.2 ton/ha

1987 – Hybrid rice seed yield more than 2 ton/ha

Hybrid rice area more than 10 million ha

National Two-line System Hybrid Rice Program established

1990 – Hybrid rice area more than 15 million ha

1995 – Two-line hybrid rice system developed

1996 – "Super Rice Breeding" national program initiated

1998 – Hybrid rice seed yield more than 2.5 ton/ha

2000 – Super hybrid rice Phase I objective (10.5 ton/ha) achieved

2004 – Super hybrid rice Phase II objective (12.0 ton/ha) achieved

2006 – Super hybrid rice Phase III objective (13.5 ton/ha) initiated

Three-line System

The three-line system (Fig. 6) includes the following lines:

- **Male sterile line** (**A line**)**:** The cytoplasmic male sterility trait is controlled by both cytoplasm and nucleus; this line is used as female in hybrid seed production.
- **Maintainer line** (**B line**)**:** This line is used as a pollinator to maintain the male sterility. The maintainer line has viable pollen grains and sets normal seed.
- **Restorer line** (**R line**)**:** Any rice cultivar that restores fertility in the F_1 when it is crossed to a CMS line.

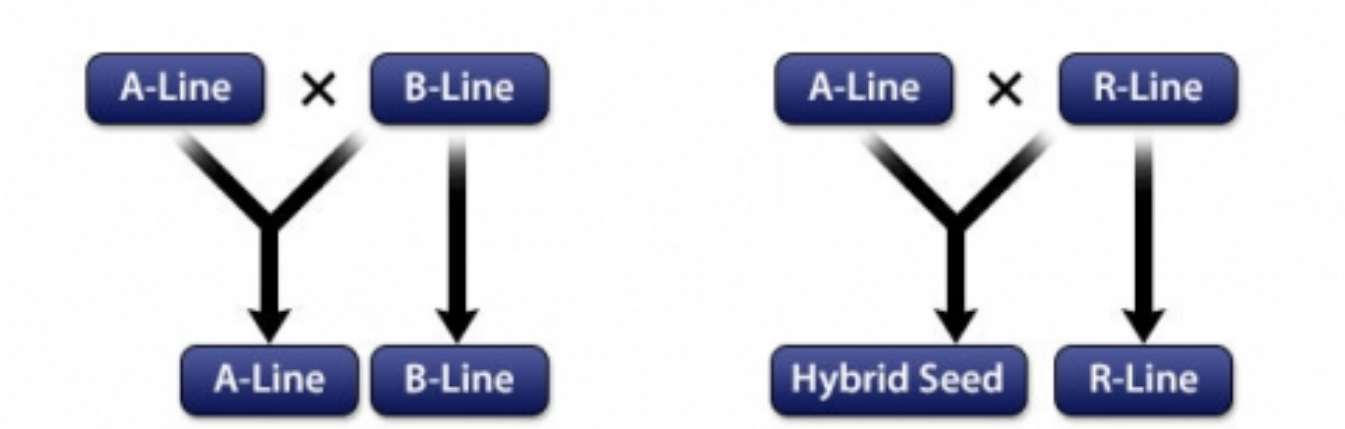

Fig. 6 Three-line system to produce hybrid rice.

Rice hybrids made with cytoplasmic nuclear male sterility have been grown for several decades in both developed and developing nations. Traditionally, three-line system was used to produce hybrid rice, but more recently an innovative kind of genetic male sterility has been used as well, to make '**two-line' hybrid rice.** Seed is produced on female inbred lines that are homozygous for environmentally sensitive (**photoperiod or temperature or both**) recessive male sterility genes. Seed production fields are planted in an environment (e.g. long day and/or high temperature) that enables expression of the male sterility gene in the female and enabling successful hybrid seed production. Seed increase fields of the female lines are grown in an environment (e.g. short day and/or cooler temperatures) that represses expression of the male sterility genes, allowing the female lines to reproduce via self-pollination.

Two-line System

Two-line system hybrid rice included the following two lines (Fig. 7):

- **Male sterile line**: nuclear gene(s) and environmental conditions such as photoperiod and/or temperature control male sterility. Male sterile lines can be environmental-conditioned genic male sterile (EGMS), photoperiod-sensitive genic male sterile (PGMS), thermo-sensitive genic male sterile (TGMs) or photoperiod- and thermo-sensitive genic male sterile (PTGMS) lines
- **Restorer line** (**R line**): any rice cultivar that restores fertility in the F_1 when it is crossed to the male sterile line.

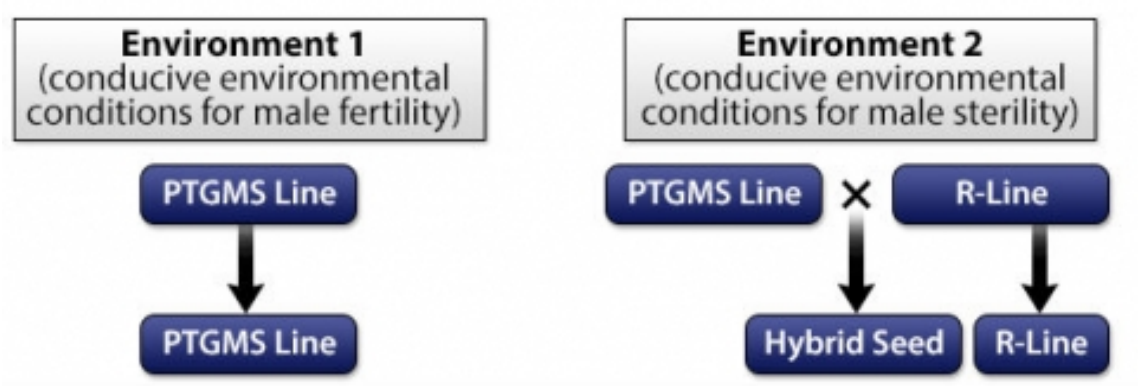

Fig. 7 Two-line system to produce hybrid rice.

Two-line system has several advantages over three-line system:

1. It is simpler as the need of maintainer is removed,
2. It is more applicable in diverse genetic background and easier to implement,
3. It has reduced cost of breeding program and seed production,
4. There is no detrimental effect of CMS system.

However, due to the dependency of trait expression to environmental conditions, problems may arise in hybrid seed production. An important requirement is that environments be chosen that have more consistent temperature and day length at critical times of crop growth.

Hybrid Maize Cultivars

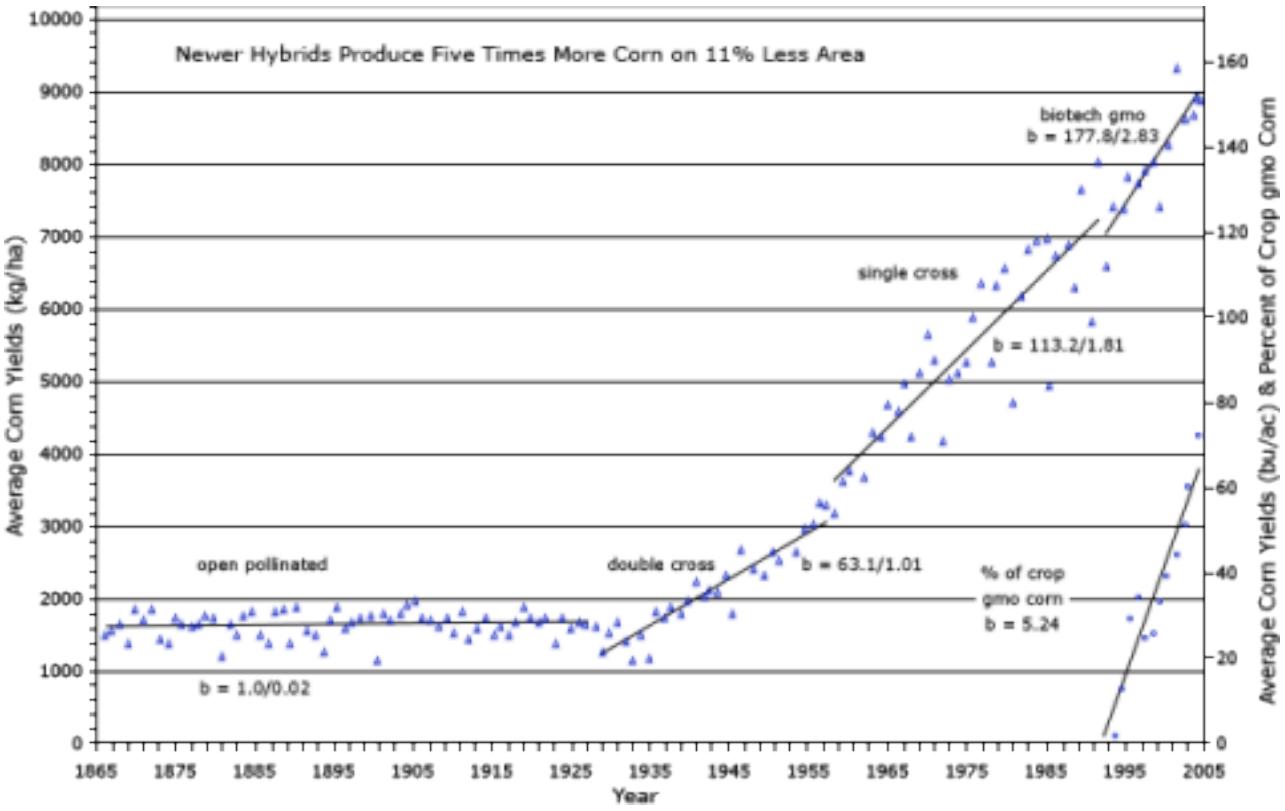

Fig. 8 Average U.S. Corn yield and kinds of corn ((1865 to 2007). Regression values, 'b', are presented as kg and bushels to demonstrate yield gain per year. Adapted from Troyer, AE. Development of Hybrid Corn and the Seed Corn Industry. In Handbook of Maize Genetics and Genomics (ed). J. Bennetzen and S. Hake, 2009.

Summary of Steps in Development of Hybrid Maize Cultivars

- **Step 1:** Development of inbred lines
- **Step 2**: Making the crosses to produce a hybrid
- **Step 3:** Testing the hybrids
- **Step 4:** Development of seed for commercial production

Inbred Lines

Inbred lines can be developed by inbreeding selected heterozygous plants until sufficient homozygosity is reached without severe inbreeding depression. Sib-mating may be used to

maintain inbred lines. With the advent of doubled haploid (DH) technology, this problem is minimized. DH lines provide genetic homozygosity in one generation. Because haploids carry only a single copy of every gene, any gene or genes that have deleterious effects for seed or plant development will have immediate genetic effects to depress or inhibit normal seed or plant development so these plants will be quickly eliminated at the haploid stage. Haploids also provide an advantage of better response to marker assisted selection as markers can be used to identify and select for desirable genes, and upon chromosomal doubling these genes are fixed. This provides an efficient and rapid tool to eliminate unfavorable genes and to enrich favorable genes to improve the genetic pool. DH lines have 100% genetic homozygosity, and the technique significantly reduces the time taken to develop inbred parents for crossing. These DH lines do not show inbreeding depression in the following generations, and in the absence of spontaneous gene mutations or transpositions that may cause certain deleterious influences and segregation, DH lines provide a powerful tool in maize breeding. In the DH process only one round of recombination happens thereby minimizes breakage of desirable linkages, as well as also reduces the chances to break undesirable linkages. Therefore a breeder needs to recognize the need for population size optimum, and so larger population sizes may be useful.

Traditionally, open pollinated varieties of maize were the source of inbred lines. However, as the cultivars moved away from OPV to double crosses, three-way crosses or single crosses, which boosted maize yields (Fig. 8), the source of inbred lines also changed. Recurrent selection programs were also a popular source of inbred lines in public breeding programs, but private programs have moved away from recurrent selection programs as sources of inbred.

Heterotic Groups

In North America, for example, heterotic groups have been developed to classify inbred lines, and modern hybrids are the result of crossing a line from one heterotic group with a line from a different heterotic group (Fig. 9). Classification of heterotic patterns is generally based on several criteria such as pedigree, molecular marker based associations, and performance in hybrid combinations. Most conventional inbred line development involves making crosses within a heterotic group and as the population (within a cross) is advancing, testers belonging to different heterotic groups are used in crosses.

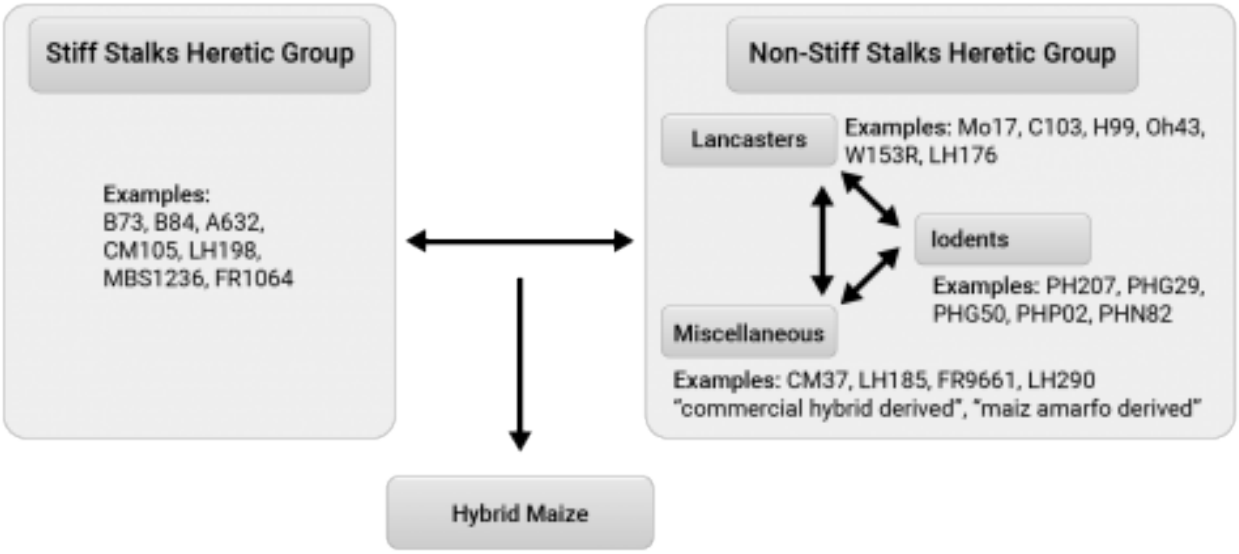

Fig. 9 Northern Corn Belt Dent heterotic patterns in North America. Adapted from Lee and Tollenaar, 2007.

Inbred Line Development

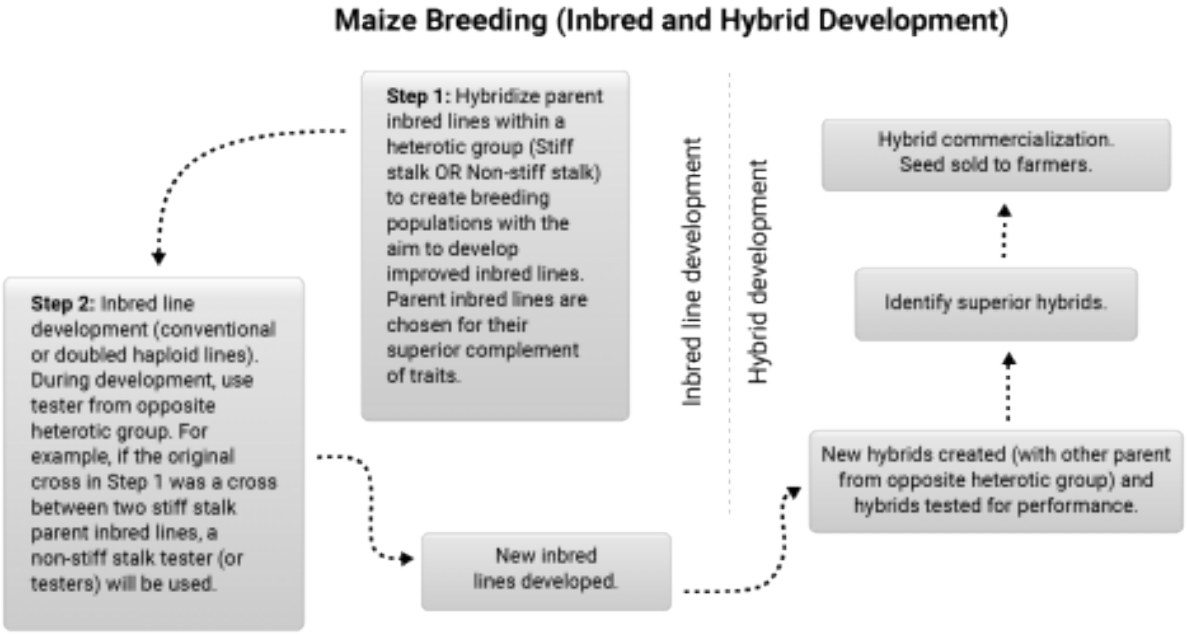

Fig. 10 Overview of a modern maize breeding program. Adapted from Lee and Tollenaar, 2007.

The majority of inbred development activities in North America involve the use of the pedigree method of breeding (Fig. 10) Breeding crosses tend to be made by crossing inbred lines within a heterotic pattern. Inbred lines from the other heterotic patterns are used to improve the heterotic pattern represented by the breeding cross.

Two-parent Cross

Two-parent cross (parents belonging to the same heterotic group) are most common in maize inbred line development. An F_2 population is formed from the breeding cross, which is then followed by several rounds of inbreeding using ear-to-row with each family tracing back to different F_2 plants. During the inbreeding process, genotypes with obvious defects are eliminated. Early generation testing occurs around the F_3 or F_4 generation, which involves forming topcross hybrids between the F_4 lines and an inbred line from a contrasting heterotic group. This cross can be made in the off-season nursery, and then in the summer (in North America), the resulting topcross hybrids can be tested in two or more environments (Fig. 11). Selection will be based on yield, lodging or stalk strength, maturity, test weight, and height, or other trait of interest. Inbred lines can be advanced in the same season through another round of inbreeding. Lines that produce hybrids considered to have merit (similar or better than commercial checks) are advanced through another round of breeding in the off season nursery and perhaps a round of crossing with inbreds of complementary heterotic group. In the summer, selection is made as described above. At this stage, the superior inbreds are forwarded to hybrid development teams for commercial testing with specific testers and in more environments. [Adapted from: Lee and Tollenaar 2007].

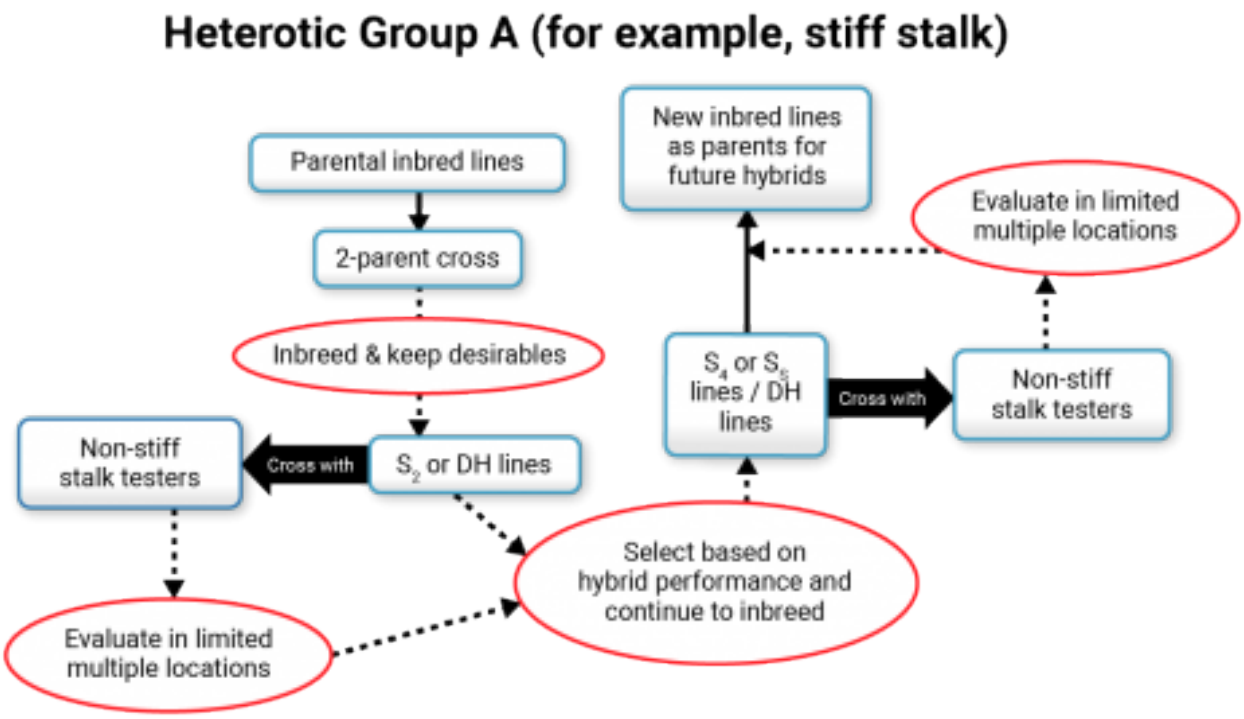

Fig. 11 Typical inbred line development scheme depicting a two-parent breeding cross involving two inbred lines from the Stiff Stalk heterotic pattern. (Information on heterotic pattern is to describe activities in a North American maize breeding programs.)

Hybrid Commercialization

In the hybrid development process, more hybrid combinations are tested in fewer environments during the early testing phase, while in the later testing phases fewer hybrid combinations are tested in more environments. Generally, testing involves growing the hybrids in more locations while reducing the number of replications, thus allowing for more vigorous evaluation for adaptation.

Requirements

Several requirements need to be met for inbred lines classified as good parents in hybrid production. For example a female parent must be vigorous and produce high quality, healthy seed, and male parents should produce abundant and good quality pollen. An important consideration for choice of testers in the pedigree type breeding approaches is that testers

be from a complementary heterotic group, maximize variance (among test crosses), as well as possess high mean (Bernardo 2010; see chapter 9).

Additional Suggested Web-Based Learning

- The History of Canola Seed Development
- Hybrid Rice and Seed
- Hybrid Rye Breeding

Steps in Development of Multi-line Cultivars

Multi-lines are generally a set of isolines (traditionally created using backcrossing; or can be through transformation) that differ for one trait or more. These are grown in self-pollinating crops, where cultivars are pure-lines, so a mixture of pure-lines (if they are isolines) can form a multi-line cultivar. This approach has been used to provide control over a prevailing pathogen, such as a multi-line cultivar with different rust resistance genes in wheat. This should theoretically provide better protection against pathogen races and prevent a total crop loss. The pure lines are phenotypically uniform for morphological and other traits of agronomic importance (e.g., height, maturity, photoperiod), in addition to genetic resistance to a specific disease (or any other trait, for example, abiotic stress). See Breth (1976).

Backcrossing is used to develop isogenic lines which are then combined in a predetermined ratio.

Steps in Development of Blends

A blend or composite cultivar, like a multiline, is a mixture of different genotypes. The difference between the two lies primarily in the genetic distance between the components of the

mixture. Whereas a multiline consists of closely related lines (isolines), a composite may consist of different types of cultivars. It is intended to pick genotypes in a blend to minimize differences in maturity, growth habit, lodging, and disease resistance in the package. This consideration is critical to having uniformity in the cultivar. However, a different approach would be to pick blends that can be phenotypically different if the intention is to maintain some level of percentages.

WB Seed Company provides additional information to familiarize yourself with examples of a blend cultivar system in commercial production.

References

Bernardo, R. 2010. Breeding for Quantitative Traits in Plants. 2nd ed. Stemma Press, Woodbury, MN.

Breth, S.A. 1976. Multilines: safety in numbers. CIMMYT Today 4.

Breth, J.R., and D.S. Virk. 2001. Number of crosses and population size for participatory and classical plant breeding. Euphytica 122: 451-463.

DePauw, R.M., R.E. Knox, F.R. Clarke, H. Wang, M.R. Fernandez, J.M. Clarke, and T.N. McCaig. 2007.

Shifting undesirable correlations. Euphytica. 157(3): 409-415.

Jan, V.V.S., C.C. de Macedo, J-M. Kinet, and J. Bouharmont. 1997. Selection of Al-resistant plants from a sensitive rice cultivar, using somaclonal variation, in vitro and hydroponic cultures. Euphytica 97: 303310.

Larkin, P.J., and S.C. Scowcroft. 1981. Somaclonal variation-a novel source of variability from cell culture for plant improvement. Theor. Appl. Genet. 60, 197214.

Lee, E.A., and M. Tollenaar. 2007. Physiological basis of successful breeding strategies for maize grain yield. Crop Sci 47(S3):S202-S215.

Li X., Y. Xin, and L. Yuan. 2009. Hybrid Rice Technology Development: Ensuring China's Food Security. 2020 Vision Initiative. IFPRI publication.

Troyer, AF. 2009. Development of Hybrid Corn and the Seed Corn Industry. In Handbook of Maize Genetics and Genomics (ed). J. Bennetzen and S. Hake.

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Chapter 6: Breeding Methods

Asheesh Singh; Arti Singh; and Anthony A. Mahama

The results of breeding and selection may be new varieties or clones that are superior to currently used standard commercially grown genotypes (checks) according to some criterion or criteria, or populations that are superior to previous ones. Several breeding strategies exist and though some methods are generally commonly accepted, different methods are applied in different crops as they are more efficient and effective based on the type of mating of different crops, resources and objectives. In other words different breeding strategies are deployed and used to maximize superiority per unit cost and time. Also depending upon the goals of the breeding program, different strategies may be used simultaneously or at different stages of the program.

Learning Objectives

- Identify and describe different plant breeding methods relevant to crops grown in Africa
- Mention and describe innovation used to enhance backcross breeding method
- Explain innovation used to enhance recurrent selection method

Methods Used in Self-Pollinated Crops

In self-pollinated crops, the following breeding methods are commonly used to develop pure-line cultivars:

- Bulk method
- Pedigree methods
- Single Seed Descent
- Doubled Haploid

Example of self-pollinated crops in which these methods are used include: common bean, soybean, cowpea, groundnut, rice, wheat, barley, millet, and sorghum.

In specific situations, for example, when a breeding program is converting pure-lines to contain a specific gene or 2-3 genes (of qualitative inheritance), the backcross breeding method is used.

The doubled haploid method is not used in legume crops as these species have so far been recalcitrant to tissue culture and haploid induction and rescue.

Methods Used in Cross-Pollinated Crops

In cross-pollinated crops, the following breeding methods are used to develop cultivars:

- Recurrent selection (for example, maize)
- Development of hybrids: a 2-step process where first inbred lines are developed and assessed for their specific combining ability, followed by crossing of the inbred lines (generally, 2 inbred lines, but can be 3 or 4) to produce hybrid, as for example, maize, rice, sorghum, cotton.

Few self-pollinated species (such as rice, sorghum, and cotton) have some level of outcrossing and expression of heterosis, which is exploited to develop hybrid cultivars.

Recurrent selection methods are used to develop open-pollinated varieties or synthetics.

Methods Used in Clonal Crops

The crop species that can be clonally propagated present unique advantages:

1. Heterosis can be fixed in F_1 and in subsequent crop production cycles, and its clones can be propagated to preserve the high yield advantage.
2. Farmers can harvest the crop and use the vegetative plant part to grow the next crop. For example, potatoes, sugarcane, cassava.

In breeding clonal cultivars, hybridization is made between two clones and a large F_1 population (remember that parental clones are heterogeneous and heterozygous) is screened as each F_1 is unique and different from other F_1s. This process is repeated over different crop cycles to identify the superior clone for release as a new cultivar.

Breeding Methods Used in Major Crops

Pedigree Method

The pedigree method of breeding is used in development of both self-pollinated (to develop pure-lines) and cross-pollinated crops (to develop inbreds). It is one of the most commonly used breeding methods. Selection of highly heritable traits is practiced in early generations on individual plants. Yield testing is generally done once homozygous lines are developed (Fig. 1). However, in an early generation testing procedure or a modified pedigree method, yield testing is done in early generations while within-family selection is still ongoing.

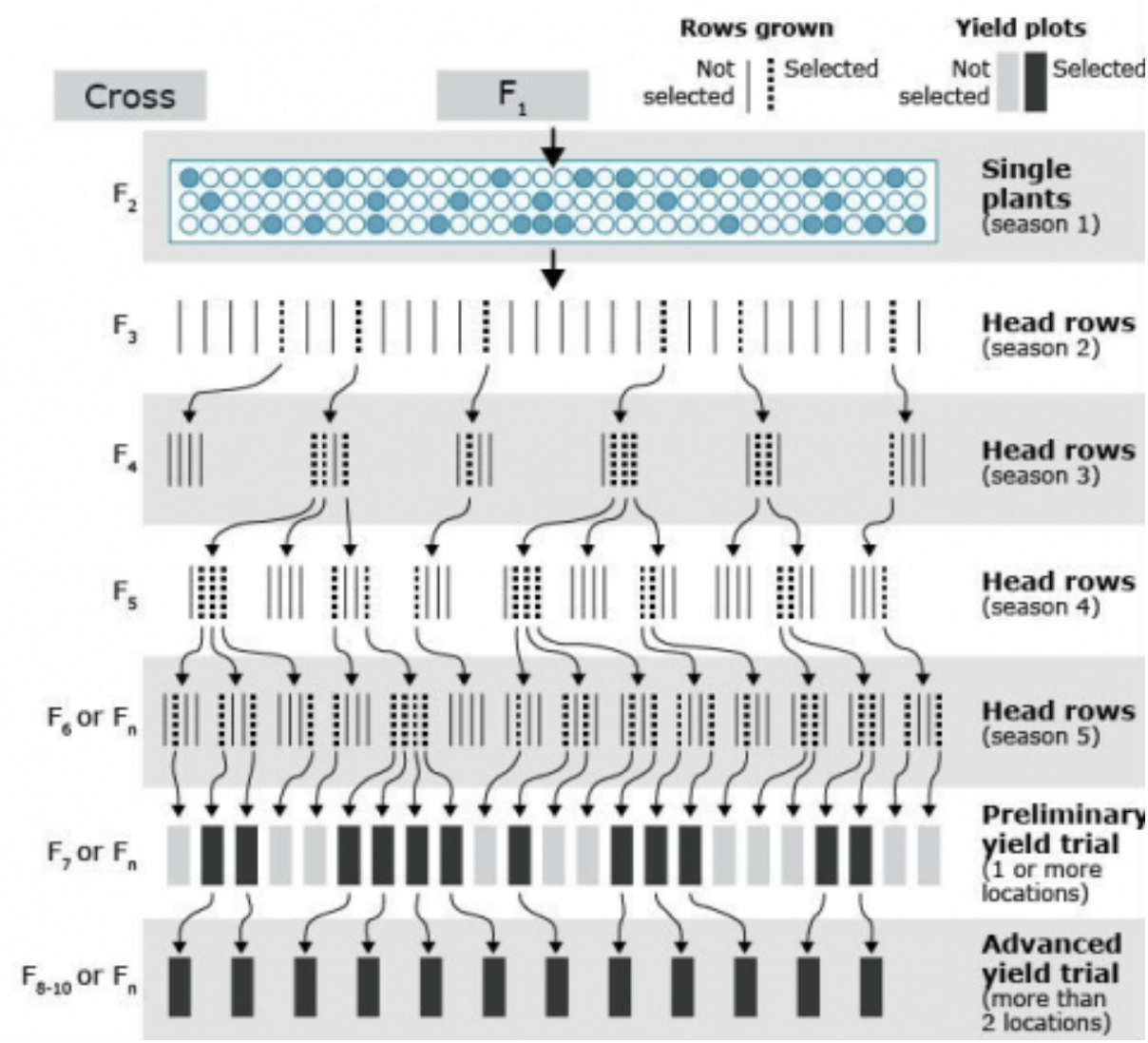

Fig. 1 Flow chart of pedigree method of breeding.

Explanation of steps in Fig. 1

    - Select in F_2 and later generations.
    - Selected F_3 plants (or seed from inflorescence of selected plants) grown in next season (in winter nursery if available).
    - Selected F_3 rows (or selected plants within rows) grown as F_4 in rows (or yield plot).
    - Selected F_4 plants (or seed from inflorescence of selected plants) grown in next season (in winter nursery if available) as F_5.
    - Repeat this process until selection is effective (remember, additive genetic variance among lines increases but decreases within lines as selfing is used).

- Bulk harvest the last generation when a row is grown (and appears homogenous), F_6 or F_n and plant in the next season as a yield plot.
- Grow through successive seasons of yield testing to select the genotypes that are superior to checks.
- Pedigree information is kept to maintain family information, which allows selecting more plants from families that are superior performing or to advance families for yield testing if those families are superior.

Additional notes

- Number of plants/row and population sizes vary between programs and some estimates can be obtained from text books or plant registration documents. These numbers will depend on the objective of the cross, number of crosses made per year, available resources (technical, infrastructure).
- Selection for other specific traits is simultaneously happening (on harvested seed, or specific nurseries).
- Single plants or inflorescence per plant are selected at each generation, but in some visibly inferior rows, breeder may not make any within rows selection (i.e., practice among row selection).
- Selection can be practiced in winter nursery if genetic correlation is high among home location and off-season location (i.e. winter/dry season nursery locations).
- A breeder may combine two or more methods of breeding and these methods will then be called modified pedigree (or modified bulk, or modified single seed descent etc.).

Bulk Method

Bulk method allows natural selection to act and remove undesirable genotypes from the population (i.e., per cross) (Fig. 2). The choice of growing environment will dictate what kinds of traits will be selected for or against, therefore care needs to be exercised to use environments that are suitable for realizing the objectives of the program.

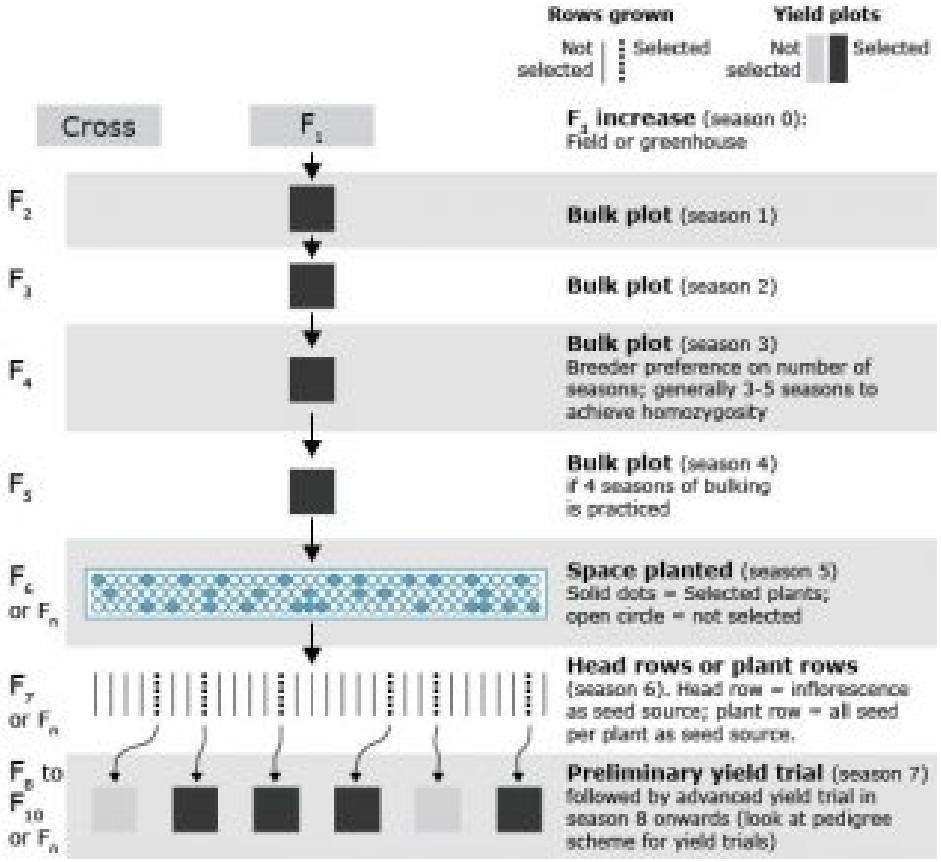

Fig. 2 Flow chart of bulk method of breeding.

Explanation of steps in Fig. 2

- Generations are advanced to homozygosity through bulks.
- It is a low cost, less technical method of breeding.
- Natural selection is used to remove undesirable plants.
- Artificial selection environment can be used to select for a trait of interest. Bulks can be

grown in a disease or another stress nursery to select for that trait. Markers can also be utilized to select for desirable traits to constitute the bulks. These variations will make the scheme as a modified bulk method.
- Early generation testing of bulk may be done for yield testing and to make a decision on retention of populations based on ranking among populations.

Additional notes

- In modified bulk method, single plants or inflorescence per plant are selected at each generation; while in bulk method, plants from the entire population are harvested and seeded (all or sub-sample of seed) in next generation.
- Lighter shade yield plot = grown, tested, not selected; darker shade yield plot = grown, tested, selected and advanced to next generation testing.

Single-Seed Descent Method

Single Seed Descent (SSD) was developed as a breeding method to rapidly advance lines to homozygosity so that selection can be practiced on homozygous lines (Fig. 3). The original intent of this method was to maintain a large population size to mimic the genetic variation in F_2 generation for effective selection. However, this method is now used to reduce the time to develop cultivars. (Sleper and Poehlman, 2006).

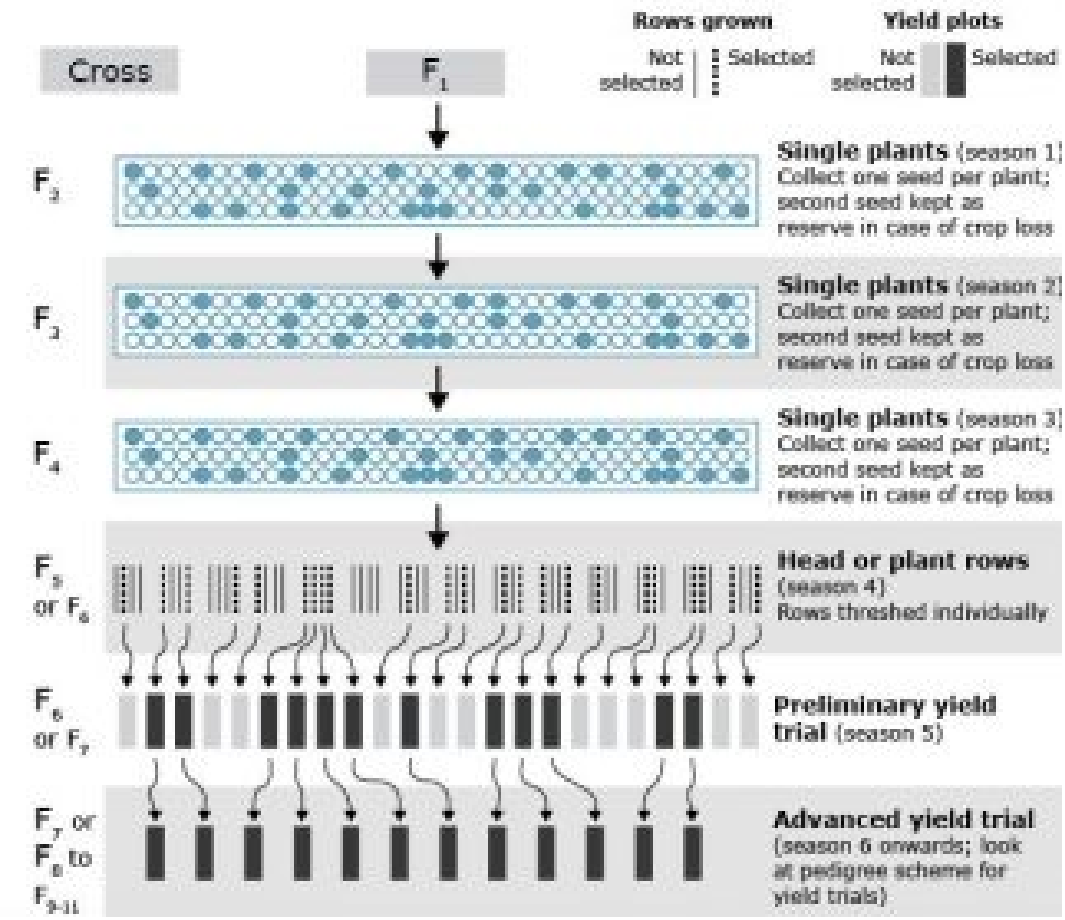

Fig. 3 Flow chart of single seed descent method of breeding.

Explanation of steps in Fig. 3

- Generations are advanced to homozygosity rapidly. In case of small grain crops (such as wheat, barley, oats), three seasons can be completed in artificial growing conditions (greenhouse etc.), and limited space is needed to keep a population size of 250-300 seed per cross.
- If true single seed descent is practiced (where one seed per plant is grown in successive generations, population size is reduced in each cycle due to losses due to no germination and emergence. As an alternative modified, single seed descent can be used where 2-3 seed per plant are planted in hill plots in each cycle, and 2-3 seed from each hill are collected from an inflorescence.
- SSD plots can be grown in a disease or another stress nursery to select for that trait.
- It is a cheaper, less technical method of breeding. Rapid inbreeding and homozygosity is

achieved.
- No need for record keeping of individual plants while advancing through SSD.
- Open circle = single plants (or hills in modified SSD) per population.

Doubled Haploid Method

Doubled haploids (DH) are created by generating haploid plants from microspores (androgenesis) or unfertilized eggs or ovules (gynogenesis). Haploid plants are then subjected to a chemical treatment (with colchicine) to double their chromosome number to produce homozygous diploid plants.

Doubled haploids are generated from heterozygous plants, typically F_1 plants derived from crossing of two pure-lines or inbred lines. DH can also be developed from selected F_2 individuals from a cross. This method is used in development of both self-pollinated (to develop pure-lines) and cross pollinated crops (to develop inbreds). Process is shown in Fig. 4.

- Generations are advanced to homozygosity in single generation. DH genotypes are true homozygous.
- Specialized lab is needed to create doubled haploids. Can be generated through a service provider.
- Population size is an important consideration because only one generation of meiosis occurs (at F_1).
- This method is suitable for marker assisted breeding to select for traits that are fixed.
- Can develop cultivars most quickly. If sufficient seed is available, can go to advanced yield trial in season 3.
- It is becoming a preferred method of inbred line development in maize.

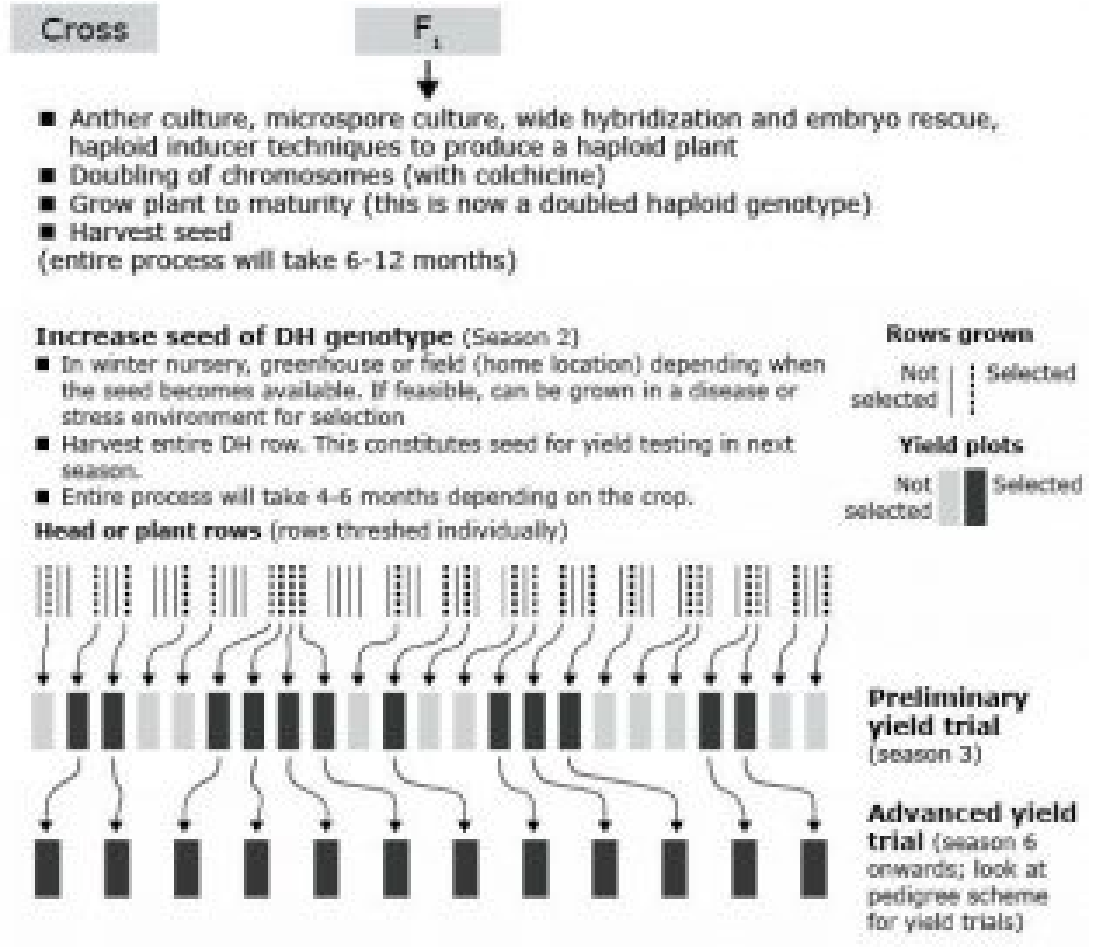

Fig. 4 Flow chart of doubled haploid method of breeding.

Backcross Breeding Method

The backcross breeding method is used if the objective is to introgress a gene into an elite cultivar or breeding line. Examples are disease resistance gene(s) and herbicide tolerance gene(s) (Fig. 5). By crossing to the recurrent (adapted) parent, the newly developed cultivar will contain the majority of the recurrent parent genome and only the gene of interest from the donor parent.

If the gene to transfer is recessive (**rr**), progeny of crossing with **RR** recurrent parent will segregate as **RR** and **Rr**, and therefore progenies are selfed for one generation to determine the **Rr** type versus **RR** types (**RR** are discarded) before making the next backcross. With the

application of molecular markers, this extra step has become redundant and F_1 plants can be grown, DNA extracted from young plant tissue to determine **Rr** and **RR** types. **RR** types can be removed and crosses can be made with **Rr** types.

For a backcross breeding program, if the gene to be moved comes from an unadapted or related species, the breeder has to be aware of inadvertently bringing in undesirable genes linked to the desired target gene (termed linkage drag). Larger population sizes will need to be grown to identify recombinants. Innovations, e.g. marker assisted backcrossing, marker assisted recurrent selection, and genomic selection, exist that reduce the need for large population sizes.

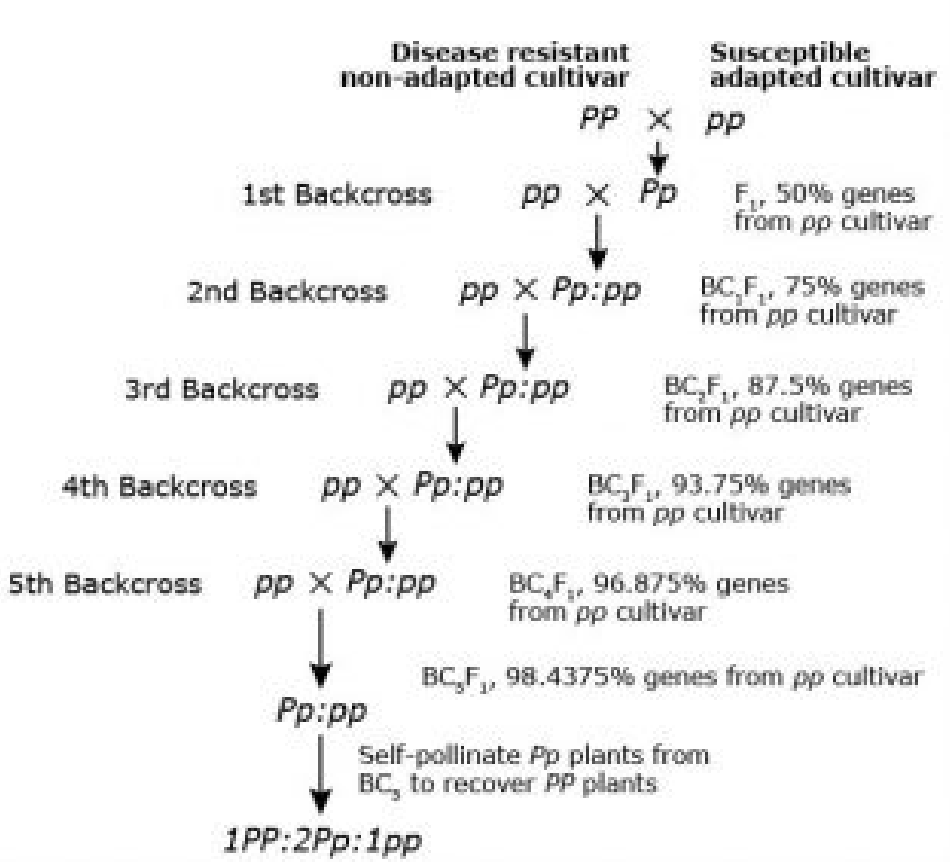

Fig. 5 Flow chart of backcross method of breeding.

Innovations in Backcross Breeding

Marker-Assisted Recurrent Selection

Reliability for Selection

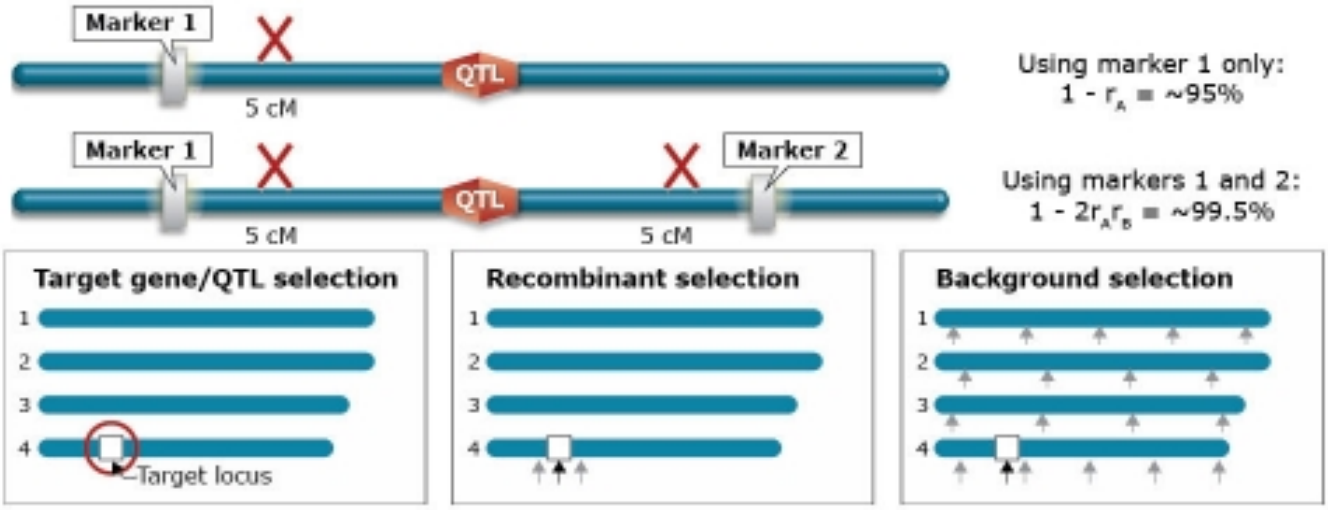

Fig. 6 Comparison of Target Gene/QTL, Recombinant and Background selection methods. Adapted from International Rice Research Institute, 2006. In *Target gene/QTL selection*, markers may be used to screen for the target trait, which may be useful for traits that have laborious phenotypic screening procedures or recessive alleles. In *Recombinant selection*, select backcross progeny with the target gene and tightly-linked flanking markers in order to minimize linkage drag. In *Background selection*, select backcross progeny (that have already been selected for the target trait) with background markers. In other words, markers can be used to select against the donor genome, and this will accelerate the recovery of the recurrent parent genome.

Steps of Marker-Assisted Recurrent Selection

1. One generation of phenotypic selection in the target environment is conducted,
2. Markers with significant effects are used to predict the performance of individual plants, and
3. Several generations of marker-only selection are performed in a year-round nursery or greenhouse

Comparison between Conventional and Marker-Assisted

Backcrossing

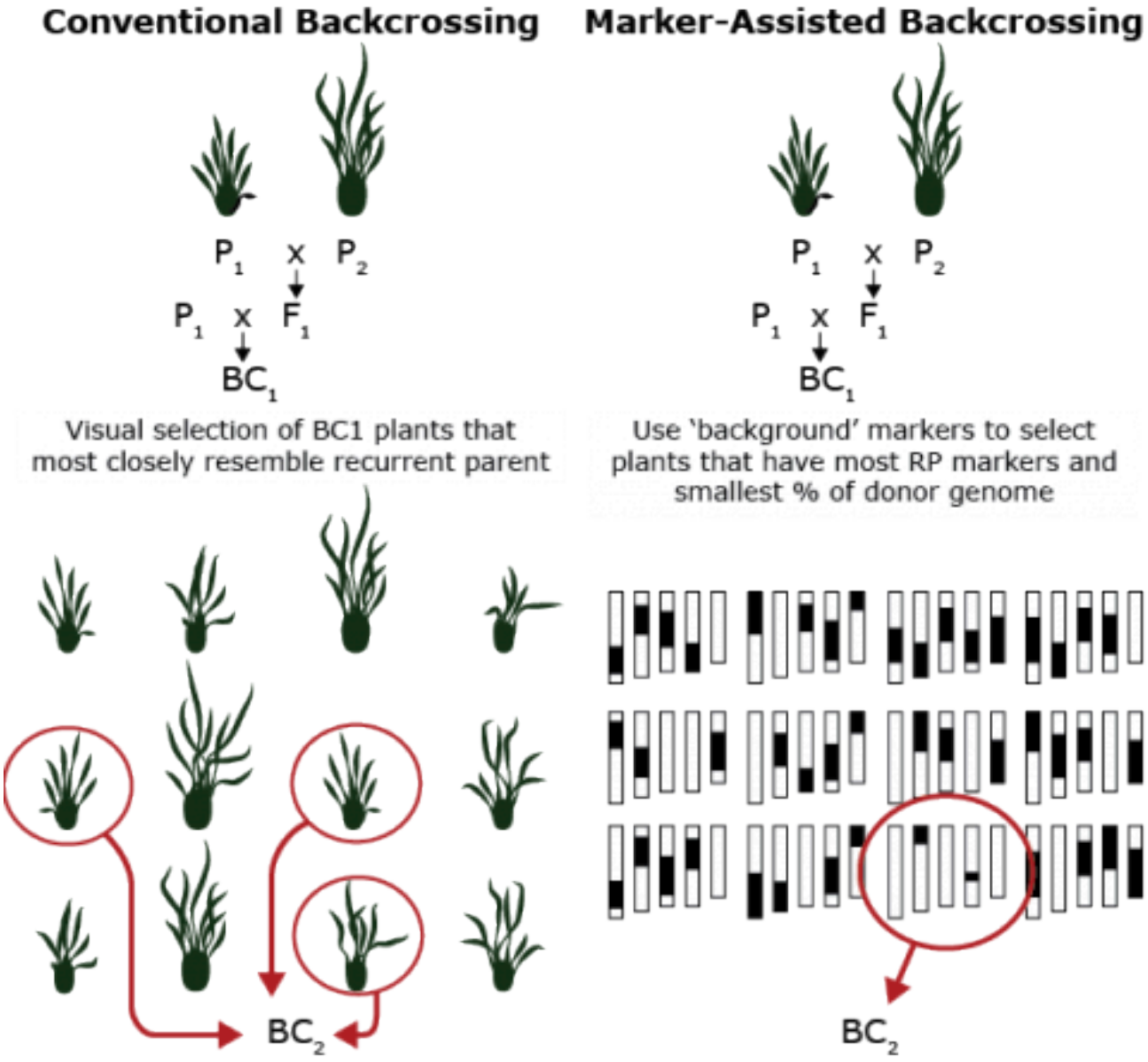

Fig. 7 Conventional versus marker-assisted backcrossing. Adapted from International Rice Research Institute, 2006.

Early Generation Testing

Early Generation Testing (EGT) describes the procedure for selecting superior lines or families before they are homozygous. It also refers to a specific use where a genetic worth of a population

is determined by analyzing yield data from a segregating (early generation) plot and removing entire populations. EGT is used in self- and cross-pollinated species.

In the pedigree breeding method we looked at individual plant selection for highly heritable traits in early generations. With high heritability, individual plant selection is still effective, for example traits such as plant height, disease resistance, and morphological traits. Several breeding programs, however, follow a modified method (such as modified pedigree method), in which yield testing is started in an early generation (for example, F_3 or F_4) to make selections. The early generation lines are grown on yield plots (2 or 4 row plots), therefore, more resources are required to handle EGT. Nonetheless, EGT allows elimination of materials (lines) that are inferior due to use of replication and multi-environment testing. Also, selection for lower heritability can be practiced to discard inferior lines.

Other breeders may choose to perform a yield test on populations derived from early generation bulks to identify superior bulks (inferior bulk populations are removed completely from further generation advancement). Thus, EGT testing in this scenario can be done for one or 2 generations followed by selection of superior plants, and then starting yield testing of these lines.

Cytoplasmic Male Sterility Systems

Plant breeders working with cytoplasmic male sterility (CMS) systems will aim to develop new 'B-lines' and 'R-lines'. In crops where CMS system is used to produce hybrids, different 'R' restorer genes are identified and breeders will improve 'R-lines' that will be used as males in creation of hybrids. 'B-lines' and 'R-lines' are developed using the self-pollinated breeding methods we learned about earlier in this module (pedigree, bulk, SSD, DH etc., or a modified method that combines more than one method in the development of breeding line of cultivar).

An outline of a CMS system is shown in Fig. 8. Note that the 'R' and 'r' genes are in the nucleus and the 'S' and 'F' genes are in the cytoplasm.

A breeder who develops 'B-lines' will use the backcross method to develop 'A-lines' using available CM sterility genes. A hybrid cultivar is produced by crossing of 'A-lines' with 'R-lines'. The A/B and R gene pools are considered separate gene pools (reproductive gene pools) similar to heterotic gene pools we learned about in maize systems.

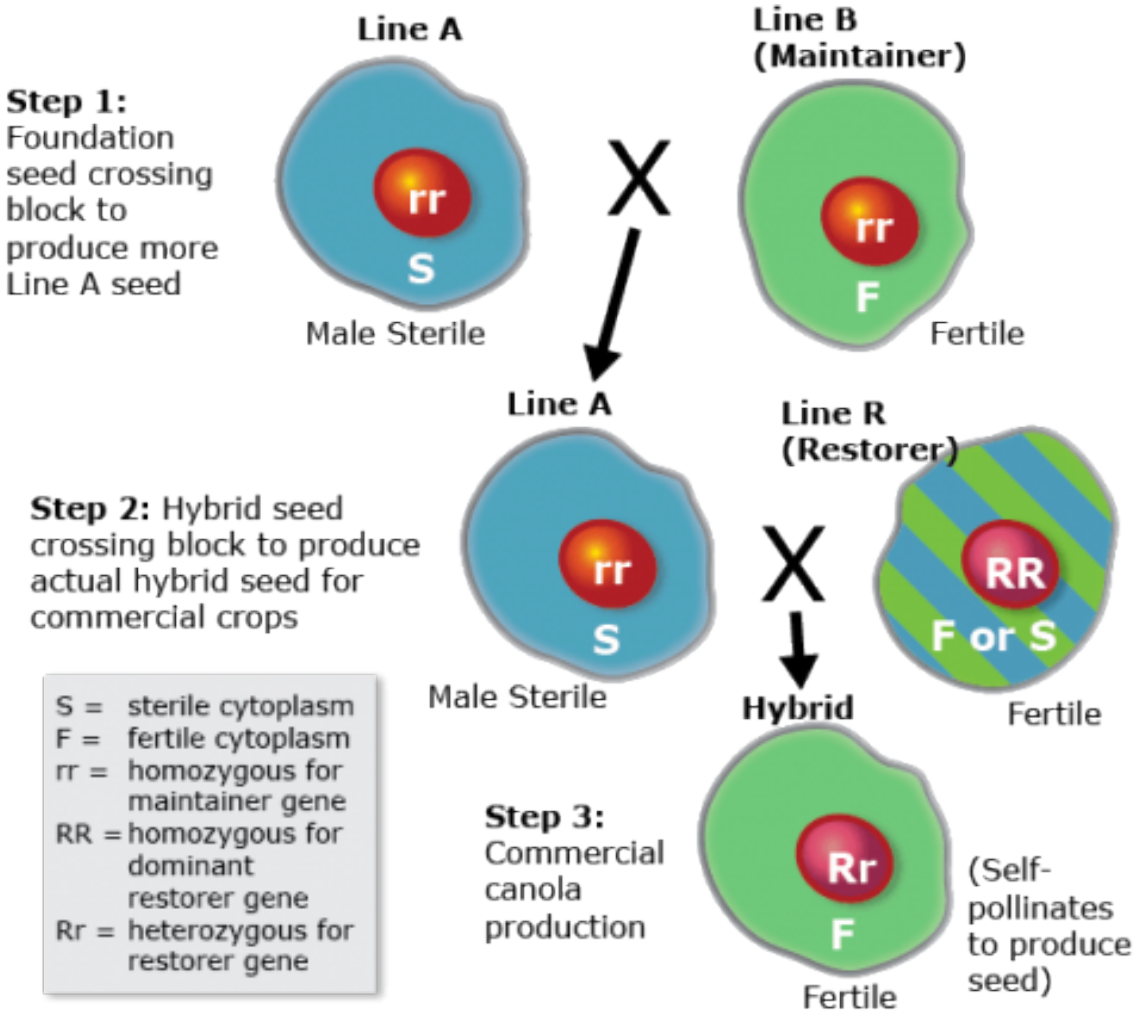

Fig. 8 Use of CMS system in hybrid seed production. Adapted from Canola Council of Canada.

Hybrid Cultivars

In the chapter on Steps in Cultivar Development, we looked at the development of maize hybrids using two-way crosses. Crosses are made within a heterotic group to develop superior inbred lines in the heterotic group. These inbred lines are crossed to testers from other heterotic groups to decide on the best specific combing ability. This process is repeated for all heterotic groups that the breeding institution or company works with internally.

For evaluation, superior inbred lines from dissimilar heterotic groups are crossed to produce hybrids. Several 100 or 1000's of hybrids are evaluated each year to finally pick the most superior

hybrid(s) for commercial release based on their performance and target area of adaptation (maturity, stress, environment etc.).

Hybrid seed is produced by growing inbred female rows (say 6 to 8) from one heterotic group and inbred male rows (1 or 2) from a dissimilar heterotic group interspersed among the sets of female rows, and de-tasseling the female rows (that is, removing male inflorescences from female plant rows) before pollen shed. Manual or mechanical tools are used to de-tassel (prior to pollen being ready or shed to avoid any selfing of plants of the inbred female line). Cobs from female rows are harvested and these constitute the hybrid seed. In some programs, but routinely done in private seed industries, the male rows are usually destroyed when pollination is completed to avoid contamination from cobs from inbred male plants if allowed to grow and produce cobs.

Recurrent Selection

In recurrent breeding and selection, parents of a crop species are crossed to develop populations using various mating designs described in the chapter on "Refresher on Population and Quantitative Genetics." Based on one or more selection criteria, and using within family and among family selection strategies, individuals are selected and inter-mated to produce the next generation. This procedure of selection can continue for an indefinite amount of time, hence the term "recurrent". Recurrent selection method is employed in order to achieve the following:

- The goal of recurrent selection is to improve the mean performance of a population of plants and to maintain the genetic variability present in the population.
- The underlying principle of recurrent selection is to increase the frequency of desirable genes that the breeder is attempting to improve.
- Recurrent selection is used to improve populations in cross pollinated species. Open pollinated varieties are one type of cultivar developed using recurrent selection.

Comparison: Mass Selection versus Phenotypic Recurrent Selection

- **Mass selection**: Female plants are selected after pollination with unselected and selected pollen source.
- **Phenotypic recurrent selection**: Male and female are both controlled. ONLY selected plants are intercrossed to obtain seed for the next cycle of selection. Expected genetic gain

from selection of only the female parent is one-half compared to expected genetic gain when both parents are selected.

Note that the terms mass selection and phenotypic recurrent selection are sometimes used interchangeably and one would have to look at the breeding scheme for details in order to determine which method is being referred to.

Comparison: Genotypic versus Phenotypic Recurrent Selection

The difference between genotypic and phenotypic recurrent selection is that Genotypic Recurrent Selection is selection **based on progeny performance** (combining ability), while Phenotypic Recurrent Selection is selection based on the **phenotype of the individual.**

Examples

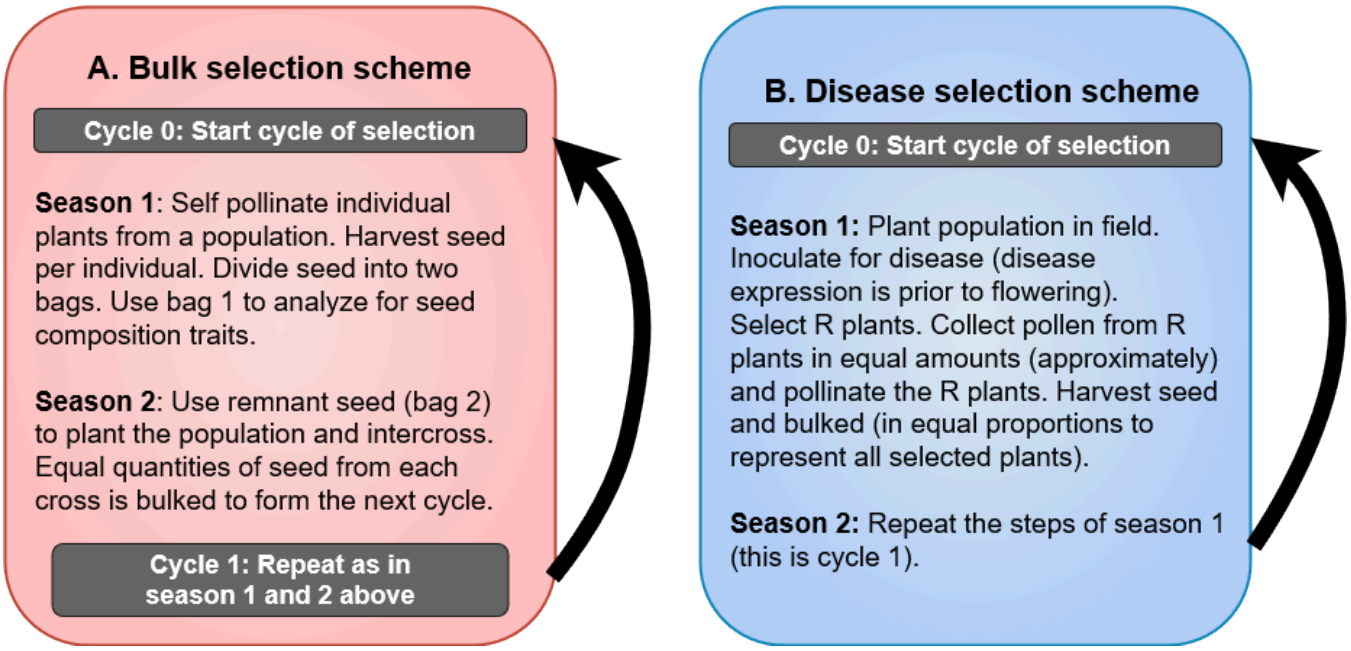

Fig. 9 Recurrent selection schemes for bulk method versus individual plant methods.

Phenotypic Recurrent Selection Issues

There are several problems with selecting individual plants in the field:

- Micro-environment variability does not permit assessing breeding value.
- Competition effect due to uneven planting.

Solutions to these problems include:

- Gridding designs (selecting plants within a grid)
- Not selecting plants that have missing neighbors

The generalized recurrent selection method consists of the following steps:

- development of a base population (for selection).
- evaluation of individuals from the population
- selection of superior individuals from the population
- intercrossing the selected individuals to form a new population.

Development of Base Population

A base population can be an existing population (for example a maize synthetic) which may not have been previously selected for your trait of interest.

More commonly, a base population will come from outstanding families from a recurrent selection program. It may also be created with elite inbred lines. Smaller number of inbred lines will ensure use of elite material that are similar morphologically, but inbreeding depression will be greater.

Superior inbred lines are identified based on their performance in multi-location tests and superior general combining ability (specific combining ability is not as important in the performance of OPV; it is most important if one is developing a hybrid cultivar).

These superior inbred lines are crossed using an appropriate mating design from among available designs (for example, diallel design).

Evaluation of Individuals

Individuals are evaluated for selection when advancing generations following crossing, and the type of cross made play a key role in the phenotypic and genotypic schemes employed in selecting individuals (Table 1).

Table 1 Comparison of phenotypic and genotypic schemes in individual selection

Phenotypic scheme	Genotypic scheme
Evaluation is based on individual plants per se	Evaluation is based on the performance of the progeny of the individual
Assessment is very variable unless species can be clonally propagated	Progeny performance strategy allows for replicated, multi-location testing.
Not easy to control environmental variability	This provides a more accurate assessment of individual's breeding value
n/a	Three types of progenies can be evaluated: self, full sib, or half sib

Progeny are produced by self-fertilizing the individuals that are evaluated for selection.

Full-sib families are created by crossing the individuals to be evaluated in pairwise combinations. Since in each pairwise cross both parents are common for that family, individuals of that family are full-sibs.

Half-sibs are formed by crossing the individuals to be evaluated to a common parent (which can be a population or an inbred line as a tester. Since all progeny have the tester as a common parent, they are half-sibs.

Population Improvement

As the name implies, the breeding populations creating from crossing parents, need to be improved in performance of the desired traits, in order to continue to make progress in breeding programs. Different methods are used for population improvement, and depending on the breeding program's specific project goals, can described as intra population or interpopulation improvement. Table 2 shows some methods that are used.

Table 2 Methods in recurrent selection.

Intrapopulation improvement	Interpopulation improvement
Mass selection (with or without pollen control)	Reciprocal half sibs recurrent
Half-sib family	Reciprocal full sib
Full-sib family	Testcross
Selfed family	n/a

Recurrent Phenotypic Selection

Steps include:

1. Plant a population (space planting individuals to facilitate note taking on individual plants).
2. Evaluate for trait of interest and identify the best individuals (higher heritability such as flowering time or morphological traits are suitable for this method).
3. Harvest seed of the best individuals and reconstitute seed to form the next cycle of recurrent selection.
4. In this example, pollen control can be exerted if the trait can be evaluated prior to flowering. Undesirables can be removed before they contribute pollen to the rest of population; and this ability to control parental pollen helps improve the response to selection.

Recurrent Half-Sib Selection

- An intra-population improvement method: cross the individuals in a population to a common tester (population per se, or inbred tester), evaluate the half-sib progeny of each plant, select the best individuals, and intercross the selected individuals.
- The main step is evaluation of individuals through their half-sib progeny. There are numerous variations within and among crops based on what is used as a tester (population vs inbred), parental control, intercrossing.
- Where possible, it is desirable to control both parents. This can be achieved by evaluating in one season and recombining in another generation (in winter nursery or second season). This necessitates an extra season but genetic gain per year will be higher. While the half-

sib are being evaluated, the remnant seed of the individual needs to be kept as reserve so that this seed can be used if the individual is selected based on the half-sib performance to intermate and create material for the next cycle of selection.

- In maize, obtaining selfed and half-sib seed from the same plant can be accomplished by self pollinating the single ear on the individual to be tested and using pollen from that individual to pollinate several individuals of the tester (bulk of population per se, or inbred line). The ears on the tester, bulked together from that individual as pollen source, represent the half-sib family to be tested for that individual.
- Recombining selfed progeny will require three seasons: (1) selfing and crossing to the tester, (2) evaluation, and (3) intercrossing selfed progeny.

Recurrent Half-Sib Examples

Female parent selected; population used as tester.

- Start with a random mating population
- Harvest ears of each plant (say, 200). Grow 200 half-sib progeny plots (with checks) at multiple locations (can be unreplicated or replicated). Traits of interest is yield (for example). At one location, grow in isolation as seed source for the next cycle. At this location, select plants within a half-sib row. At other locations, use for testing.
- At the location with isolation, grow the male rows (bulk seed of all half-sib families) adjacent to female half-sib rows. De-tassel the female rows.
- At the location where grown in isolation harvest ears from each selected plant by hand. Make selections to pick the best half-sib families. These ears will form the next cycle seed.
- Season 2, conduct random mating of selected plants.
- Repeat steps
- One can use an inbred line as tester instead of bulk seed of population used as male.

Female and male parent selected; population used as tester.

Cycle 0: (intermate population)

- Harvest ears from each plant (selection may be performed)
- Divide the seed of half sib plants into two: part 1 for next season field testing, part 2 for remnant to reconstitute selected half-sibs.

Season 1: Each half sib (using part 1 seed) is a separate entry in replicated or unreplicated trials with 2 or more locations, with checks.

- Select superior half-sib families based on performance. These selections will be used in crossing.

Season 2: remnant seed (part 2 of seed bag) of selected individuals is used for intercrossing to form next cycle.

Cycle 1: Seasons 3 and 4 – repeat as above.

- One can use an inbred line as tester instead of bulk seed of population used as male.

Recurrent Half-Sib (Testcross Progeny)

- Start with an intermated population
- Season 1: plants in an intermated population are selfed and pollen used for selfing and pollinating a tester.
- Season 2: testcross progeny are evaluated in replicated tests. Selections made to identify superior performing progenies.
- Season 3: selfed seed of selected families are used to form the next intermating cycle. Cycle is repeated as above.

Recurrent Full-Sib Process

The main steps are listed below.

End of first year:

- Season 1: Make paired crosses between individuals in the population.
- Season 2: Evaluate the full-sib families in the field and identify the best families.
- Season 3: Recombine (intercross) the best families using remnant seed from the first season.

Start of second year:

- Season 4: Begin the second cycle with paired crosses between individuals in the population.

An advantage is the completion of one cycle per year. A disadvantage is less recombination between cycles of selection.

Recurrent Full-Sib Example

Start with an intermated population. Make selections.

- Season 1: paired crosses are made between pairs of selected plants in a population. Seed is divided into two parts: Part 1 is for field testing, and Part 2 is to reconstitute next cycle.
- Season 2: Part 1 seed used to plant field tests. Evaluate full-sib in field tests (single or multiple locations, unreplicated or replicated, with checks). Select superior families based on performance.
- Season 3: Part 2 seed used to intercross selected families. Intermated seed is used to form the next cycle.
- Cycle 2: Seasons 4, 5, 6.

Recurrent Selection Among Selfed Families

- Season 1: S_0 plants from the population are selfed to produce $S_{0:1}$ lines.
- Season 2: Evaluate the selfed progenies in field (for trait of interest).
- Season 3: Use the remnant S_1 seed from season 1 to intercross selected lines.

This completes cycle 1 and S_0 plants are obtained. The cycle is repeated as described above in season 4-6 for cycle 2, and so on.

Variation can include more than one generation of selfing if more seed is required for evaluation.

Reciprocal Recurrent Selection

Reciprocal recurrent selection (RRS), as a breeding method for open-pollinated crops was first proposed by Comstock et al. 1949 to take advantage of both additive and dominance genetic

effects. In brief, plants from one population are mated to plants of another population, and selection of individuals for the next cycle of selection is based on the performance of progeny in hybrid combination. For this breeding method, each cycle requires one generation for selection of individuals and a second generation for intermating of selected individuals to produce materials for the next generation. RRS is a procedure to improve both the general and specific combining ability of two populations simultaneously, and steps involved are as below:

- Plants are selected in each of two populations
- Plants of population#1 are selfed and outcrossed as the tester to the selected plants in population#2 to generate test cross progeny.
- Plants of population#2 are selfed and outcrossed as the tester to the selected plants in population#1.
- The resulting test cross progenies are evaluate in each season. Superior plants are identified based on their test cross performance. Selfed seed from these selected plants are used to intercross within each population to generate materials for the next generation.
- Cycle is repeated.

Maize Open Pollinated Varieties (OPV)

Development of a Maize OPV

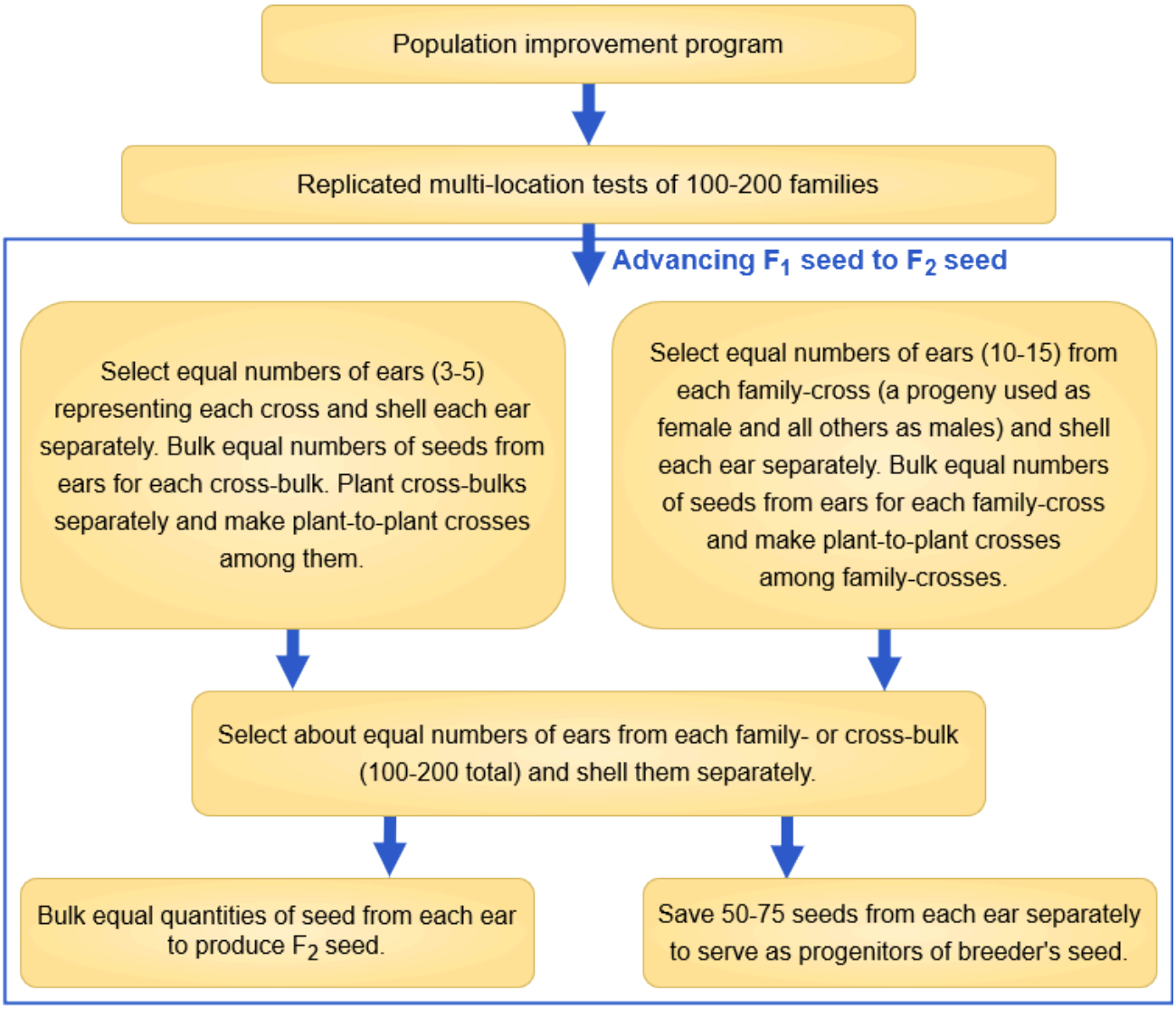

Fig. 10 Flow chart of development of an OPV of maize. Adapted from The Maize Program, 1999.

Maize OPV Cultivar Evaluation

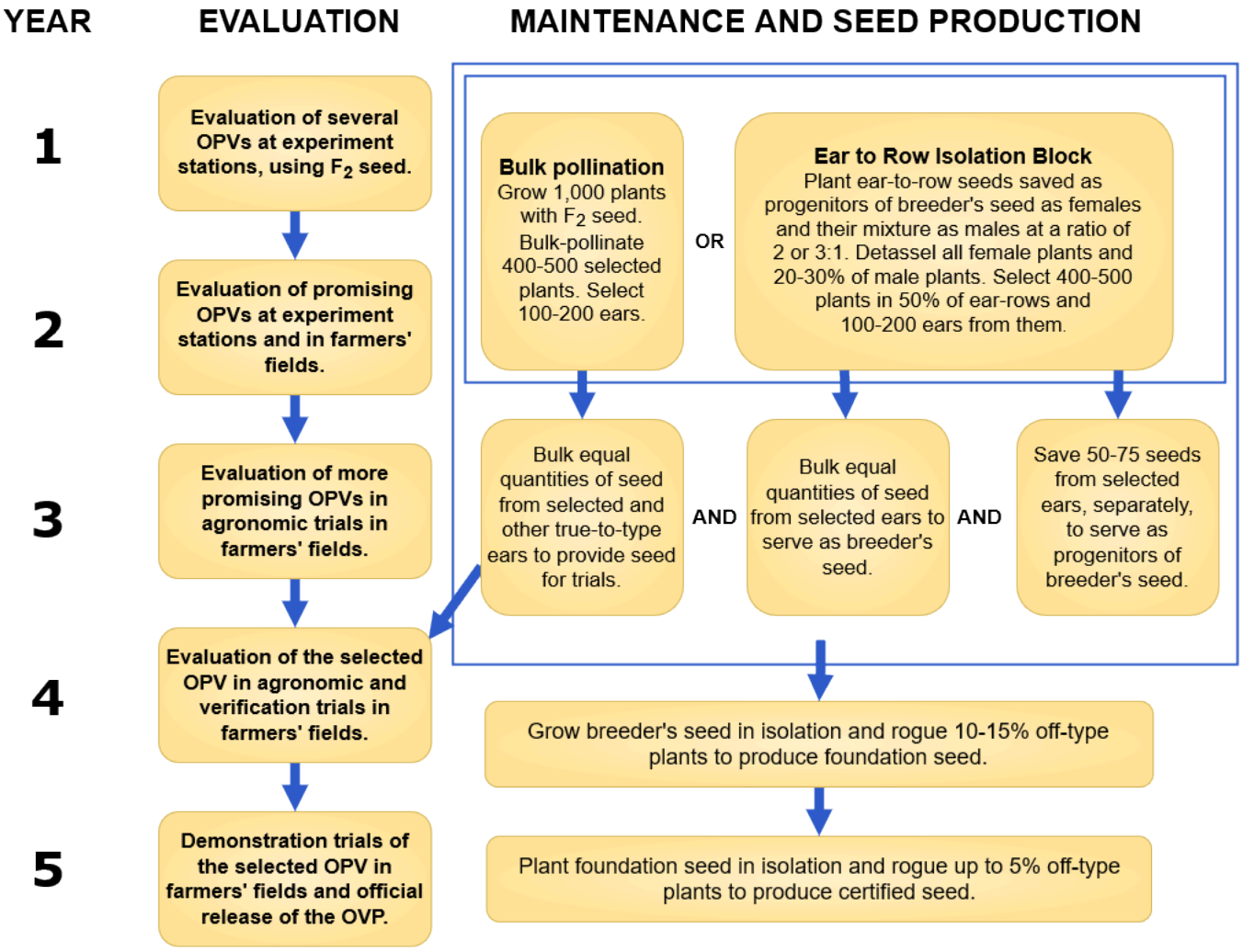

Fig. 11 Flow chart of cultivar evaluation, maintenance, and seed production of maize.

OPV Advantages and Disadvantages

Table 3 OPV advantages and disadvantages.

ADVANTAGES	DISADVANTAGES
Seed can be re-cycled (if grown in isolation or middle field harvested without a significant yield reduction due to inbreeding depression)	Yields lower than hybrids
Can have much more broader adaptability compared to hybrids (that are developed for targeted areas)	Is not comparable to hybrids in areas where land is fertile and inputs are available to maximize yield
May be less costly than hybrid	Plants are less uniform
May require less inputs than hybrids	Seed needs to be harvested properly to use for next year, and even then there will be a yield reduction.
OPV may be more accessible in areas where no hybrids are available or seed availability channels are poor	n/a

Clonal Cultivar Methods

Since each clone breeds true (i.e., no gene segregation because no sexual recombination), breeding programs can evaluate a clone in several different tests simultaneously (field testing, disease nursery etc.). In clonal crop breeding, each cross produces unique and distinct F_1 seed (true seed). True seed plants are transplanted into field testing and selection commences to identify which F_1 of F_1's are suitable for cultivar release. Step-wise reduction process is used to discard undesirable F_1 clones each testing season (remember, clones can be propagated for more extensive testing once smaller number of desirable clones are identified. Shown in Table 4 below is an example of sugarcane cultivar CP 03-1912 developed in Florida.

Table 4 Summary of process followed in the release of sugarcane cultivar CP 03-1912 in Florida. Data from Gilbert et al., 2011.

Year	Month	Stage and activity completed	Number of genotypes in stage	Locations
2000	Dec	Cross made at USDA–ARS sugarcane field station	No data	Canal Point, FL
2002	May	Germinated true seed transplanted into field (seedlings)	100,000	Canal Point, FL
2003	Jan	Advanced from plant–cane seedlings to stage 1	15,000	Canal Point, FL
2003	Sep	Assigned name CP 03-1912 in stage 1	15,000	Canal Point, FL
2003	Nov	Advanced from plant cane stage 1 to stage 2	1,496	Canal Point, FL
2004	Nov–Dec	Advanced from plant cane stage 2 to stage 3	135	Four farms in Florida
2006	Nov–Dec	Advanced from plant cane stage 3 to stage 4 sand soils	13	Four farms in Florida
2011	Feb	Cultivar release	1	No data

Approximately 10% culling rate was practiced in each season after 2003. As seasons advance, clones are grown in replicated yield trials at several locations and comparisons with standard checks is made to identify which clones to advance to the next stage of testing.

Synthetic Cultivar

Synthetic cultivars are formed by using clones of inbred lines in pre-determined proportions for released to farmers. Farmers can use a synthetic for several generations (as open-pollinated population) but once inbreeding depression causes yield reduction, farmers need to use seed from the breeding institution or company. Therefore synthetics are reconstituted regularly by the breeder. Maize is an example where synthetics have been developed. In crops with self-incompatibility, synthetics are the preferred types of cultivars as the method exploits heterosis for a few generations.

Clones or inbred lines used in the formation of synthetic are chosen on the basis of their general

combining ability. Crossing is made to ensure random pollination allowing gametes of each component (clone of inbred line) to be equally represented.

References

Canola Council of Canada. 2014. Canola varieties.

The Maize Program. 1999. Development, Maintenance and Seed Multiplication of Open-Pollinated Maize Varieties – 2nd edition. Mexico, D>F.: CIMMYT.

Comstock, R. E, H.E. Robinson, and H.P. Harvey. 1949. A breeding procedure designed to make maximum use of both general and specific combining ability. J. Am. Soc. Agron. 41: 360-367.

Gilbert et al. 2011. Registration of 'CP 03-1912' sugarcane. JPR 5(3): 319-324.

International Rice Research Institute. 2006. Molecular breeding, Lesson 1: Marker assisted breeding for rice improvement.

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Chapter 7: Participatory Plant Breeding and Participatory Variety Selection

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Formal or conventional plant breeding programs (centralized breeding programs) are often designed to meet specific requirements of different groups of farmers in different growing environments (regions, countries, soil, or climatic conditions). Formal or conventional plant breeding programs have generally been more beneficial to those farmers who either have good crop growing environments or have the capacity to modify growing environments through the application of additional inputs such as fertilizer, pesticides, and irrigation to create more favorable growing conditions for new varieties. However, the results of formal plant breeding may sometimes not meet the requirements of those farmers who grow their crops under marginal soils and high-stress environmental conditions (Sperling et al., 2001) thus necessitating different breeding approaches to be created to meet the needs of poor farmers.

Participatory plant breeding (PPB) and participatory variety selection (PVS) have been developed and implemented over the past 10 years as an alternative and integral part of the breeding approach in traditional plant breeding. It has been mainly implemented in developing countries where farmers with limited resources grow their crops in marginal lands of remote regions. It is practically implemented in areas where the technology transfer or adoption of modern cultivars is low (as farmers are not comfortable with taking the risk to replace their well-known and reliable traditional varieties with new varieties) or where modern cultivars are not available. Therefore PPB has emerged to address the agricultural problems of poor farmers in developing countries where resources and modern technologies are limited. PPB has been widely considered to be more advantageous to use in areas where low yield potential, high stress (drought), and heterogeneous environments exist. The various aspects of PPB described above are depicted in Fig. 1 below.

Learning Objectives

- Know the goals of participatory plant breeding
- State the different types, stages, and requirements of participatory plant breeding
- Describe the roles farmers play in participatory plant breeding
- Articulate the outcomes and impact of participatory plant breeding and participatory variety selection

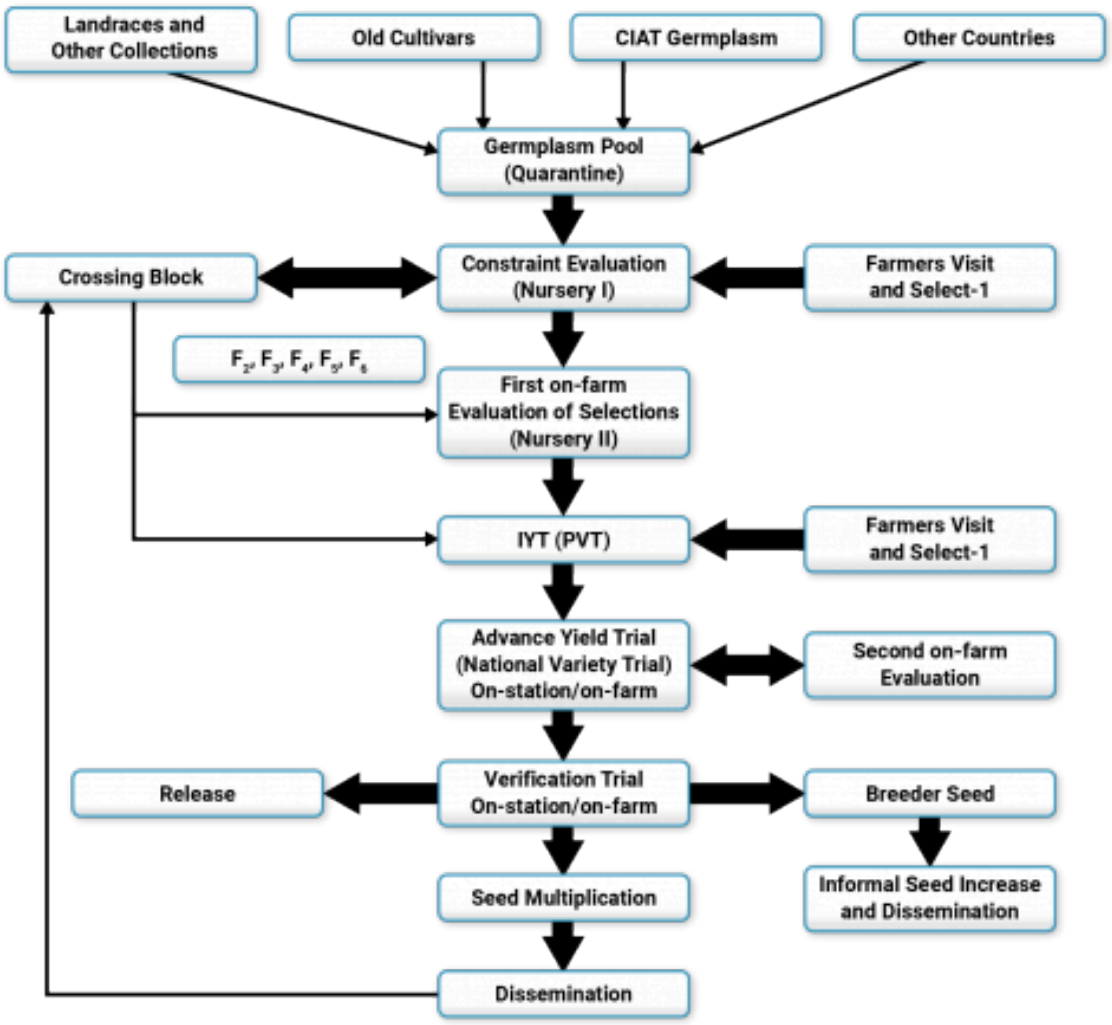

Fig. 1 A generalized scheme linking formal and participatory bean breeding (CIAT, 2001).

Participatory Plant Breeding (PPB)

PPB Categories

It is an approach involving different participants including scientists, farmers, along with consumers, extension agents, farmers' cooperatives, vendors, traders, processors, government and non-government organizations in plant breeding research (Sperling et al., 2001). It is considered as "participatory" because of the mixture of different people from different organizations involved, especially end-users, having significant research roles in all major stages of the breeding, evaluation and selection process. These different actors participate in setting PPB goals, setting breeding priorities, selecting genotypes from a heterogeneous population, helping in evaluation and selection of cultivars in the farmers' fields and on research stations, releasing and popularizing high yielding cultivars, and helping in seed multiplication and distributing (McGuire et al., 2003). Participatory plant breeding is grouped into the following categories:

1. **Formal-led Participatory Plant Breeding** describes a situation when farmers are asked to join in PPB activities which have been initiated, managed and executed by formal breeding programs such as National Agricultural Research System (NARS) or International Agricultural Research Center (IARC).
2. **Farmer-led Participatory Plant Breeding** describes a situation when scientists and/or development workers seek to contribute or support famers own controlled, managed and executed breeding systems. Scientists can support their own varietal selection and seed system.

Goals of PPB

In any PPB approach, the first activity involves carrying out a diagnostic survey. The diagnostic survey allows an effective discussion between breeders and farmers and also enables breeders to better understand:

1. agricultural problems of the local farming conditions,
2. farmers' crop management practices,
3. farmers' specific needs and preferences.

The goals of PPB are to:

1. Increase production and productivity in non-commercial crops in environments that are unpredictable and under abiotic and/or biotic stress.
2. Enhance biodiversity and increase germplasm access to local farmers. This provides benefit to local farmers, especially to disadvantaged user groups (women and poor farmers), for developing adapted genotypes. It also makes the breeding program cost-effective and output-oriented through decentralization that can address more niches.
3. Increase farmer skills to speed up farmer selection and seed production efforts.

Types of Participation

The types of participation in PPB are:

- **Conventional:** In this approach, there is no farmer participation.
- **Consultative:** Farmers are consulted at every PPB stage but the breeder makes the decisions. The consultation of farmers starts from identifying breeding objectives and selection of appropriate parental materials. In this approach, farmers participate in making joint selections with a breeder among genotypes in breeders' plots on station.
- **Collaborative:** In this approach, decisions are made jointly by farmer and breeder. Farmers and breeders know each other regarding selection criteria and their priorities for their research through two-way communication. To revoke or override the joint decision made earlier, both farmer and breeder need to agree on the change(s). Usually this type of participation is effective for self-pollinated crops.
- **Collegial participation:** Farmers grow genotypes in their farm fields and make their own plant or genotype selections. In this approach farmers can make decisions in a group or individually but in an organized communication with the breeder. In this approach farmers voluntarily supply some of the seeds of selected genotypes to the breeder for further evaluation and seed multiplication.
- **Farmer experimentation:** In this approach breeders do not participate in selection of genotypes or in any farmers' research activities. Farmers make their own decision either in a group or as individuals on how to implement their research activities with new genotypes without organized communication with breeders.

Stages of Participation

In general, participation approaches to choose and implement depends on the resources availability and type of the crop which could be used in PPB.

Stages of Participation In PPB Process

1. Set the breeding objectives/targets: Farmers' participation in setting breeding objectives begins from the participatory rural appraisal.
2. Generate (access) genetic variability from local landraces or using collections for testing with complementary characteristics.
3. Determine the approach (consultative/collaborative). This depends on the availability of resources and on the type of the crop (it is more easily done for collaborative participation if the crop is self-pollinated species) and selecting among segregating populations.
4. Evaluate cultivar and discard inferior genotypes (culling) (this is participatory variety selection if the farmer is involved in selection of genotype).
5. Collaborating with seed system (cultivar release, popularization, diffusion and seed multiplication and finally distribution).

Essential Requirements for Success

For PPB to be successful, the following requirements must be met:

1. The local farmers should be interested in active participation during plant breeding/ selection process
2. Breeders and farmers have to collaborate with each other during each stage of PPB
3. Importantly, PPB has a better chance of success if:

    - locally adapted parents are used in the development of crosses made for PPB
    - selection of desirable or superior genotypes is made in the local environments
    - cultivars that are selected by farmers should have traits important to the farmers

Roles And Contributions of Farmers in PPB Work

Farmers:

1. provide technical leadership role in testing cultivars to specific environmental niches. They also contribute their knowledge and experiences.
2. play a role in organizing farmer research groups.
3. provide information on cultivar preferences and important traits that could be maintained or introduced to the existing land races.
4. are involved in skill building through farmer-farmer interactions.
5. provide their landraces or their genetic materials that could be used for further breeding work.
6. provide land for testing the PPB genotypes.

Major Possible Outcomes

1. *Production gain*: significant production gains would be expected through increased yield, increased stability of crop yield, faster uptake of released cultivars, wider diffusion of the varieties and better identification of farmer-preferred quality traits (e.g. taste, ease of processing, etc.).
2. *Biodiversity enhancement*: Farmers communities get more access to different germplasm, more information related to germplasm as well as getting related knowledge, increases access to inter and intra cultivar diversity.
3. *Cost-efficiency and cost effectiveness*: The time of selection is short so cultivars are identified within shorter timeframe (3-4 years), i.e. cultivars identified faster. This reduces research cost. The released cultivars do not take a long time to disseminate to the farmers so less expensive for diffusing cultivars. Figure 2 is a timeline comparing conventional and PPB systems in bean breeding and clearly shows the fewer number of years involved in selection for the next cycle or variety release with PPB system.
4. *Farmer knowledge increase and capacity is enhancement*: this facilitates the development of more PPB lines, gain in extensive experience and increase in agricultural knowledge dissemination, including agronomic practices.
5. *Farmers' needs are met*. Farmer satisfaction increases due to fulfillment of their demand. A broader range of users, such as women, men, elders and young, is reached.

Impacts of PPB

1. Higher adoption rate of PPB products such as new cultivars, agronomic and crop protection practices.

2. Improved cultivars acceptable by farmers for highly stressed marginal areas.
3. In most remote areas of developing countries where soil is degraded and drought is a major production problem, new varieties developed and immediate adoption of the new technologies and yield increase is achieved.
4. Significant changes in cultivar release procedure and seed multiplication system. In PPB, time for testing to release of cultivar is shorter than conventional breeding.

Participatory Variety Selection (PVS)

Introduction

Generally, participatory variety selection (PVS) is a continuation of PPB. Once potential cultivars are identified through PPB process, farmers can test those cultivars using PVS approach. Usually farmers participate at the end of the cyclical process.

More specifically, PVS is an approach where selection of finished or near finished cultivars is made by the farmer on her/his own fields. The finished products/genotypes include released cultivars, advanced stage cultivars, advanced non-segregating lines in self-pollinated crops or advanced populations in cross pollinated crops.

PVS includes research and extension methods that help to deploy genotypes (promising advanced lines/released cultivars) on farmers' fields. Therefore, cultivars that are developed through PVS, would meet the demand of different farmers (men and women, old and young).

Participatory variety selection comprises three phases to select farmer preferred cultivars.

1. Clear identification of farmers' needs.
2. Search for suitable advanced lines or cultivars to test in farmers' conditions.
3. Implementing the experiment on farmers own fields and dissemination of preferred cultivars.

Importance of PVS

1. Provide access to local farmers' choice of a large number of cultivars and increase in crop diversity.

2. Increase production and productivity which helps to ensure food security.
3. It helps to speed up dissemination and enhances adoption of pre-released and released cultivars in diversified environments.
4. It enables cultivar selection in targeted environmental niches in a short period of time with less cost.

Conventional and Participatory Timeline

In Fig. 2, conventional and participatory breeding methods are shown, comparing the steps and duration for cultivar release, using beans as an example.

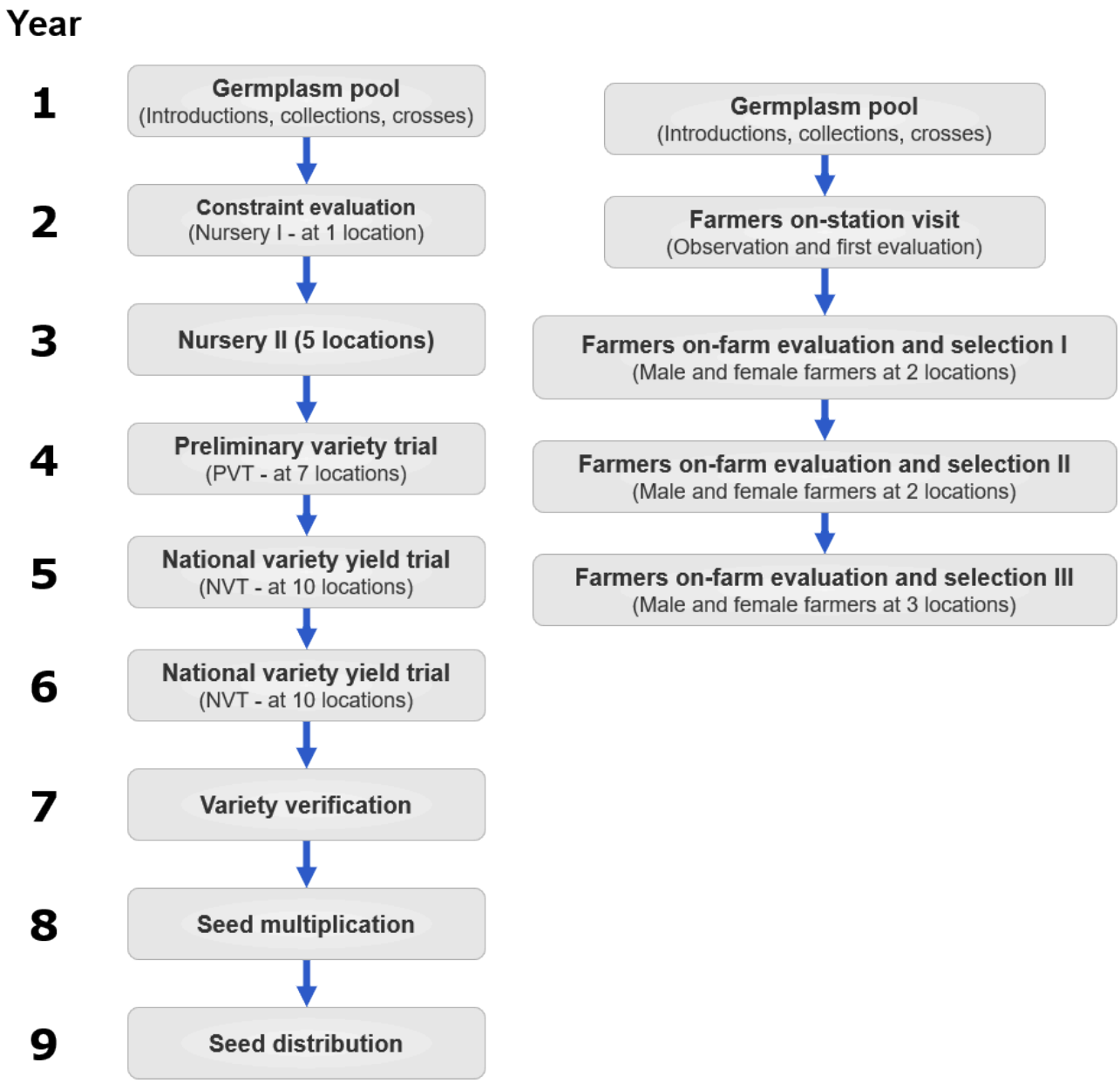

Fig. 2 Timeline of conventional & participatory bean breeding. Adapted from Assefa, 2005.

Impact Pathway

The impacts of participatory breeding are shown in Fig. 3 below.

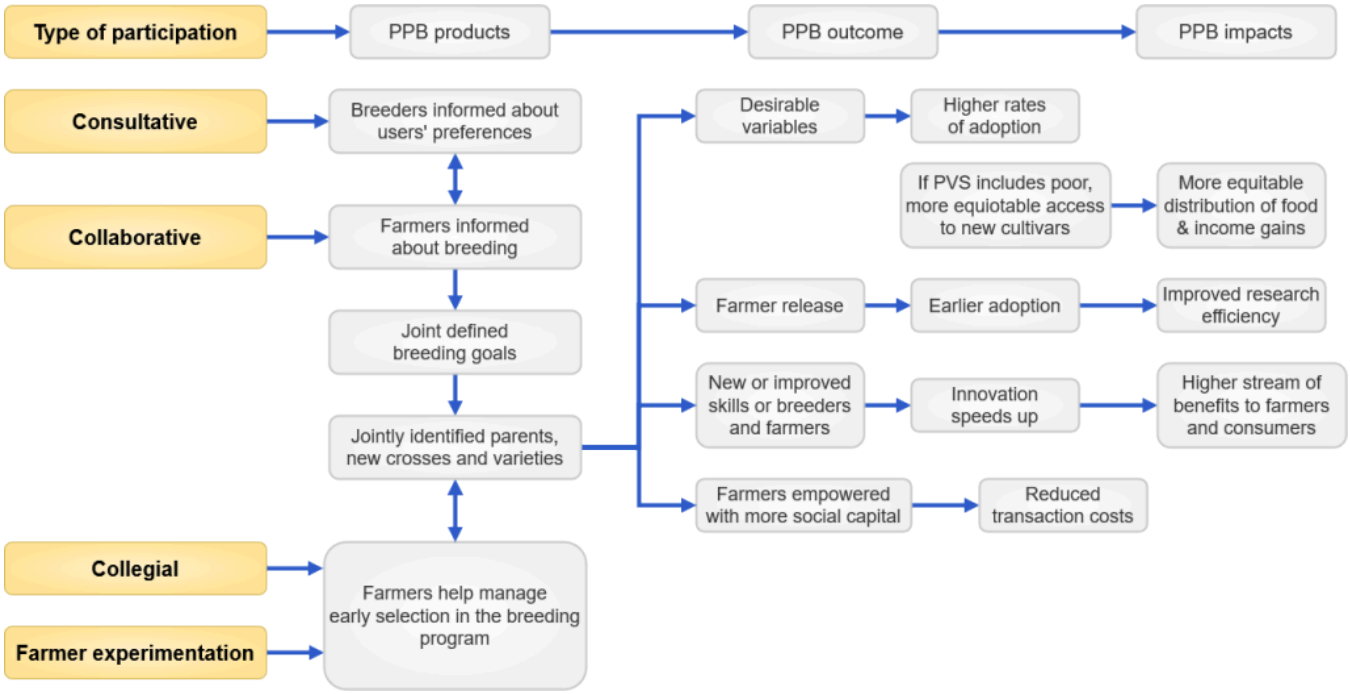

Fig. 3 Impact Pathway for Participatory Plant Breeding. Click the image to see a larger version. Adapted from Jacqueline A. Ashby, 2009.

References

Ashby, J. A. 2009. The impact of participatory plant breeding pp. 649-671. In: S. Ceccarelli, E. P. Guimaraes, and E. Weltzien, editor. Plant breeding and farmer participation. Food and Agriculture Organization of the United Nations, Rome, 2009.

Assefa, T., A. Girma, F. Chemeda, T. Bulti, and R. Abdel. 2005. Participatory bean breeding with women and smallholder farmers in eastern Ethiopia. World Journal of Agricultural Sciences 1 (1): 28-35, 2005.

McGuire, S. J. 2003. Path-dependency in plant breeding: Challenges facing participatory reforms in the Ethiopian Sorghum Improvement Program. Agricultural Systems 96 (2008) 139-149. ScienceDirect

Sperling, L., J. A. Ashby, M. E. Smith, E. Weltzien, and S. McGuire. 2001. A framework for analyzing participatory plant breeding approaches and results. Euphytica 122: 439-450.

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Chapter 8: Common Bean Breeding

Teshale Mamo; Asheesh Singh; Arti Singh; and Anthony A. Mahama

Common bean, *Phaseolus vulgaris*, (belongs to the *Fabaceae* family) is an annual plant that is grown in many parts of the world for the seed (bean) that is eaten immature or mature (after shelling and drying). Common bean is an important plant protein source in human diet.

The genus Phaseolus originated across a wide geographical area in the tropics and subtropics of Latin America, from north-central Mexico to northwest Argentina (Fig. 1), and comprises more than 30 species. Among these species, the most widely cultivated species are:

- P. *vulgaris* L. (common bean),
- P. *coccinus* L. (runner bean),
- P. *accutifolius* L. (tepary bean),
- P. *polyanthus* Greenman (year-long bean) and
- P. *lunatus* L. (Lima bean).

Among these five species, P. *vulgaris* (common bean) is the most widely grown (~ 85% of total world production planted).

Learning Objectives

- Become familiar with the Common bean crop
- Know crop biology and classification system
- Describe adaptation and usage
- Outline production constraints
- List breeding institutions working on the crop
- Discuss the breeding methods used to develop common bean cultivars

Domestication and Diversity

Ancestral Origins

Common beans were domesticated in two major centers, the Andean and Middle America (Fig. 1). Plants of the wild bean ancestor of *Phaseolus vulgaris* L. grow as viny herbaceous annual plants found from northern Mexico to northern Argentina (Fig. 1).

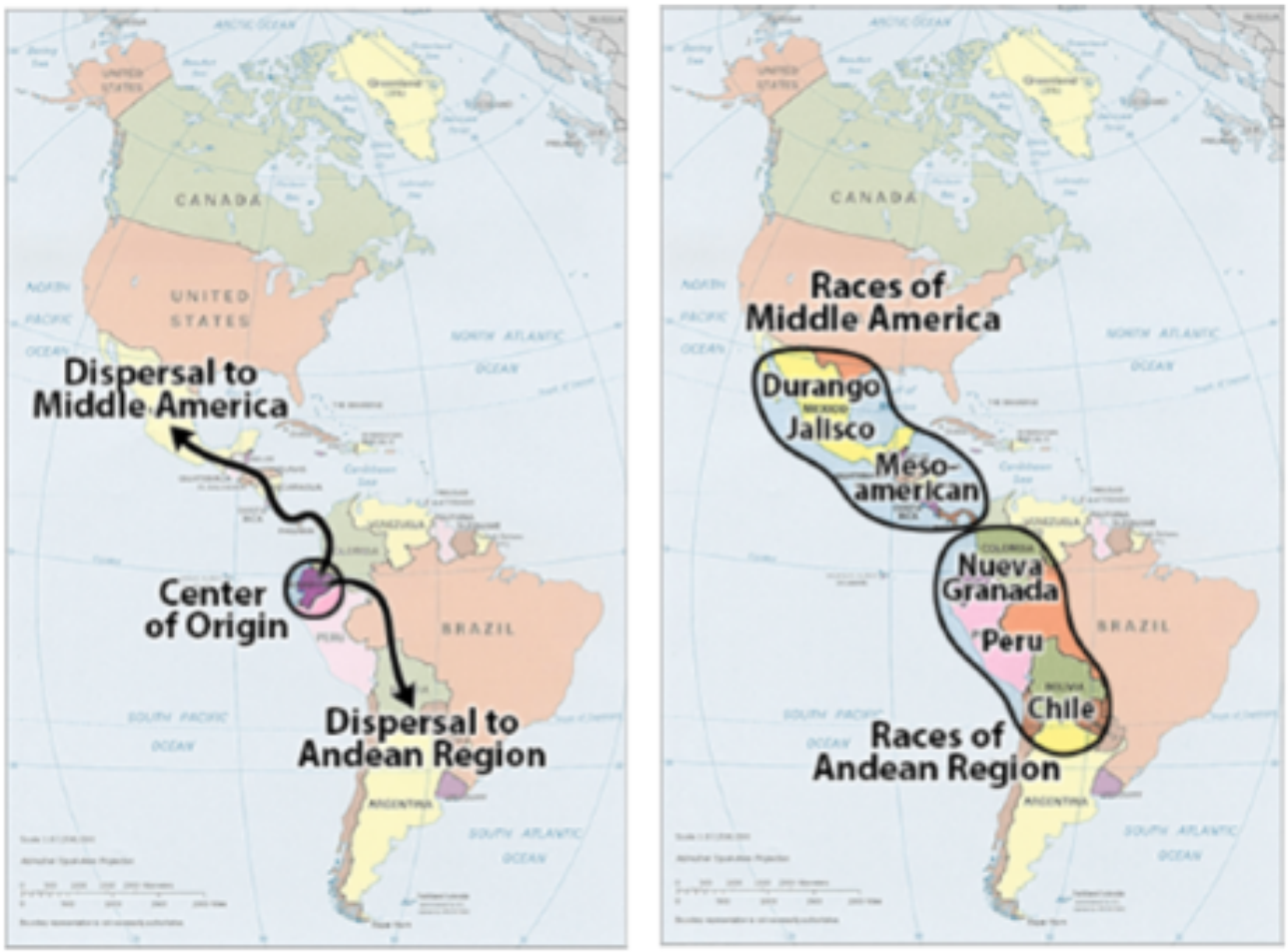

Fig. 1 Center of origin and dispersal of wild bean and domesticated races of common bean within the two major gene pools. Adapted from P. McClean in Kelly J.D., 2010.

Bean Gene Pool

Genetic analysis using DNA marker diversity and Amplified Fragmented Length Polymorphism (AFLP) suggest that there are four wild bean gene pools, centered in: (1) Middle America (Mexico and Central America); (2) Colombia; (3) Western Ecuador and Northern Peru; and (4) the southern Andes (Tohme et al., 1996). The cultivated bean gene pool is derived mainly from the southern Andean wild bean gene pool and the Middle American gene pool. Wild stands are shown in Fig. 2.

Fig. 2 A wild *P. vulgaris* population in Argentina. Photo by Gepts.

Different Races

The major gene pools in turn have been divided into different races based on plant morphology, adaptation range and agronomic traits (Fig. 3).

Fig. 3 Characteristics of dry seeds of different races of cultivated common bean. Photo by Singh et al., 1991.

Biology of the Crop

General Characteristics of the Development of the Bean Plant

The biological cycle of the bean plant is divided into a vegetative phase and a reproductive phase following that. The vegetative phase starts from germination of the seed and ends when the first floral buds appear in cultivars of determinate growth habit, or the first racemes in cultivars of indeterminate habit. The reproductive phase ranges from the moment the first floral buds or racemes appear, to maturity. In plants of indeterminate growth habit, vegetative structures continue to appear when the vegetative phase has ended, which makes it possible for a plant to

produce leaves, branches, stem, flowers and pods simultaneously. In determinate growth habit, the vegetative phase ends when the floral buds appear.

Growth stages of common bean are categorized into four groups (Schwartz et al., 2010).

Group 1: *Emergence & Early Vegetative Growth*

- VE: Emergence of hypocotyl from soil (crook stage)
- VC: two cotyledons & primary leaves at nodes 1 & 2
- V1: 1st trifoliolate leaf unfolded at node 3
- V2: 2nd trifoliolate leaf unfolded at node 4
- V3: 3rd trifoliolate leaf unfolded at node 5

Group 2: *Branching & Rapid Vegetative Growth*

- V4: 4^{th} trifoliolate unfolded at node 6 + branching
- Vn: n^{th} trifoliolate leaf unfolded at node (n+2)

Group 3: *Flowering & Pod Formation*

- R1: one open flower (early flower) on the plant
- R2: 50% open flowers (mid flower)
- R3: one pod at maximum length (early pod set)
- R4: 50% of pods at maximum length (mid pod set)

Group 4: *Pod Fill & Maturation*

- R5: one pod with fully developed seeds
- R6: 50% of pods with fully developed seeds (mid seed fill)
- R7: one pod at mature color (physiological maturity)
- RH: 80% of pods at mature color (harvest maturity)

Photosynthesis

Beans are classified in a C_3 photosynthetic pathway. The maximum leaf photosynthetic rates at

ambient carbon dioxide (CO_2) concentrations is estimated from 12 mg CO_2 dm^2 h^{-1} to 35 mg CO_2 dm^2 h^{-1}. Recent report showed relatively high photosynthetic rates in common beans, and this might be due to improved measurement techniques, but still lower photosynthetic rates than soybean (White and Juan, 1991).

Photoperiod and Temperature

Common bean, like most plants, flowers only in response to a certain amount of exposure to sunlight or photoperiod (termed the critical photoperiod), and are described as being photoperiod-sensitive, while others flower regardless of exposure time, and are described as photoperiod-insensitive or day neutral. Whereas day neutral genotypes occur, most common bean cultivars show a short day response for flowering (i.e. plants of such cultivars flower when the length of night exceeds their critical photoperiod). Genotypes of a high proportion of large seeded and highland germplasm are photoperiod-sensitive. The International Center for Tropical Agriculture (CIAT) reported that the photoperiod effects on common bean phenology increases with temperature. Higher temperatures cause a greater overall rate of growth and development. In general, both temperature and photoperiod have strong effects on growth and development of the bean plant. The inheritance of photoperiod-temperature response of flowering is controlled by few major genes.

General Classification System of Beans

Classification by Utilization or Mode of Consumption

Common beans may be grouped based on the stage of plant maturity when they are consumed:

- green or snap beans are horticultural beans grown for, and consumed as, fresh or processed pods
- green shell or fresh beans are grown for, and consumed as, fresh, full-sized seeds
- dry beans are grown for dried ripe seeds.

Classification by Seed Characteristics

Dry common beans are primarily characterized by the great diversity of seed types within the

species: a rainbow array of colors and color patterns, varying degree of brilliance, and several seed shapes and sizes exist as shown in Fig. 4.

- Seed type (color, size, shape, and surface texture) is the character most commonly used to classify beans. Seed size of commercial cultivars may vary from 17 grams (navy beans) to 100 grams per 100 seeds (Faba beans).
- Seed shape varies from round to oblong to kidney-shaped with many combinations of color patterns. Surface texture may be shiny (brilliant), opaque, or intermediate.

Fig. 4 Diversity of bean seed size, shape and color in major commercial classes grown in the U.S. Photos by L. Copeland in Kelly J.D., 2010.

Classification By Growth Habit

Growth habit in beans varies from determinate dwarf beans to very vigorous indeterminate

climbing beans. Common classification often divides beans into two or three groups: bush and climbing beans, or bush, semi-climbing, and climbing beans (Fig. 5).

Fig. 5 Bush bean (left) and climbing bean (right). Photos by Iowa State University.

Classification by Duration of Growth Period

Bean varieties are usually grouped as early or late, however, the range of duration of growth-period varies from one region to another, or among varieties of different growth habits. According to growth habit and region, days to maturity among bean cultivars range from 60 to 300. The difference is not only varietal but also environmental, especially for the factors of day-length and temperature.

Adaption, Economic Importance, and Uses

Adaption

Common bean is a widely cultivated grain legume crop in tropical and subtropical areas of the world. Bean is adapted to a wide range of environments, and grows in latitudes between 52^{o}N to 32^{o}S in humid tropics, in the semi-arid tropics, and even in the cold climatic regions (Fig. 6).

It is a short-day tropical crop that requires between 300-600 mm precipitation to complete its life cycle, depending on soil, climate, and cultivar (Beebe et al., 2013).

Optimum crop production requires temperatures of between 21-24^{o}C during the growing season and soil pH of between 6.3-6.7.

According to figures from FAO, world production is around 27.7 million tons (FAO, 2021). Latin America is the largest common bean-producing region, followed by the continent of Africa. Brazil, Mexico, and the USA are the three largest common bean-producing countries in the western hemisphere. In Africa, the majority of bean production is concentrated in the eastern and southern highlands extending from Ethiopia to South Africa. In this region, Kenya is the largest common bean-producing country. Common Bean production mainly occurs on dryland (i.e., depending on rainfall), with smaller land area under irrigated systems.

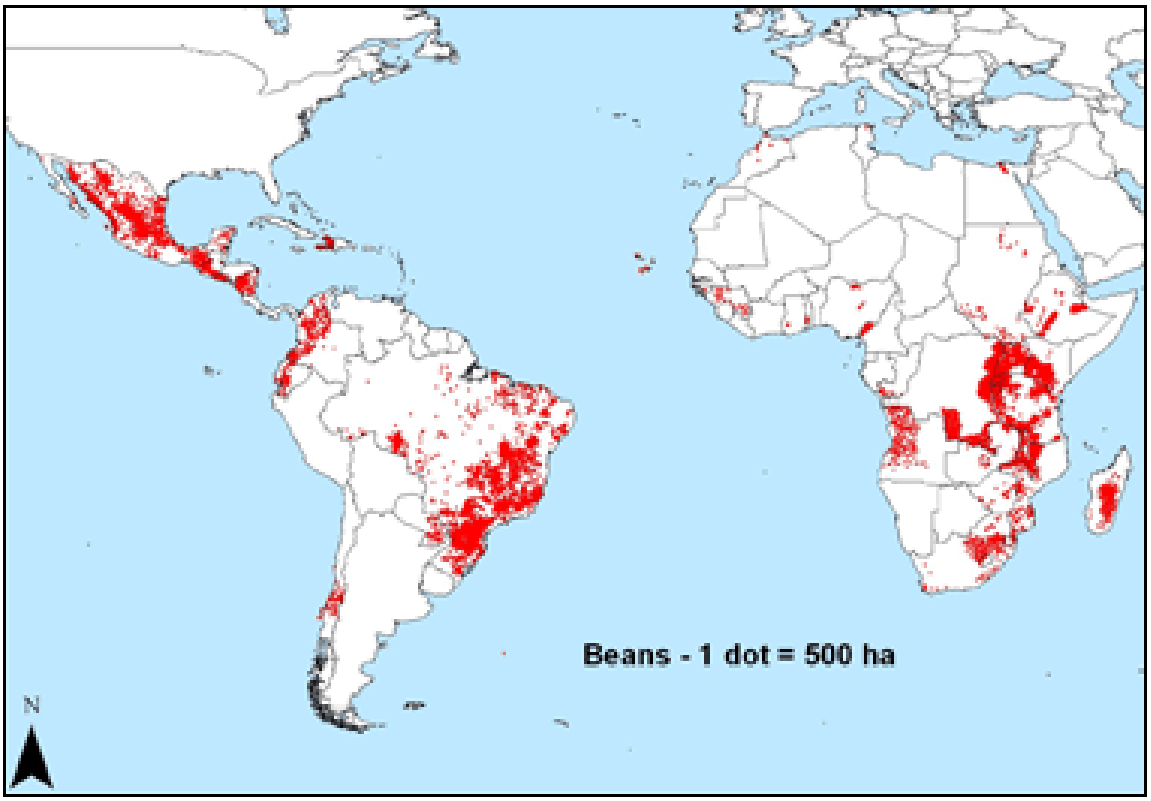

Fig. 6 Distribution of bean production in the tropics and sub-tropics.

Common Bean in the Human Diet and Nutrition

Common bean is mainly grown for human consumption. In some countries it is one of the food security crops providing protein and fiber to more than 100 million people in Africa

(Kimani et al. 2001). Common bean is mainly consumed as a mature grain in most parts of the world. Immature seeds, young pods and leaves are also consumed as a vegetable by some communities in sub-Saharan Africa and Latin America. Plant protein is the largest source of protein in human diet of poor people in the developing countries. Common bean therefore plays an important role in human diet due to its high protein content. Common beans are also a key source of minerals in human diet, especially iron.

Common Bean in Cropping Systems

In Africa, common beans are traditionally grown by farmers with small land holdings. This crop is often grown in complex farming systems as intercropped or in rotation with maize, sorghum, bananas, or other crops (Fig. 7).

The range of growth habits (from determinate bush types to vigorous climbers), and the range of growth cycles (from 3 to 10 months in length) allow common beans to fit many production niches.

In East and Central Africa, 23% of the production area is monocropped and 77% is grown in association, that is intercropping, with other crops (Katungi et al. 2010).

Monocropping is dominant in southern Africa with only 47% of the production area assigned to intercropping with other crops. In this cropping system, common bean has the capacity to break disease and pest cycles usually associated with cereals.

Atmospheric Nitrogen

The ability to fix atmospheric nitrogen (N) for subsequent crops has made common bean a valuable crop in many smallholder cropping systems. Lunze and Ngongo (2011) reported that climbing beans have the capacity to fix 16-42 kg ha^{-1} of atmospheric N per season and this can be further increased with good agronomic and cultural practices, thus boosting yields of non-legume crops. For example, in East Africa, sorghum yield improvements of 40-57% were reported when sorghum was grown in rotation with climbing beans. In the eastern region of Central Africa, yield of cereal crops grown after climbing beans increased by 25-40%. In this region farmers have no capacity to purchase inorganic fertilizers, neither do they have enough animals to supply organic fertilizer in the form of manure. As a result, common bean acts as a source of N supply to primary cereal crops. Common bean is therefore important in improving the soil health and helps maintain soil fertility.

Fig. 7 Intercropping of bean with sorghum. Photo by Iowa State University.

Production Constraints

Biotic Constraints

Biotic stresses such as diseases and pests are universal constraints to common bean production, especially fungal pathogens. Under favorable disease conditions, fungal pathogens cause significant yield losses. Yield losses also occur due to insect damage (Table 1).

Anthracnose, rust, and angular leaf spot are widely distributed, while rhizoctonia web blight and ascochyta blight can be locally intense in warm-moist environments, respectively. In recent years, root rots have emerged as a significant problem for common bean production, especially

those caused by *Pythium* spp. and Fusarium spp. Insects are occasional problems. In Central America the bean pod weevil, *Apion godmani* and *A. aurichalceum*, is the most important pest, while in East Africa the bean stem maggot, aphids, and pod borers cause the most serious problems.

Abiotic Constraints

Abiotic stress is the major constraint to bean productivity in most tropical countries. Abiotic factor such as extreme limited water stress (drought) cause yield loss in Mexico, Brazil, Central America, and Eastern and Southern Africa. Heat stress adversely affects the cultivation of beans in Central and Southern America and Africa (Beebe et al. 2011). Nutrient deficiencies of phosphorous (P) and nitrogen (N) also reduces yield, while Aluminum and Manganese toxicity associated with acid soil, as well as low Calcium availability, cause significant common bean yield loss (Table 1).

Table 1 A schematic comparison of different bean production limitations, classified for their frequency, likely intensity, and risk to farmers.

Limitation	Frequency	Intensity	Risk
Pests and diseases	+++	+++	++++
Drought	++	++++	
Low soil fertility	+++++	+++	+
High temperatures	+++++	+++	+
+ : very low +++++ : very high *Source*: Adapted from Beebe et al. 2006b			n/a

International Breeding Centers

The International Center for Tropical Agriculture (CIAT) was established in Cali, Colombia, under the Consultative Group on International Agricultural Research (CGIAR) system (in 1971) with the mandate to work on common bean (*Phaseolus vulgaris* L.). CIAT coordinates all common bean research programs at the national level. Strong collaborative and active breeding programs are found in many countries throughout the tropics of the Americas and Africa, with interchanging of improved germplasm among countries.

The primary mission of CIAT's bean program is to contribute to global food security. Their goal includes making bean production more profitable for small scale farmers in Africa, Latin America and the Caribbean countries.

CIAT has successfully developed bean varieties with genetic resistance to major diseases and pests, which have helped to minimize yield losses for farmers. More recently breeding programs have focused on breeding for improved bean tolerance to abiotic stresses such as drought and soil problems. These efforts have gained more significance due to more erratic climatic conditions that change the patterns and intensity of both abiotic and biotic stresses. CIAT's breeding strategy for beans focusses on priority bean grain (market class) types.

Supporting Broad Goals

The CIAT's bean program uses tools that allow them to support the broad goals including exploiting the biodiversity of more than 35,000 accessions in CIAT collection, biotechnology, particularly marker assisted selection, and gene discovery.

CIAT outlines the technical contributions and responsibilities for various regional bean breeding centers such as ECABREN (East and Central Africa Bean Research Network) and SABREN (South African Bean Research Network), and national bean breeding programs, universities and advanced research institutions.

Breeding Methods

Improving Seed Yield

Common bean is a self-pollinating crop, and thus breeding methods to improve seed yield and other important traits have followed methods similar to those applied to autogamous crops. These include *pedigree selection* (most commonly used breeding system in common bean improvement), *back cross* (for highly heritable traits, usually under single gene control), *inbred back cross* (1 or 2 back cross, followed by selfing), *congruity back crossing* (alternate crossing to each parent in alternate generations – maintains heterozygosity), *recurrent selection* (Fig. 8), *single seed descent* (among closely related elite lines), *gamete selection* (individual F_1 plants of multiple parent crosses give rise to families) have been used. The breeding strategies of common bean have also followed approaches similar to those applied to other crops (Gepts, 2002). These approaches

are described in Fig. 9. In addition, a three-tiered breeding strategy has been proposed to accommodate gene exchange between distantly related parents and to have more success for integrated genetic improvement of common bean (Fig. 10).

Fig. 8 Common bean yield trial at Melkassa, Ethiopia. Photos by Iowa State University.

General Steps

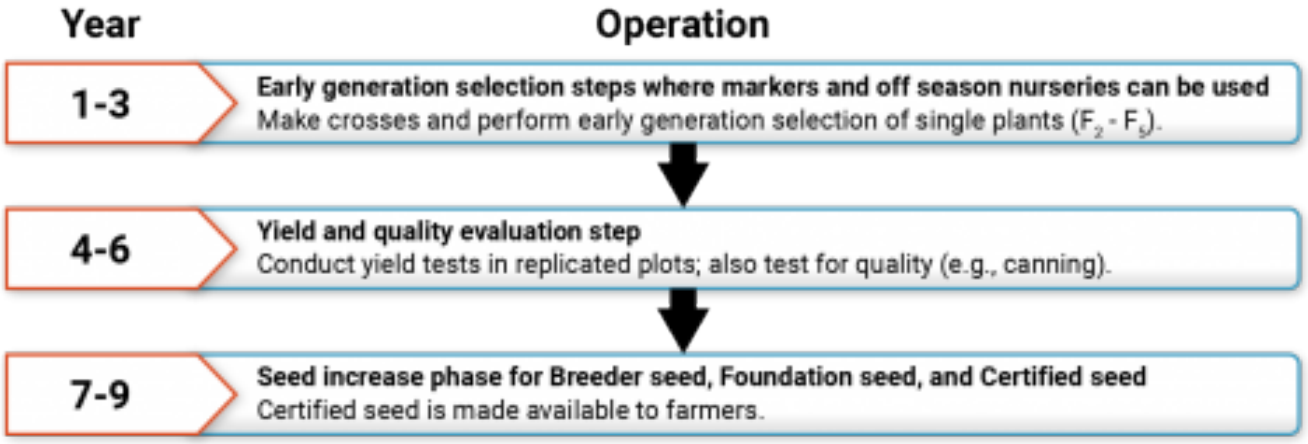

Fig. 9 General steps in common bean breeding.

Breeding Pyramid

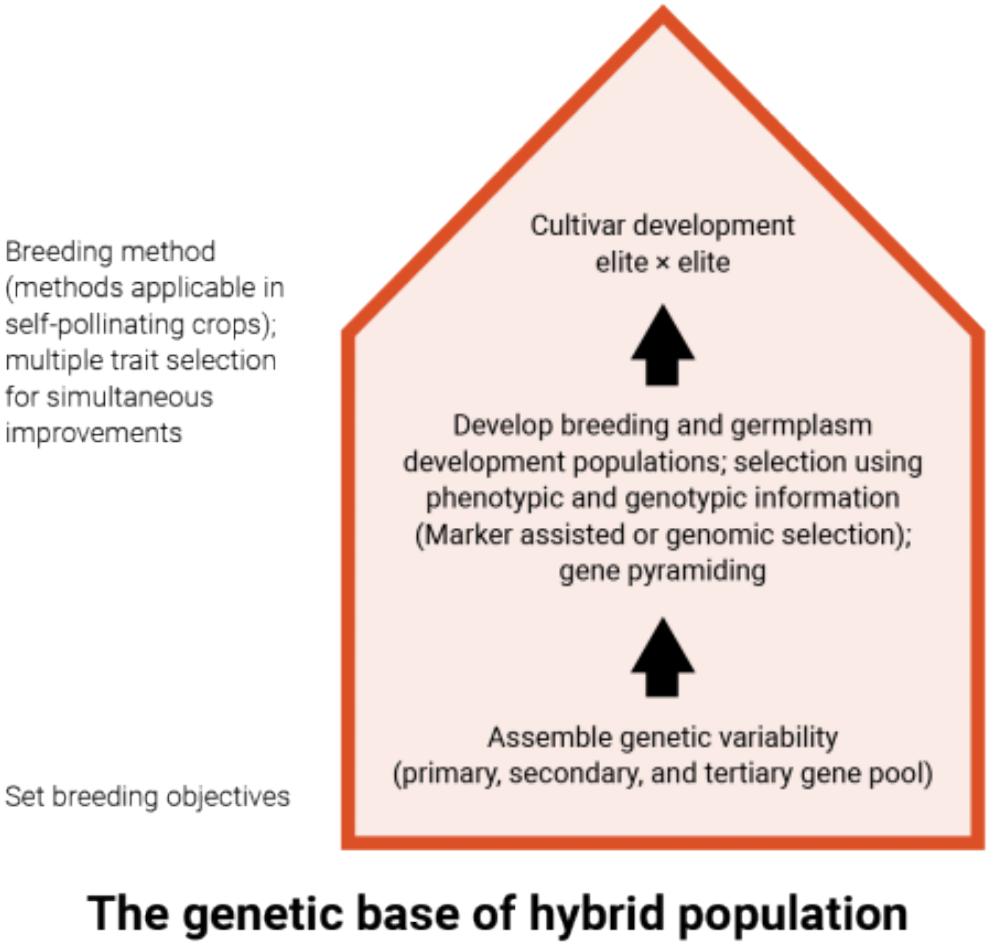

Fig. 10 Summary of integrated genetic improvement of common bean.

References

Beebe, S., I. Rao, H. Terán, and C. Cajiao. 2006b. Breeding concepts and approaches in food legumes: The example of common bean. p. 23-29. In: K. Ali, G. Kenneni, S. Ahmed, R.S. Malhotra, S. Beniwal, K. Makkouk, and M.H. Halila (eds.), Food and forage legumes of Ethiopia: Progress and prospects. Proceedings of the Workshop on Food and Forage Legume, 22-26 Sept. 2003. Addis Ababa, Ethiopia. EIAR and ICARDA, Allepo. Syria.

Beebe S., J. Ramirez, A. Jarvis, I. M. Rao, G. Mosquera, J. M. Bueno, and M. Blair. 2011. Genetic improvement of common beans and the challenges of climate change. In: Yadav SS, Redden RJ,

Hatfield JL, LotzeCampen J and Hall AE (eds), Crop Adaptation to Climate Change. John Wiley and Sons, Ltd. Published by Blackwell Publishing Ltd., pp. 356-369.

Beebe. S., I. Rao, M. Blair, and A. Jorge. 2013. Phenotyping common beans for adaptation to drought. Front Physiol. 2013; 4: 35.

FAO. 2021. FAOSTAT: FAO Statistical Databases. Available online at: http://faostat.fao.org/

Gepts, P. 2002. A Comparison between Crop Domehttp://faostat.fao.org/stication, Clasical Plant Breeding, and Genetic Engineering. Crop Science 42(6) 1780-1790.

Katungi E., A. Farrow, T. Mutuoki, S. Gebeyehu, D. Karanja, F. Alamayehu, L. Sperling, S. Beebe, J.C. Rubyogo, and R. Buruchara. 2010. Improving Common Bean productivity. An Analysis of socio-economic factors in Ethiopia and Eastern Kenya. CIAT Working document #217.

Kelly, J.D. 2010. The Story of Bean Breeding. Michigan State University (Ed.). U.S.A. 30 pp.

Kimani, P.M., R. Buruchara, K. Ampofo, M. Pyndji, R. M. Chirwa, and R. Kirkby. 2001. Breeding beans for smallholder farmers in Eastern, Central, and Southern Africa: Constraints, achievements, and potential.

Lunze, L., and M. Ngongo. 2011. Potential nitrogen contribution of climbing bean to subsequent maize crop in rotation in South Kivu province of Democratic Republic of Congo. *In* A. Bationo, B. Waswa, J. Okeyo, F. Maina & J. Kihara, eds. *Innovations as key to the Green Revolution in Africa*, pp. 677681. Dordrecht, The Netherlands, Springer Science.

Singh S.P., P. Gepts, and D.G. Debouck. 1991. Races of common bean (*Phaseolus vulgaris*, Fabaceae). Econ. Bot. 45, 379396.

Schwartz, H.F., and M.A.C. Langham. Growth Stages of Common Bean. 2010. Legume ipmPIPE Diagnostic Pocket Series (2 page diagnostic cards, distributed nationally), http://legume.ipmpipe.org/cgi-bin/sbr/public.cgi.

Tohme J., D. O. González, S. Beebe, and M.C. Duque. 1996. AFLP analysis of gene pools of a wild bean core collection. Crop Sci. 36, 13751384.

White. J., and I. Juan. 1991. Physiology of yield potential and stress tolerance. Pages 287-382 in A. van Schoonhoven and O. Voysest, eds., Common beans: research for crop improvement. C.A.B. Int., Wallingford, UK and CIAT, Cali, Colombia.

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Chapter 9: Cowpea Breeding

Arti Singh; Teshale Mamo; Asheesh Singh; and Anthony A. Mahama

Cowpea (*Vigna unguiculata* L. Walp.) (2n=2x=22) belongs to the Leguminosae family. Cowpea is an important legume crop ranked second after groundnut. It is grown for food and feed in multiple continents (Africa, Asia, Europe, the United States, and Central and South America). The center of origin and domestication is Southern Africa from where is was later carried to East and West Africa and Asia. Wild relatives of cowpea are found all over Africa. With grain comprised of 25% protein and several minerals and vitamins, it is another important crop that is vital for tackling current global food security challenges facing the world.

Learning Objectives

- Become familiar with the Cowpea crop
- List breeding institutions working on it
- Know classification system
- Describe adaptation and usage
- Outline production constraints
- Discuss breeding method used to develop pureline cowpea cultivars
- Outline a step by step breeding procedure using CB-27 cowpea cultivar as an example

Domestication and Diversification

Cowpea was domesticated in southern Africa and later spread to East and West Africa and Asia. Baudoin and Marechal (1985) classified domesticated cowpea into five cultivar groups (cultigroups).

1. Unguiculata (seed testa thick and shiny) is the major group.
2. Textilis (long inflorescence peduncle) is mostly found in West Africa.
3. Sesquipedalis (fleshy pod, wrinkled when ripe) is mainly found in East Africa.
4. Melanophthalmus (seed testa thin & often wrinkled, flower & seed partly white) originated

in West Africa.

5. Biflora (seed testa thick and shiny, flower and seed most often colored) is grown in South East Asia.

Biology of the Crop

General Characteristics and Development of the Crop

Cowpea is a warm-season, annual, herbaceous and similar in appearance to common bean (Phaseolus vulgaris L.) except that the leaves are generally darker green, shinier, and rarely pubescent. It has twining stems varying in erectness and bushiness. The trifoliate leaves develop alternatively, and petioles are 2 to 12 cm long. A wider range exists for leaf shape and size in cowpea than in common bean.

Fig. 1 Cowpea exhibits diverse seed color, shape, size, and texture. Photo by J.D. Ehlers; cited in M.P. Timko et al., 2007.

Plant growth habit is categorized as erect to semi-erect, prostrate (trailing type) or climbing, and indeterminate to determinate, depending on the genotype. However, most cowpea accessions have the indeterminate type of growth habit. Cowpea has a strong taproot system and the depth of the root has been measured up to 95 inches after 8 weeks of seeding. Flowers are born in axillary racemes on stalks with 15 to 30 cm peduncles. Usually, a single peduncle has two to three pods, however, under favorable growing conditions, a single peduncle often carries four or more pods. The presence of long peduncles is a unique feature of cowpea among legumes, and this characteristic facilitates hand harvesting. The cowpea flowers vary in color from white, cream and yellow to purple, and the seeds, which are smooth or wrinkled, range in color from white, cream or yellow to red, and are characterized by a marked hilum surrounded by a dark arc (Fig. 1).

Photosynthesis, Photoperiod, and Temperature

Cowpea is a short-day plant and like other grain legumes, cowpea processes its food using a C3 photosynthetic pathway. Different cowpea genotypes show photoperiod sensitivity in connection with floral bud initiation and development. Some genotypes are day-neutral, while other genotypes display a wider range of photoperiods (Craufurd et al. 1997). In addition, few cowpea genotypes exhibit various degrees of sensitivity to photoperiod (extent of delay in flowering) and temperature (Ehlers and Hall 1996). Warmer temperatures speed up flowering time in both photoperiod sensitive and insensitive cowpea genotypes. The development of improved cowpea genotypes for warm environments requires an understanding of the developmental responses to heat and photoperiod. Cowpea cultivars show a wide range of reproductive characteristics. The flower initiation ranges from 30 to 90 days after planting, and attaining physiological maturity (dry seed maturity) ranges from 55 to 240 days after planting (Wien and Summerfield, 1984). Wien and Summerfield (1984) reported that cowpea cultivars that flower early have a shorter or more concentrated flowering period than cultivars that flower late. In Sub-Saharan Africa, selection for different degrees of photosensitivity has occurred in different climatic zones and this resulted in pod ripening coinciding with the rainy season in some given locations. This condition helps the plant during pod set and ripening to escape damage from excessive rainfall and diseases attack. Therefore, photoperiod and temperature responses of particular cowpea genotypes allow cowpea breeders to make parental choices to best utilize exotic and adapted germplasm to serve particular environments.

General Classification

Classification by Utilization or Mode of Consumption

Cowpea is used as food as well as feed, including forage, hay and silage for livestock in Sub-Saharan Africa, Asia, Europe, USA and Central and South America. In Africa, people consume young leaves, immature pods, immature seeds and dried seeds. The stems, leaves, and vines of the cowpea serve as animal feed. Cowpea is also used as green manure and cover crop for maintaining the productivity of the soil. The grain contains 25% protein and several vitamins, minerals and fibers. Breeding efforts at the International Institute of Tropical Agriculture (IITA) and national programs have resulted in dual-purpose varieties (with good grain and fodder yields). The dual-purpose varieties have provided both grain and fodder while fitting

the different cropping systems, economic, and climatic conditions encountered in Africa. In addition, cowpea has great flexibility in terms of its use as farmers can choose to harvest the cowpea for grains or for forage to feed their livestock, depending on economic or climatic conditions.

Classification by Seed Characteristics

Cowpea seed size ranges from small wild types to 0.5-1 cm long. The 1000 seed weight of cowpea is 150-300 grams. Most of the time, seeds develop a kidney shape if not restricted within the pod. If the development of seed is restricted by the pod, the seed becomes more globular. The seed coat in cowpea can either be smooth or wrinkled and an assortment of colors has been observed (including white, cream, green, buff, red, brown and black). Sometimes, the seed is either speckled or mottled. Many of the cowpea seeds are also referred to as eye bean (black eye, pinkeye purple hull) (Fig 2) where they are covered with a white tissue, with a blackish rim-like aril. In cowpea, the seed size is important because it directly influences productivity, and together with different color standards, can determine grain quality for the market. Therefore, seed size and color should also be considered as major traits of interest for breeding programs.

Fig. 2 Popular blackeye bean seed (Credit Toby Hudson, in Heuzé V., Tran G., 2015)

In the United States, different cowpea cultivar classes with a broad range in characteristics are grown for horticultural use. All cultivars that are grown in USA are day neutral members of the subspecies Unguiculata cultivar group Unguiculata. The cultivars grown for seed are classified as Blackeye beans, are known for good yield production), the Crowders type are known for their largest peas, and are often used for canning. Cream peas are the most popular and have become increasingly important for home gardening, while field types have few popular cultivars and most cultivars are old agronomic types.

Classification by Growth Habit

Cowpea has substantial genetic diversity for growth habit. The major growth habits are categorized as erect to semi-erect, prostrate (trailing) or climbing types. Growth habit in cowpea ranges from indeterminate to fairly determinate with the non-vining types tending to be more determinate. Meanwhile, some of the early maturing groups have a determinate growth types.

Classification by duration of Growth Period

Cowpea is grouped into early, medium and late maturity group. However, the range for growth-period duration varies from one region to another or among varieties of different growth habits. According to growth habit and region, cowpea cultivars range from 55 to 240 days to physiologically mature. The difference is not only varietal but also environmental, especially for the factors of day-length and temperature.

Adaptation and Economic Importance and Uses

Adaption

Cowpea is widely cultivated throughout the tropics and subtropics between 35°N and 30°S, across Africa, Asia and Oceania, the Middle East, Southern Europe, Southern USA and Central and South America. Cowpea is a crop adapted to hot and dry tropical conditions. It is also considered drought tolerant compared to other legumes. They grow best at low altitude with a precipitation of 400 to 700 mm per annum. Optimum crop production requires temperatures between 20-35°C during the growing season, and soil pH between 5.5 and 8.3. Cowpea is grown on a wide range of soil textures but the crop shows preference to sandy soil. It has low tolerance to salt but somewhat tolerant to aluminium. Like other legumes, the crop does not withstand waterlogged or flooded conditions. Cowpea is sensitive to chilling conditions. The crop is grown in 45 countries across the globe. An estimated 14 million ha is planted to cowpea each year across the globe with total annual production of about 6 million MT, the current average is estimated at about 0.45 tonnes/ha (FAOSTAT, 2010). The production trend of cowpea across the world is shown in a Fig. 3. Cowpea is primarily an African crop. The largest producers are Nigeria, Niger, Brazil, Haiti, India, Myanmar, Sri Lanka, Australia and the United States.

Among these high cowpea producing countries, Nigeria and Niger each grow over 4 million ha and account for 45% and 15%, respectively, of the total world production (FAOSTAT, 2010).

Cowpea in the Human Diet and Nutrition

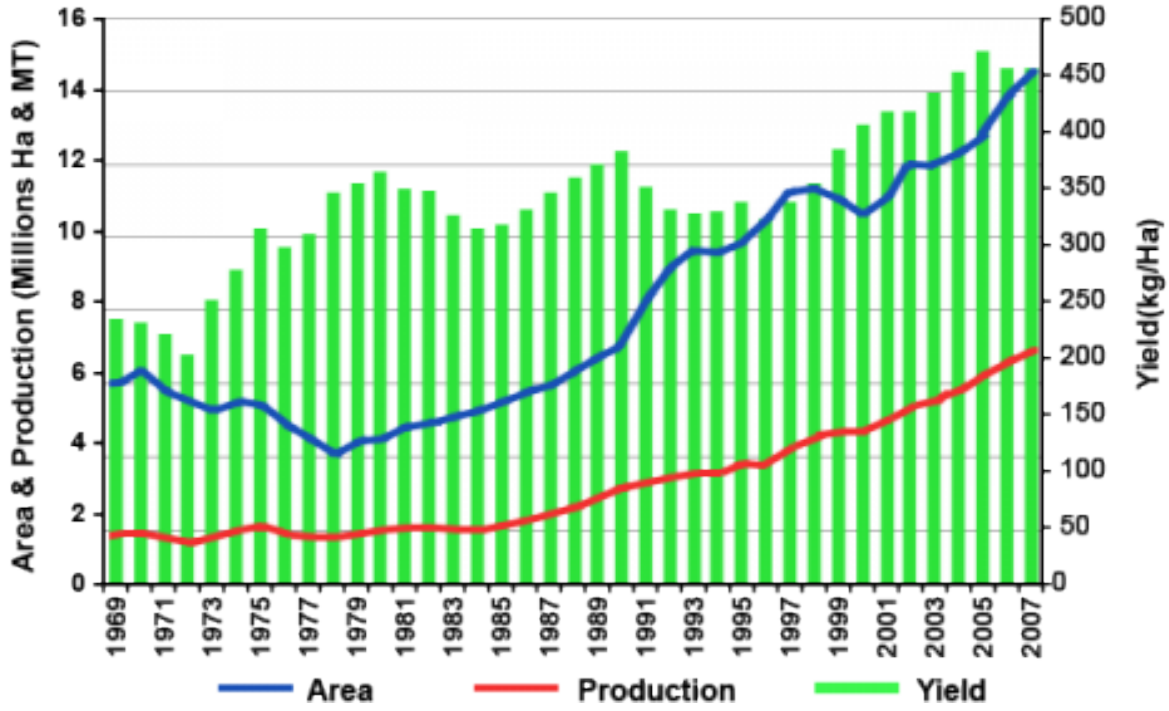

Fig. 3 Cowpea world trends. Illustration by Abate et al., 2012.

Cowpea is one of the most widely used legumes in the tropical parts of the world. It can be used at all growth stages as a vegetable crop. The grain is mainly used for human nutrition, making cowpea one of the most important dual purpose legumes. The nutritional content of cowpea grain is comparable to common beans, with relative low fat content. The protein in cowpea grains is rich in tryptophan compared to cereal grains. In Africa, immature green pods are used similar to snap bean in common bean.

Cropping System

Cowpea grows well in association with cereal crops through intercropping. Cowpea is a major component of the traditional cropping system in Africa, Asia, and Central and South America, where it is mainly grown with other crops in various combinations. It is grown as a millet-

cowpea mixture (exhibit 22% of the field sampled), a predominant crop mixture system in the Sudan savanna of Nigeria (Henriet et al., 1997). In the dry savanna cropping system, millets have been grown with different crop mixtures including millet-sorghum-cowpea (represent 19%), sorghum-cowpea (10%) and millet-cowpea-groundnut (8 %) (Olufajo and Singh, 2002). Cowpea grain yield in the mixture is lower than under sole crop condition. The factors contributing to low yields under intercropping systems include low plant population, shading effects, and competition for nutrients. Cowpea is also used as green manure, where it is incorporated into soil and can provide nitrogen to subsequent crops, minimize soil erosion and suppresses weeds.

Production Constraints

Biotic Constraints

Several biotic factors that cause yield reduction in cowpea include insect pests, fungal, bacterial, viral diseases, plant parasites, other organisms.

- **Insect Pests** – Aphids are the main insect pests of cowpea, and are important vectors of cowpea mosaic virus. Other insect pests attacking cowpea are flower thrips and pod borers.
- **Diseases** – Cowpea diseases are due to fungi, bacteria and viruses. Examples of diseases include, Cercospora leaf spot, ashy stem blight, bacterial blight, blackeye cowpea mosaic polyvirus (BICMV), and cowpea mosaic comovirus.
- **Plant Parasites** – Certain weeds are important in cowpea production and most notable examples are the parasitic weedy plants Striga and Alectra.
- **Nematodes** – Nematode also causes root damage to the crop and result in significant yield loss.

Abiotic Constraints

Extreme drought and heat, soil acidity, low phosphorous are some of the abiotic factors that limit the yield of cowpea.

International Breeding Centers

The International Institute of Tropical Agriculture (IITA) has a global mandate for the development and improvement of cowpea. Its main duty and responsibility is to develop and distribute improved cowpea varieties to over 65 national cowpea research programs in Africa. Variety requirements for cowpea differ from region to region in respect of the seed color preference, use patterns, maturity and growth habit. Therefore, IITA located additional scientists and breeding centers in Philippines, Nigeria, Burkina Faso, Cameroon, Congo and Brazil in order to address the regional constraints in cowpea production at the global level.

A general strategy for IITA is to develop different cowpea breeding lines with diverse maturity (to feed specific adaptation across wide agro-ecological zones where cowpea is grown), plant type, and seed types combined with resistance to major biotic (diseases, insect-pests, and weeds) and abiotic (drought, heat and low phosphorous) stresses.

IITA's genetic resources account for the world's largest and most diverse pool of cowpea germplasm. The collection consists of over 15,000 cultivated varieties from over 100 countries, and 560 accessions of wild cowpeas (Singh et al., 1997). The IITA collection constitutes a valuable resource for the cowpea improvement worldwide. Scientists from IITA center and regional centers have identified various cowpea genotypes with numerous desirable genes, which govern plant architecture and physiological traits (like plant type, root architecture, growth habit, pod traits, seed traits, photosensitivity, maturity and nitrogen fixation), quality traits (fodder quality and grain quality), abiotic stress (heat and drought tolerances), biotic stress (resistance to major bacterial, fungal and viral diseases, resistance to rootknot nematodes, resistance to aphids, bruchid, thrips, and resistance to parasitic weeds such as *Striga gesneriodes*, and *Alectra vogelii*).

Breeding Methods and Strategies

Introduction

Cowpea is a true diploid species with a chromosome number of 2n = 2x = 22. It is primarily a self-pollinating crop in most production environments, although up to 5% outcrossing can occur in some environments, possibly associated with pollen transfer by insects. Different cowpea breeding programs have their own priority of target production zones including the cropping

systems, consumption preferences and major constraints to cowpea production in their agro-ecological zones.

Most Cowpea breeders at IITA and National programs use bulk, backcross, and pedigree breeding methods to deal with large numbers of segregating populations because cowpea is an autogamous crop and most cultivars grown by farmers are pure lines. The primary objective in all cowpea breeding programs is higher grain yield and improved grain quality. In addition, to yield and quality traits, most breeders seek to breed in a wide range of abiotic and biotic stress resistance traits. The breeding strategy of IITA and regional breeding program is to develop broad range of breeding lines with high yield and adapted to different agro-ecological zones that possess regionally preferred characters for plant type, growth habit, days to maturity, seed type, combined with resistance to biotic and abiotic stress, along with quality. In general, the main focus of breeding programs is to develop extra early maturity (60-70 days) and medium maturity (75-90 days), non-photosensitive lines with good grain quality and possibility for dual purpose use, either for use as sole crop or as intercrop in multiple cropping systems.

Example of Cultivar Development

Development of Blackeye Cowpea Cultivar "CB27" at University of California Riverside

California Blackeye 27 (CB27) was developed by the University of California, Riverside (UCR) following the protocol shown in Fig. 4, and released in 1999 for its better performance in the following characteristics:

1. High yielding
2. Reproductive-stage heat tolerance
3. Broad-based resistance to Fusarium wilt
4. Broad-based resistance to root-knot nematodes
5. Semi-dwarf and less vegetative shoot biomass
6. Bright white seed coat
7. Good seed weight
8. Non-leaky pigments during boiling and excellent canning quality.

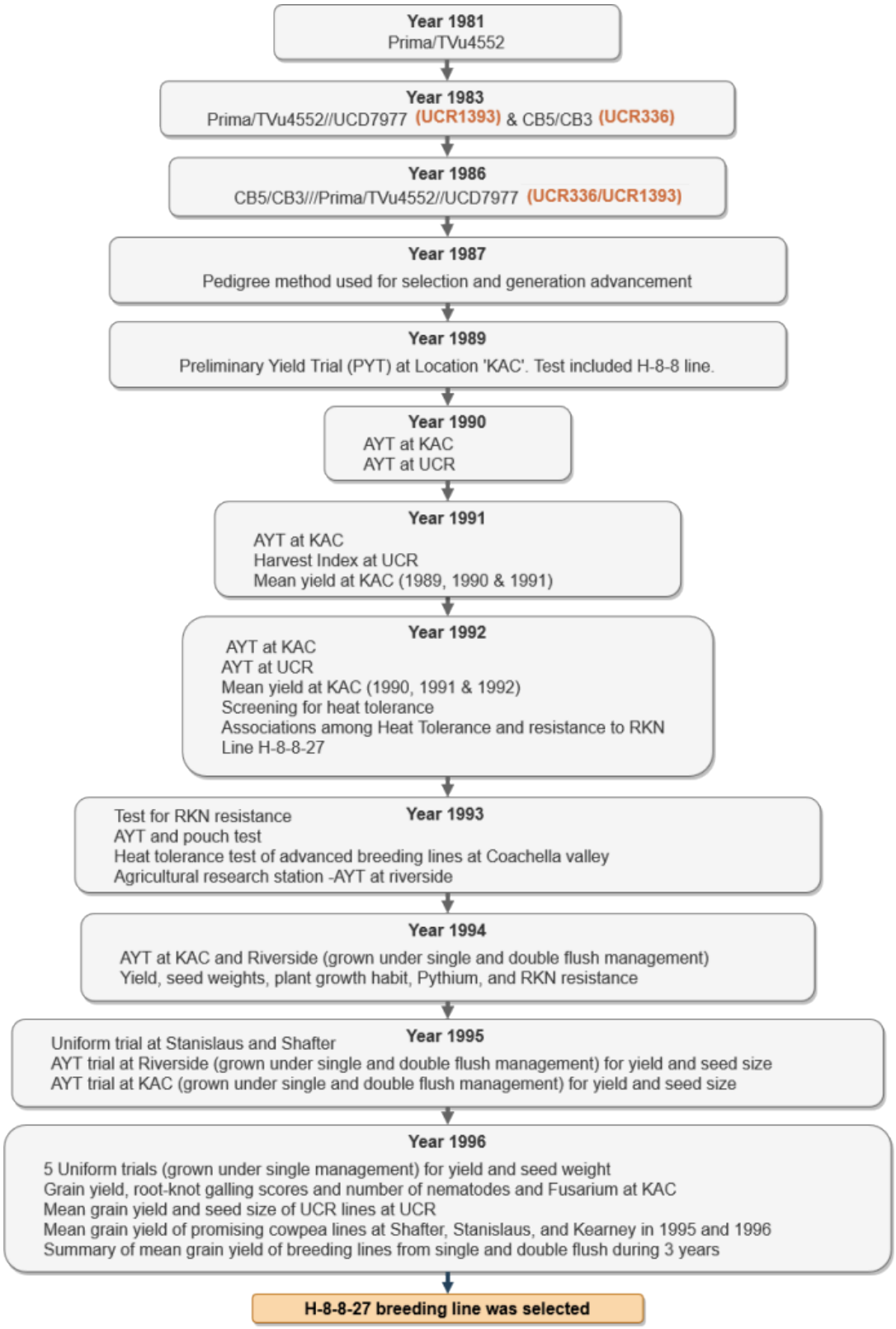

Fig. 4 Flow Chart for the Development of Blackeye Cowpea Cultivar "CB27"

Actual data from Preliminary Yield Trials (PYT), Advanced Yield Trials (AYT) and Uniform Yield Trials (UYT) along with different test conducted on agronomic, disease and quality traits (from 1989 – 1998) led to the development of CB-27. Tables 1 – 20 show the results of various trials and years in which they were conducted to eventually release CB-27.

Note: all cells in Tables 1-20 with "n/a" are blank cells.

Year – 1989

Table 1 Preliminary Blackeye Trials at Kearney Agricultural Center (KAC), 1989; H = Heat Tolerance.

Entry	Origin	Score	Yield (lbs/ acre)	Seed weight g/100
H8-14	336 x 1393	H	3281	24.5
H8-9	336 x 1393	H	3236	24.7
H8-8	336 x 1393	n/a	3152	23.5
H8-7	336 x 1393	H	3022	25.9
H8-4	336 x 1393	H	2861	23.6
CB5	n/a	n/a	2995	26.3
CB46	n/a	n/a	3017	21.5
LSD	n/a	n/a	715	13
CV (%)	n/a	n/a	16.2	3.4

Year – 1990

Table 2 Advanced Blackeye Trials at University of California Riverside (UCR), 1990.

Entry	Origin	Yield (lbs/ acre)	Seed weight (mg)	Seed density g/cm^3	Lodging	Earliness	Vigor
H8-14	336 x 1393	1805	235	1.10	erect	early	compact
H8-9	336 x 1393	1805	248	1.09	erect	early	compact
H8-8	336 x 1393	1497	233	1.11	erect	early	compact
CB5	CB x Iron	1889	254	1.06	erect	med	moderate
CB46	CB5 x 166146	2274	224	1.09	erect	med	moderate
$LSD_{.05}$	n/a	266	10	0.03	n/a	n/a	n/a
CV (%)	n/a>	10	3	2	n/a	n/a	n/a

Table 3 Advanced Blackeye Trials at Kearney Agricultural Center (KAC), 1990.

Entry	Origin	Yield (lbs/ acre)	Seed weight (mg)	Seed density g/cm^3	Lodging	Earliness	Vigor
H8-14	336 x 1393	2855	208	1.11	erect	med	moderate
H8-9	336 x 1393	3007	223	1.14	slight	med	moderate
H8-8	336 x 1393	2744	217	1.12	erect	early	compact
CB5	CB x Iron	2389	241	1.11	erect	early	compact
CB46	CB5 x 166146	2688	202	1.17	erect	med	moderate
$LSD_{.05}$	n/a	420	9	0.02	n/a	n/a	n/a
CV (%)	n/a	11	3	1	n/a	n/a	n/a

Year – 1991

Table 4 Advanced Blackeye Trials at Kearney Agricultural Center (KAC),1991.

Entry	Origin	Yield (lbs/ acre)	Seed weight (mg/ seed)	Seed density g/cm^3
H8-14	336 x 1393	2825	195	1.10
H8-9	336 x 1393	3647	227	1.09
H8-8	336 x 1393	306	217	1.10
CB5	CB x Iron	2244	234	1.09
CB46	CB5 x 166146	2514	200	1.15
$LSD_{.05}$	n/a	374	15	0.01
CV (%)	n/a	10	5	1

Table 5 Advanced Blackeye Trials at Kearney Agricultural Center (KAC) over several years, 1991.

A. Entry	**Mean Grain Yield (lbs/ acre) 1990 & 1991**	**Seed weight (mg)**
H8-14	2840	202
H8-9	3327	225
H8-8	2902	217
CB5	2316	238
CB46	2601	201
CB88	2662	221
B. Entry	**Mean Grain Yield (lbs/ acre) 1989-1991**	**Seed weight (mg)**
H8-14	2987	216
H8-9	3297	232
H8-8	2985	223
CB5	2543	246
CB46	2740	206

Table 6 Harvest Index (Ratio of grain weight to total shoot biomass x 100) and Seed Weight data at University of California Riverside (UCR), 1991.

Entry	Harvest Index %	Seed weight (mg/ seed)
H8-14	43	247
H8-9	51	232
H8-8	43	234
CB5	43	252
CB46	47	203
$LSD_{.05}$	4	16
CV (%)	7	5

Year – 1992

Table 7 Advanced Blackeye Trials at Kearney Agricultural Center (KAC), 1992.

Entry	Origin	Yield (lbs/ acre)	Seed weight (mg/ seed)	Seed density g/cm^3
H8-14	336 x 1393	2949	213	1.01
H8-9	336 x 1393	2805	219	1.02
H8-8	336 x 1393	2865	209	1.02
CB5	CB x Iron	2831	229	1.00
CB46	CB5 x 166146	3164	194	1.06
$LSD_{.05}$	n/a	404	11	0.02
CV (%)	n/a	10	3.5	1.3

Table 8 Advanced Blackeye Trials at University of California Riverside (UCR), 1992.

Entry	Origin	Yield (lbs/ acre)	Seed weight (mg/ seed)	Seed density g/cm^3
H8-14	336 x 1393	2777	209	0.98
H8-9	336 x 1393	2684	207	0.97
H8-8	336 x 1393	2615	216	0.97
CB5	CB x Iron	2373	225	0.95
CB46	CB5 x 166146	2461	193	0.99
$LSD_{.05}$	n/a	318	19	0.03
CV (%)	n/a	85	6.0	2.0

Table 9 Advanced Blackeye Trials over several years at Kearney Agricultural Center (KAC), 1990, 1991, and 1992

Entry	Yield (MEAN of years 1990, 1991 and 1992)	Seed weight (mg)
H8-14	2876	206
H8-9	3153	223
H8-8	2890	214
CB5	2488	235
CB46	2789	199

Table 10 Screening for Heat Tolerance data, 1992.

Line	Total # of Sub-lines	# Heat Tolerance – Flowering (CVARS & GH)	Heat Tolerance – % Podding (Hot Glasshouse at UCR)	# Selected sublines	Average Podding
H8-14	45	26	57	9	4.7
H8-9	54	54	92	14	9.4
H8-8	46	24	70	12	5.0

Table 11 Summer glasshouse evaluation results for associations among heat Tolerance and Resistance to Root Knot Nematode (non-aggressive *Meloidogyne incogonita*); day/ night temperatures of 34/30 degree centigrade, 1992.

Line	[1]Nematode Resistance	#[2]Heat Tolerance – Floral buds	#[2]Heat Tolerance – Pod set
H8-8-1	R	N	3
H8-8-2	S	S	–
H8-8-3	R	N	6
H8-8-4	S	S	–
H8-8-5	R	N	2
H8-8-6	S	S	–
H8-8-8	R	N	0
H8-8-9	R	N	7
H8-8-10	R	N	0
-to-	n/a	n/a	n/a
—-	n/a	n/a	n/a
H-8-8-27	R	N	n/a

Year – 1993

The blackeye cowpea cultivators follow three management schemes:

1. Single-flush main crop cut after ~ 100 days
2. Single-flush double crop, sown later and cut after ~ 100 days
3. Double-flush main crop, sown early and cut after ~ 140 days

Short-term goal – to develop blackeye varieties with resistance to the:

1. common race of Fusarium wilt in California (race #3)
2. wide range of root knot nematodes
3. heat tolerance
4. increased yield potential

Medium-term goal – to develop blackeye varieties that have resistance to early cut-out and greater ability to produce pods over an extended season (140 days from planting to cutting)

Long-term goal – resistance to lygus, resistance to cowpea aphid

Table 12 Mean non-aggressive *Meloidogyne* inc*ognita* egg mass count from four to five replicates on breeding lines using "pouch" tests, 1993. R indicates resistant, S indicates susceptible, and – indicates not tested.

Line	Date of Test			
	13-May	27-Feb	25-Aug	R/S
CB5	2	n/a	n/a	R
CB46	2	n/a	n/a	R
H-8-8-2	2	0	n/a	R
H-8-8-4	4	<1	n/a	R
H-8-8-6	<1	<1	0	R
H-8-8-8	2	0	n/a	R
H-8-8-13	0	0	0	R
H-8-8-15	0	0	0	R
H-8-8-16	10	0	0	R
H-8-8-27	<1	<1	<1	R
H-8-8-28	5	n/a	n/a	R
H-8-8-30	0	n/a	n/a	R
H-8-8-32	43	n/a	n/a	S
H-8-8-35	0	<1	<1	R

Table 13 Mean non-aggressive *Meloidogyne incognita* egg mass count from four to five replicates on breeding lines using "pouch" tests, in Advanced Yield Trial, 1993. R indicates resistant, S indicates susceptible, and – indicates not tested.

Line	Date of Test		Classification	
	May 13	April 9	Aggressive	Non-aggressive
CB3	32	190	S	S
CB46	15	24	S	R
H-8-8-2	6	n/a	R	R
H-8-8-4	n/a	4	R	R
H-8-8-6	7	12	R	R
H-8-8-8	n/a	17	R	R
H-8-8-13	2	29	R	R
H-8-8-15	6	7	R	R
H-8-8-16	11	20	R	R
H-8-8-27	2	31	R	R
H-8-8-31	n/a	85	S	S
H-8-8-35	8	22	S	R

Table 14 Heat–tolerance results of advanced blackeye breeding lines evaluated in a hot glasshouse (day/night temperature of 35/30 degree Celsius) and Coachella Valley Research Station, 1993. – indicates not tested.

Entry	Grain Yield g/ plant	Plots/ Plant	Seeds/ Pod	Seed Weight Mg/ seed	Flower Production	Pods/ Peduncle #
CB5	0	0	n/a	n/a	NO	n/a
CB46	2	4	2.7	166	NO	n/a
H8-8-6	22	28	4.2	192	YES	2.5
H-8-8-13	21	27	4.1	190	YES	2.75
H-8-8-15	22	27	4.1	196	YES	3.00
H-8-8-16	30	34	4.6	195	YES	2.75
H-8-8-27	26	30	4.2	207	YES	2.75
H-8-8-35	28	30	4.6	201	YES	2.75

Table 15 Advanced Blackeye Trial at Riverside, 1993. (Sown June 14, cut September 17 (95-day season))

Entry	Grain Yield lbs/ac	Seed weight mg/ seed	Heat tolerance	Root Knot Resistance	
				Non-aggressive	Aggressive
CB5	1975	260	SUS	RES	SUS
CB46	1996	225	SUS	RES	SUS
H8-8-6	1631	240	TOL	RES	RES
H-8-8-13	1951	228	TOL	RES	RES
H-8-8-15	1938	227	TOL	RES	RES
H-8-8-16	2156	231	TOL	RES	RES
H-8-8-27	1767	246	TOL	RES	RES
H-8-8-35	2049	229	TOL	RES	RES
$LSD_{.05}$	405	22	n/a		
CV%	15	6			

Year – 1994

Table 16 Mean Grain Yields (lbs/ac) under single and double management in multilocation Advanced Yield Trials at UCR and KAC. a – indicates the top yielding group based on statistical analysis.

Entry	Riverside Single Flush	Riverside Double Flush	Kearney Single Flush	Kearney Double Flush	Mean
CB-46	1860	2996	3046a	3916	2955
CB-5	2063	2869	2222	3479	2658
H8-8-1N	1985	3199	2629	3786	2900
H8-8-6	2039	2677	2051	3366	2531
H8-8-13	1800	2318	2637	2717	2668
H8-8-15	1703	3040	2589	3175	2627
H8-8-27	1742	2979	2398	3728	2712
H8-8-35	1771	2658	2414	3399	2561
$LSD_{(.05)}$	NS	581	245	NS	288
CV(%)	13	14	15	16	16

Table 17 Seed Weights (mg/seed) under single and double management in multilocation Advanced blackeye Trial at UCR and KAC, 1994.

Entry	Riverside Single Flush	Riverside Double Flush	Kearney Single Flush	Kearney Double Flush	Mean
CB-46	234	211	221	211	219
CB-5	273	255	265	246	260
H8-8-1N	249	233	242	225	237
H8-8-6	243	238	230	220	233
H8-8-13	231	206	209	213	215
H8-8-15	239	227	240	218	231
H8-8-27	240	229	242	221	233
H8-8-35	237	220	234	222	228
$LSD_{(.05)}$	11	12	6	13	6
CV(%)	3.1	3.6	3.9	3.9	3.6

Table 18 Plant growth habit, plant size and Pythium incidence (no. of infected plants/plot) in of entries in advanced trials at KAC and UCR and double flush advanced trial at UCR, 1994. M = medium; M-L = medium-large; L = large plants; L = low; M = medium; H = high; M-L = moderately low in vinyness.

Entry	Growth habit Plant Size	Growth habit vinyness	Pythium
CB-46	M	M-L	5.3
CB-5	L	H	7.5
H8-8-1N	M-L	H	2.3
H8-8-6	L	M	2.5
H8-8-13	M	M	4.3
H8-8-15	M	L	1.3
H8-8-27	M	L	1.5
H8-8-35	M	L	1.3
$LSD_{(.05)}$	n/a	n/a	4.3

Table 19 Average grain yields (lbs/ac) and seed size under single and double management systems for the resistance to three nematode strains in Advanced blackeye trials at KAC, 1994. a – indicates the top-yielding group based on statistical analysis.

Entry	Grain yield lbs/ac	Seed weight mg/ seed	RKN resistance *non-aggr*	RKN resistance *aggr*	RKN resistance *javanica*
CB-46	3481**a**	216	R	S	S
CB-5	2851	255	R	S	S
H8-8-1N	3208**a**	234	R	S	S
H8-8-6	2709	225	R	R	R
H8-8-13	2677	211	R	S	S
H8-8-15	2882	229	R	R	R
H8-8-27	3063**a**	231	R	R	R
H8-8-35	2907	228	R	R	R
H8-8-35	2907	228	n/a	n/a	n/a
$LSD_{(.05)}$	472	9	n/a		
CV(%)	16	3.9			

Year – 1995

Table 20 Grain Yields (cwt/ac) in Uniform Trial at Stanislaus and Shafter, 1995

Entry	Stanislaus	Shafter	Mean
H8-8-27	24.5	55.5	40.2
H8-8-15	23.0	55.2	39.1
CB46	20.8	55.9	38.4
CB88	11.5	54.3	32.9
$LSD_{(.05)}$	3.4	3.8	2.5
CV(%)	14.8	6.1	8.5

Table 21 Grain Yields (cwt/ac) of UCR Advanced Blackeye Trials at UCR, 1995.

Entry	Origin	Single Flush	Double Flush	Mean
H8-8-27	CB5/CB3//1393	22.3	29.6	26.0
H8-8-15	CB5/CB3//1393	23.9	29.2	26.6
CB46	CB5/ CB3//PI1166146	23.3	25.9	24.6
CB88	CB5/ CB3//PI1166146	24.5	29.6	27.1
$LSD_{(.05)}$	2.1	NS	3.4	
CV(%)	8	14	16	

Table 22 Grain Yields (cwt/ac) of UCR Advanced Blackeye Trials at KAC, 1995.

Entry	Origin	Single Flush	Double Flush	Mean
H8-8-27	CB5/ CB3//1393	38.3	42.8	41.3
H8-8-15	CB5/ CB3//1393	38.3	41.9	40.1
CB46	CB5/CB3/ PI1166146	31.7	47.2	39.4
CB88	CB5/CB3/ PI1166146	34.0	44.4	39.2
$LSD_{(.05)}$	4.6	8.0	4.8	
$CV_{(\%)}$	12	16	15	

Table 23 Rootknot nematode infestation from UCR Advanced Blackeye Trials at KAC, 1995.

Line	Nematodes *Non-aggres*	Nematodes *aggres*	Nematodes *M.jay.*	*Fusarium* Race 3	*Fusarium* Race 4	Heat
H8-8-27	Yes	Yes	Yes	Yes	Yes	Yes
H8-8-15	Yes	Yes	Yes	Yes	Yes	Yes
CB46	Yes	No	No	Yes	Yes	No
CB5	Yes	No	No	No	Yes	No

Notes: Types of RKN; Non-aggres = Non-aggressive *M. incognita* – not able to overcome standard 'Rk' gene resistance.
aggres = strain of *M. incognita* – able to overcome 'Rk' resistance

Table 24 Seed Size (mg/seed) from UCR Advanced Blackeye Trials at KAC and UCR, 1995.

Line	Kearney	Riverside	Mean
H8-8-27	208	215	212
H8-8-15	207	209	208
CB46	203	201	202
CB88	216	210	213
$LSD_{(.05)}$	11	19	11
CV(%)	4.4	7.7	6.3

Table 25 Comparison of seed size (mg/seed) of high-yielding line, at locations KAC and UCR, 1994 and 1995.

Line	1994	1995	Mean
H8-8-27	231	212	222
H8-8-15	229	208	219
CB46	216	202	209
CB88	231	213	222
$LSD_{(.05)}$	6	11	n/a

Table 26 Mean Yields of high-yielding lines at locations KAC, UCR, SHAFT and STANI, 1994 and 1995.

Line	Mean
H8-8-27	33
H8-8-15	33
CB46	33
CB88	32

Year – 1996

Table 27 Grain Yields (cwt/ac) of high yielding breeding lines and check varieties CB46 and CB88 in 5 uniform trials and Individual Seed Weight from single flush trial at KAC–1996.

Entry	Shafter	Tulare	Kearney double	Kearney single	Westside	Mean	Seed weight mg/ seed
H8-8-27	53.1	42.6	38.0	26.1	24.5	36.9	213
H8-8-15	45.8	40.3	33.3	23.9	23.8	33.4	207
CB46	46.6	49.1	40.5	25.5	24.6	37.3	215
CB88	46.2	41.1	37.0	23.4	20.9	33.7	215
$LSD_{.05}$	8.3	8.1	NS	2.7	1.4	2.7	7
CV(%)	11.7	12.5	13.8	7.7	4.0	12.1	2.1

Table 28 Grain Yields (cwt/ac), root-knot galling scores and number of nematodes (juveniles per liter of soil) of high yielding UCR blackeye breeding lines in a field at KAC and at the Muller Farm-Chance Field, Stanislaus Co. that are infested with Rk gene virulent stains of M.javanica and M.incognita. Fusarium wild races 3 and 4 from the Chance Field alone, 1996.

Entry	Grain Yield (KAC)	Grain Yield (Muller)	Galling (KAC)	Galling (Muller)	No. juveniles (KAC)	No. juveniles (Muller)	Resistance Nematode	*Fusarium* Wilt
H8-8-27	21.1	21.0	2.3	2.1	1672	328	Rk+	3 & 4
H8-8-15	19.9	23.1	1.6	2.0	1061	239	Rk+	3 & 4
CB46	21.2	16.5	4.9	4.7	1833	572	Rk	3
CB88	22.9	10.2	4.5	5.1	2478	572	Rk	3
$LSD_{.05}$	2.9	3.1	0.6	0.9	750	NS	n/a	n/a

Table 29 Grain Yields (cwt/ac) and seed size (mg/seed) of high-yielding UCR blackeye breeding lines and checks (C46 and CB88) under single and double flush management at UCR, 1996.

Entry	Grain Yield (Single)	Grain Yield (Double)	Seed Size (Single)	Seed Size (Double)	Means Yield	Means Seed Size
H8-8-27	20.8	32.7	223	249	26.8	236
H8-8-15	22.4	36.1	226	245	29.3	236
CB46	24.7	34.9	223	234	29.8	228
CB88	22.7	34.7	226	232	28.7	229
$LSD_{.05}$	2.5	NS	9	9	2.5	6
CV(%)	7.5	9.0	2.7	2.5	8.7	2.6

Table 30 Grain Yields of promising blackeye breeding lines and checks (CB46 and CB88) over years and locations in the Central Valley. Overall mean includes data from Tulare and Westside Field Station trials, 1996.

Entry	Shafter mean Yield 1995 and 1996	Stanislaus mean Yield 1995 and 1996	Kearney Mean Yield 1995 and 1996	Overall Mean
H8-8-27	55	23	34	36
H8-8-15	50	23	33	34
CB46	51	19	36	36
CB88	50	11	33	31

Table 31 Summary of grain yields (cwt/ac) of selected blackeye bean breeding lines and check cultivars (CB46 and CB88) from single-flush and double-flush trials at KAC, 1994, 1995, 1996.

Entry	1994 Single flush	1994 Double flush	1995 Single flush	1995 Double flush	1996 Single flush	1996 Double flush	Mean Single	Mean Double
H8-8-27	30.5	39.2	31.7	47.2	25.5	40.5	29.2	42.3
H8-8-15	25.1	35.5	34.0	44.4	23.4	37.0	27.5	39.0
CB46	25.9	31.8	38.3	42.8	23.9	33.3	29.4	36.0
CB88	24.0	37.3	38.3	41.9	26.1	38.0	29.5	39.1
$LSD_{.05}$	2.4	NS	4.6	8.0	2.7	NS	2.2	4.2
CV(%)	15	16	12	16	8	14	10	13

Year – 1997

Table 32 Grain yield, bean weight and bean quality grade of selected H8-8-27 cowpea breeding line in a Strip Trial near Wasco, CA, 1997.

Entry	Grain Yield (Dirt Wt) Cwt/ ac	Grain Yield (Clean Wt) Cwt/ ac	Clean Out %	Bean Size Gm/ 100 seeds	Total Damage (%)	Splits	Grade
H8-8-27	49	44	8.1	24.1	2.0	0.3	UN No. 1
CB46	48	44	7.7	21.7	4.2	0.3	US No. 3

Table 33 Grains yield of selected H8-8-27 cowpea breeding line in Uniform Trials, 1997.

Entry	Westside	Riverside	Tulare	Shafter	Mean
H8-8-27	1790	3491	2840	4446	3147
CB46	1990	3772	3769	5231	3638
$LSD_{(.05)}$	NS	472	302	NS	241
CV(%)	11.4	7.8	7.0	16.6	11.3

Table 34 Grain Yield and bean size of selected H8-8-27 cowpea breeding line and check (C46) at Shafter and KAC, 1997.

Entry	Shafter Mean Yield 1995, 1996, and 1997	Kearney mean Yield 1994, 1995, 1996, and 1997	Overall Mean
H8-8-27	51	35	42
H8-8-15	51	33	41
Seed size			
CB46	n/a	n/a	21.1
CB88	n/a	n/a	22.1

Table 35 Grain Yields and rating for 'greenness' after the first pod plush of selected H8-8-27 cowpea breeding line and check (C46) in Uniform Trials at UCR and Tulare, 1997.

Entry	Riverside Yield	Riverside Greenness	Tulare Yield	Tulare Greenness	Mean Yield	Means Greenness
H8-8-27	3491	0	2840	0.7	3166	0.4
CB46	3772	0	3769	2.2	3771	1.1
$LSD_{.05}$	472	n/a	699	n/a	302	n/a
CV(%)	7.8	n/a	7.0	n/a	7.6	n/a

1998 – Uniform Blackeye Trials

Table 36 Grain yields (lb/ac) H8-8-27 and check (CB46) in Uniform Blackeye Trials, 1998.

Entry	Shafter	Tulare	Kearney	Riverside	Mean
H8-8-27	5156	4967	4629	3113	4466
CB46	4732	5178	4268	2938	4271
$LSD_{(.05)}$	521	478	806	529	295
CV(%)	8	6	13	13	10

Table 37 Individual seed weights (grams/100 seeds) and % split seedcoat in Uniform Blackeye Trials, 1998.

Entry	Shafter	Tulare	Kearney	Riverside	Mean	% Split
H8-8-27	22.8	4967	22.6	24.9	23.0	13
CB46	22.6	5178	21.9	25.8	23.0	18
$LSD_{.05}$	1.3	478	1.1	1.7	0.7	6
CV(%)	4	6	3	5	4	42

Table 38 Effect of row spacing and play type on yield and yield contributing traits of cowpea lines at UCR, 1998.

Genotype	Spacing	Yield	HI %	Seed weight g/100 seed	Seeds/ pod	Pods/ peduncle
Compact type						
H8-8-27	30″	3642	47.0	24.7	8.4	1.7
n/a	40″	3407	50.6	24.6	8.7	1.5
n/a	40″ x 2	4111	47.1	23.8	8.4	1.5
n/a	**Mean**	**3721**	**48.2**	**24.4**	**8.5**	**1.6**
CB46	30″	3583	47.0	23.6	8.5	2.1
n/a	40″	3081	45.8	24.1	8.3	1.9
n/a	40″ x 2	3692	42.7	24.2	8.6	1.6
n/a	**Mean**	**3665**	**45.2**	**24.0**	**8.5**	**1.9**

Table 39 Effect of row spacing and plant type on yield and yield contributing traits of cowpea genotypes at Shafter, 1998.

Genotype	Spacing	Yield	HI %	Seed weight g/100 seed	Seeds/ pod	Pods/ peduncle
Compact type						
H8-8-27	30″	2717	48	23.8	8.4	2.1
n/a	40″	2399	48.1	23.3	8.8	2.2
n/a	40″ x 2	2643	48.2	23.7	8.0	2.0
n/a	**Mean**	**2587**	**48.1**	**23.6**	**8.4**	**2.1**
CB46	30″	2472	42.0	23	9.5	1.9
n/a	40″	2328	45.5	21.9	8.0	2.0
n/a	40″ x 2	2498	44.0	23.3	8.1	1.8
n/a	**Mean**	**2432**	**43.8**	**22.8**	**8.5**	**1.9**

Year – 1999

Table 40 Performance of CB27 compared to checks CB5 and CB46 for disease (Fusarium), pest (nematode) and Agronomic performance (heat and chill tolerance), 1999.

Entry	Fusarium wilt Race 3	Fusarium wilt Race 4	RKN (M. incognita) Avirulent	RKN (M. incognita) Virulent	RKN (M. javanica)	Heat Tolerance	Chill Tolerance
CB5	No	No	Yes	No	No	No	No
CB46	Yes	No	Yes	No	No	No	No
CB27	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Example Using Participatory Varietal Selection

Recent example of Cowpea cultivar released by IITA in parts of Africa using participatory varietal selection.

1. In Burkina Faso, two improved cowpea varieties developed by IITA have been released.
   i. IT99K-573-2-1 and
   ii. IT98K-205-8,
2. Using participatory varietal selection approach, local farmers and researchers choose varieties from various options after two years of trial in the central and northern regions of Burkina Faso.
3. Selected varieties are early maturing (60 days), high yielding (2170 kg/ha), resistant to parasitic weed striga along with big size, preferred color, and cooking qualities pertaining to farmers' taste.
4. New cowpea varieties also have better adaptability to climate change and can be grown successfully in drier regions with low rainfall.

Important Traits

Example of Participatory market-led cowpea breeding in Sub-Saharan Africa (Tanzania and Malawi) in assigning importance to traits (Fig. 5)

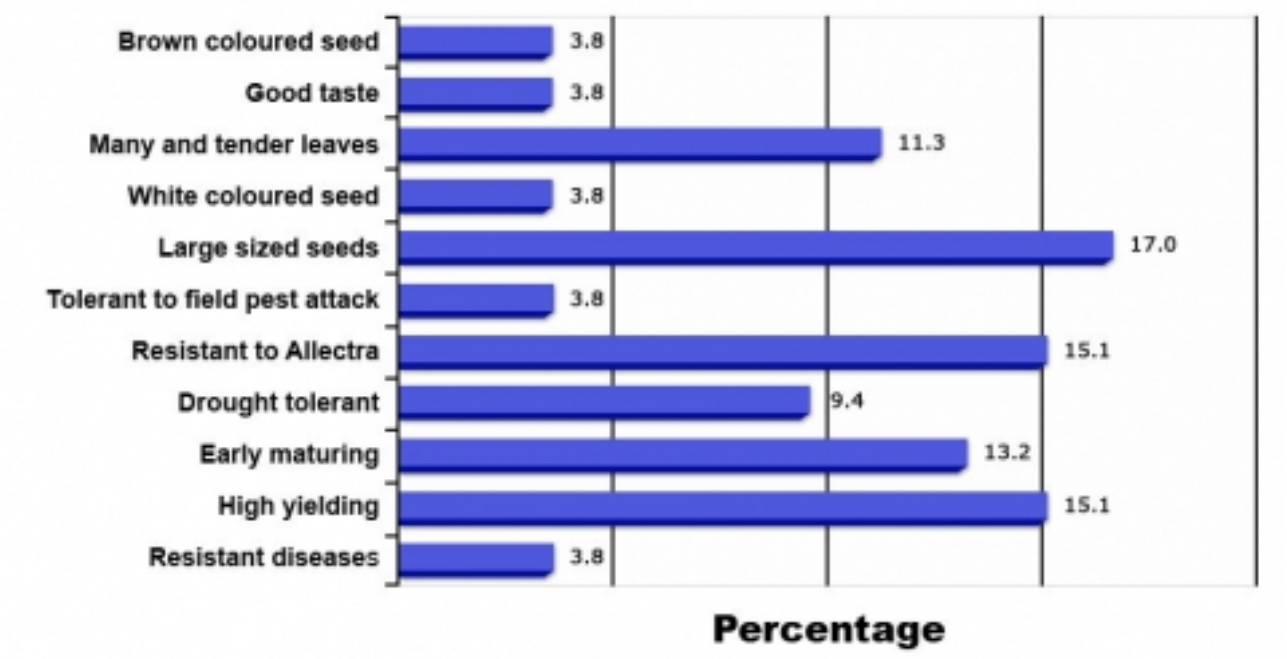

Fig. 5 Important traits of Cowpea required by farmers (%). Illustration by Hella et al, 2013, Merit Research Journal of Agricultural Science and Soil Sciences.

Pathway Based on Preferences

Farmers' and consumers' preferences of traits in a variety or cultivar play a critical role on the release and adoption of new varieties. It is important to note that the preferences of the two groups differ and therefore, require the close attention needed to address those preferences (Fig. 6).

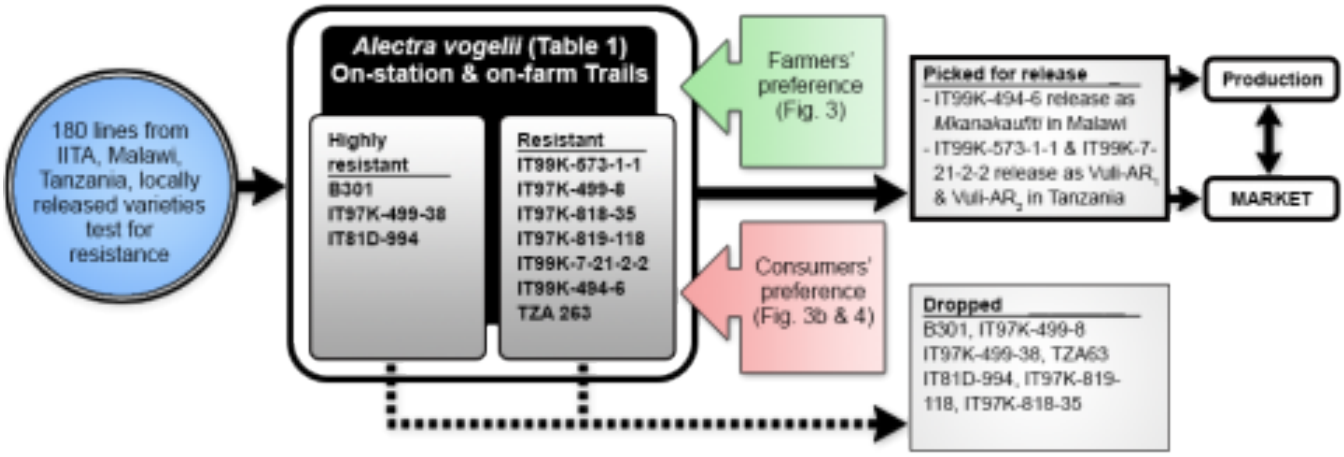

Fig. 6 Pathway showing the release of cowpea cultivar based on farmers' and consumers' preference along with resistance to *Alectra vogelli.* Click the image to see a larger version.

Notes to Consider

1. *Alectra vogelii* is a parasitic weed that causes considerable damage to cowpea plant by attaching to it and tapping nutrients.
2. In Tanzania and Malawi, Alectra is one of the major weed growing in almost all cowpea growing areas.
3. In Figure 4 is shown important traits of cowpea required by farmers. Out of 11 traits used in selection of best cowpea lines by farmers, only five traits (brown seed color, white seed color, good taste, large seed, many leaves and tender leaves) are specific to the final consumer, while the other six traits (early maturity, high yield, resistance to Alectra, resistance to diseases, tolerant to pest, drought tolerance) are agronomic traits. Large seed size is the most important trait from marketing perspective, whereas high yield, early maturity, and resistance to *A. vogelli* are the main agronomic traits which are the deciding criteria used by farmers to select varieties for growing on their farm.
4. In Figure 5 is shown an example of value chain approach used to develop cultivars (for example-IT99K-573-2-1)
5. This approach resolves biases and takes care of farmers, consumers and market preference and will not let breeders effort go waste like in past where outstanding varieties with excellent agronomic traits failed due to inability to satisfy needs of farmers, consumers and market at the same time.

Marker-Assisted Selection

Marker-assisted selection approaches are being developed in cowpea with high-density marker maps and SNP markers becoming available. As cowpea is gaining acreage globally more investment is being made for breeding and marker development. This will assist in further development of MAS in cowpea. Genetic loci controlling important pest and disease resistance genes and agronomic traits have been placed on the genetic map (for example, Kelly et al, 2003). Closely linked markers to some of the biotic traits have been identified (Gowda et al., 2002). Most of these traits are governed by major genes and are potentially good candidates for MAS. Along with MAS for simply inherited traits, the genomic selection approach offers usefulness in future breeding efforts. Currently, joint efforts are being made by IITA, Bean/Cowpea Collaborative Research Support Program (Bean/Cowpea CRSP), advanced laboratories in the USA, Australia, African Agricultural Technology Foundation (AATF), Network for Genetic Improvement of Cowpea for Africa (NGICA) and Monsanto Corporation to exploit

biotechnology tools to complement conventional breeding methods for improving resistance to diseases and insects.

References

Abate T., A.D. Alene, D. Bergvinson, B. Shiferaw, S. Silim, A. Orr, and S. Asfaw. 2012. Tropical Grain Legumes in Africa and South Asia: Knowledge and Opportunities. PO Box 39063, Nairobi, Kenya: International Crops Research Institute for the Semi Arid Tropics. 112 pp. ISBN: 978-92-9066-544-1. Order Code: BOE 056.

Baudoin J.P., and R. Maréchal. 1985. Genetic diversity in Vigna. In: Singh SR, Rachie KO (eds) Cowpea research, production, and utilization. Wiley, New York, pp 3-11.

Ehlers J.D. and A.E. Hall. 1996. Genotypic Classification of Cowpea Based on Response to Heat and Photoperiod. Crop Science 36: 673-679.

Hella, J.P., T. Chilongo, A.M. Mbwag, J. Bokosi, V. Kabambe, C. Riches, and C.L. Massawe. 2013. Participatory market-led cowpea breeding in Sub-Saharan Africa: Evidence pathway from Malawi and Tanzania. Merit Research Journal of Agricultural Science and Soil Sciences (ISSN:2350-2274) Vol. 1(2) pp. 011-018.

Henriet, J., G.A. van EK, S.F. Blade, and B.B. Singh. 1997. Quantitative assessment of traditional cropping systems in the Sudan savanna of Northern Nigeria, I. Rapid survey of prevalent cropping systems. Samara Journal of Agricultural Research 14: 37-45.

Heuzé V., Tran G., 2015. Cowpea (Vigna unguiculata) seeds. Feedipedia, a programme by INRAE, CIRAD, AFZ and FAO. https://www.feedipedia.org/node/232 Last updated on May 11, 2015, 14:31

Kelly, J.D., Gepts P, Miklas PN, Coyne DP. (2003). Tagging and mapping of genes and QTL and molecular marker-assisted selection for traits of economic importance in bean and cowpea. Field Crops Res. 82:135-154.

Olufajo, O.O., and B.B. Singh. 2002. Advances in cowpea cropping systems research. Pp 267-277 in challenges and opportunities for enhancing sustainable cowpea production. Proceedings of world cowpea conference III held at International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. 4-8 September 2000. IITA. Ibadan Nigeria.

Singh, B.B., O.L. Chamblis, and B. Sharma. 1997. Recent advances in cowpea breeding. Pages 3049 in Advances in Cowpea Research, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. IITA, and Japan International Research Centre for Agricultural Sciences (JIRCAS) copublication. Available at IITA, Ibadan, Nigeria.

Timko, M.P., J.D. Ehlers, and P.A. Roberts: Cowpea. In Genome Mapping and Molecular Breeding in Plants, Pulses, Sugar and Tuber Crops. Volume 3. Edited by Kole C. Berlin: Springer-Verlag; 2007: 49-68.

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Chapter 10: Millet Breeding

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Millets are tall and vigorous grasses with panicles containing small seeds, and are grouped in the cereal family Gramineae, same category as sorghum and maize. Millets are adapted and used as staple food in the semi-arid tropics of Africa and Asia where other crops generally cannot be grown. Pearl millet *(Pennisetum glaucum L.*), finger millet (*Eleusine coracana L.*), foxtail millet *(Setaria italic L.*) and proso millet (*panicum miliaceum*) are among the millet species grown widely in Africa, Asia, Europe and North America.

Learning Objectives

- Become familiar with the Millet crop
- List breeding institutions working on this crop
- Know crop biology and classification system
- Describe adaptation and usage
- Outline production constraints
- Discuss breeding method used to develop pearl millet cultivars

Origin and Domestication

History

Fig. 1 A field of millet in the Upper East Region of Ghana. Photo by Abejaobrera, licensed under CC BY-SA 3.0 via Wikimedia Commons.

Millet is one of the ancient staple human foods and believed to be the primary domesticated cereal crop. Although the exact origin and domestication of millet remains unclear, it is believed that millet was domesticated and cultivated over 7000 years ago during the Neolithic era in Africa and then distributed throughout the world as human food. To date, a total of 161,708 accessions of millet species have been collected and preserved in gene banks across the globe, and these collections comprise 98.1% of cultivated types (Sangham et al., 2012). Fig. 1 shows a field of millet with a path through it, in the Upper Region of Ghana, which is characterized by generally sandy soils and limited rainfall regimes.

Global Production

Globally, millets are grown in over 90 countries from 2004 to 2008 and on average contribute

32.3 million tons of food production annually (http://faostat.fao.org/). Major producers of millet include India, China, Nepal, Pakistan, and Myanmar in Asia, and Nigeria, Niger, Senegal, Cameroon, Burkina Faso, Mali, Uganda, Kenya, Namibia, Tanzania, Togo, Senegal, Chad and Zimbabwe in Sub-Saharan Africa (Sangham et al., 2012). Pearl millet is mainly grown in South Asia and Sub-Saharan Africa while Finger millet is grown mainly in South and Southeast Asia and East Africa. Foxtail millet is grown mainly in South and Southeast Asia, while Proso millet is grown mainly in Asia, Europe, and North America.

Finger millet, foxtail millet, Pear millet, and Proso millet are the largest collection of cultivated millet germplasm. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is one of the Consultative Group on International Agricultural Research (CGIAR) centers that has a mandate to work on millet, and ICRISAT coordinates all millets research programs in the semi-arid tropics. A large number of millet germplasm is preserved in the ICRISAT gene bank in Patancheru, India. In addition, East African countries (Ethiopia and Kenya), and West African countries (Nigeria and Senegal) preserved significant numbers of millets in their gene banks. In the USA different species of millet are also preserved in gene banks based in Fort Collins, Colorado; Griffin, Georgia; Ames, Iowa; and Pullman, Washington.

Morphology

Fig. 2 Pearl millet grain in a bag. Photo by Surya Prakash.S.A. Licensed under CC BY-SA 3.0 via Wikimedia Commons.

Pearl millet (*Pennisetum glaucum*), commonly known as bulrush millet, is a member of the grass family and originated in the semi dry land tropics of western Africa. The diversity and current distribution of the crop indicate that a large number of cultivated and wild forms of pearl millet is found from western Sudan to Senegal. In western Africa, a higher morphological diversity of pearl millet is found particularly in the south of the Sahara desert (Harlan, 1971; Harlan et al., 1975; Tostain et al. 1987). It is believed that the evolution of the crop under the pressures of high temperature and drought made pearl millet tolerant to moisture stress and high temperatures, in addition to low soil fertility. Such tolerance makes pearl millet a very important crop to farmers in the hot dessert parts of Africa and Asia.

Races

Pearl millet is grouped into four races based on grain shape.

- **Race typhoides:** This race is typically identified by obovate caryopsis in which the cross sections are obtuse and terete. The shape of the inflorescences is cylindrical and has more diverse morphology among the four races. This group occurs widely in all Africa and it is widely grown in India (Brunken et al. 1977).
- **Race Nigritarium:** the cross-section in caryopsis is angular with three and six facets in each grain. It has candle-like inflorescence and mature grain is longer and protrudes beyond the floral bracts compared to other group of races. Western Sudan and Nigeria are the main places where this race is found.
- **Race Globosum:** It has spherical caryopsis with candle shape inflorescence. It is mainly found in central Nigeria, Niger, Ghana, Togo and Benin
- **Race Leonis:** It has an acute and terete caryopsis. The unique character of this race is its acute apex, which is ended by the remnants of the stylar base. It has candle-like inflorescence shape. It is mainly grown in Sierra Leone but also produced in Senegal and Mauritania.

Biology of the Plant

Pearl millet is a warm season grass that uses the high efficiency C_4 type of photosynthesis to fix carbon and thus has the ability to produce high dry matter. It is a short day plant requiring long nights before flower initiation. It is produced mainly for grain and forage in the semi-arid tropics of Africa and the Indian subcontinent. The crop grows on different types of soil including, light textured soils, sandy, and on acidic less fertile soils.

In Pearl millet, a wide variation is observed for vegetative, reproductive and physiological features, and these variations are advantageous in the development of cultivars adapted to different climates, environments and cropping systems. Knowledge of pearl millet biology has enabled breeders to develop different cultivars that are adapted to varied environmental conditions (Khairwal et al. 1990).

Growth and Development

Vegetative Phase

Pearl millet has three well defined growth phases: the vegetative phase; reproductive phase; and the grain filling phase.

This phase is from emergence to floral (panicle) initiation of the main stalk. The seed of pearl millet takes 2-3 days to germinate under optimum temperature and moisture. The root has monocotyledonous type of root system consisting of a primary root, and adventurous type roots. It has deep root system penetrating up to 180 cm below the soil surface to absorb water. A research report indicated that in heavy-tillering pearl millet plants, the root system tends to have more horizontal spread than deep penetration. Similarly, those cultivars tolerant to early season moisture stress (3-15 days after sowing) have a 35% more root length than the susceptible cultivars.

Pearl millet is an upright annual grass that tillers from the base. The main stems are 1-2 cm in diameter and are solid, attaining a height of 2-4 m with a round and oval shape. Pearl millet stem has slightly swollen nodes with a ring of adventitious roots at the basal end. Usually the internodal length increases upwards from the base of the stem. Pearl millet leaves appear as single leaf on each node in alternate orientation with leaf sheaths open and hairy ligules. Pearl millet has high potential to produce effective tillers enhancing the probability of producing more seeds from the same plant if flowering of tillers is synchronized with that of the main shoot. Different tillers arise from different branches and all can potentially bear productive panicles, a situation that can be important during unfavorable environmental conditions such as extreme drought.

Reproductive Phase

Fig. 3 Panicles of pearl millet. Photo by Iowa State University.

Reproductive stage is started by the formation of a dome-like structure which leads to the development of spikelets, florets, glumes, stigma and anthers, and finally stigma emergence (flowering) and pollination occurs. This is the time that marks the end of the reproductive stage. The critical time for grain number determination in pearl millet is the period between panicle

initiation to anthesis. The inflorescence in pearl millets is a compound terminal spike known as panicle and is often similar in size and shape for a particular genotype.

Usually the panicle is compact and cylindrical or conical in shape, 2-3 cm in diameter, and usually 15-45 cm long.

Grain Filling Phase

This is the stage that fertilization taking place in the panicle of the main shoot and continues until the plant matures. During this phase, plant dry weight increases in the grain (seed). However, in some cultivars, elongation and flowering of tillers takes place during this time and in this case there is some dry matter translocation to the non-grain components, mainly to stems of the tillers. The end of physiological maturity or grain filling stage is clearly marked by the development of a dark layer of tissue on the grain. For most cultivars, this dark layer of tissue occurs in an individual panicle 20-25 days after flowering.

Adaptation, Economic Importance and Uses

Fig. 4 Rotalo is a bread made from pearl millet. Photo by Ashok Modhvadia. Licensed under CC BY-SA 4.0 via Wikimedia Commons.

Pearl millet is produced annually on about 29 million ha in the dry land tropical regions of which 16 million hectares are grown in Africa), 11 million hectares in Asia, and 2 million hectares in Latin America (FAO data, 2005). Pearl millet accounts for about half of the world's millet production. Africa accounts for about 60% of the area under millet cultivation, followed by 35% in Asian countries (primarily India), 4% European countries, and 1% in North America where millet is mainly used as forage and for poultry feed. Pearl millet is the third major cereal crop produced and used as staple food in sub-Saharan Africa which spans Nigeria, Niger, Burkina Faso, Chad, Mali, Mauritania, Senegal, Sudan, and Uganda.

Pearl millet is referred to as subsistence staple food of the poor people living in semi-arid and arid environments in Asia and Africa. Ninety-three percent of pearl millet grain is used as food in developing countries of Africa and Asia while the rest (7%) is used for animal and poultry feed in USA, Australia and South Africa (Sangham et al., 2012). The crop is traditionally used to

prepare food products such as flat bread, stiff roti and porridge. It is used for bakery products and snacks.

Soil Types

Pearl millet is grown on wide range of soil types, but light sandy soil is the best suited for the crop with rainfall of 350 to 700 mm per annum. Its high tolerance to drought allows pearl millet to regrow and produce tillers to compensate for losses due to drought stress thereby resulting in faster regeneration of yield of about 4000-5000 kg/ha when conditions are favorable. However, severe drought conditions result in yield reduction in the range between 500 to 600 kg/ha. In marginal areas, pearl millet is more reliable than other cereals such as sorghum and maize. In some parts of the world, pearl millet is produced in warm areas overlapping with other cereals such as sorghum, but it is less tolerant to waterlogging and flooding.

Production Constraints

Biotic stress: Diseases and insect pests are the major biotic factors significantly reducing grain yield and quality in pearl millet. Among bacterial diseases, bacterial spot (caused by *Pseudomonas syringae*) and bacterial leaf streak (caused by *Xanthomonas campestris pv. pennamericanum*) are the major causes of yield loss in pearl millet. Fungal diseases including downy mildew (caused by *Sclerospora graminicola* and *Plasmopara penniseti*), blast (caused by *Pyricularia grisea*), smut (caused by *Moesziomyces penicillariae* or *Tolyposporium penicillariae*) and rust (caused by *Puccinia substriata* var. *penicillariae*) cause more yield loss than other fungal diseases. Among insect pests, millet head miner and stalk borer cause serious problems to pearl millet plants. Parasitic weeds such as *Striga hermonthica* and *Striga asiatica* are major plant pests contributing to yield reduction in pearl millet. These two parasitic weeds are serious problems in sub-Saharan African countries. Parasitic nematodes are also major problems in pearl millet production regions.

Abiotic stress: Significant yield losses can result due to abiotic stresses which include drought (in all pearl millet growing regions), high soil salinity and soil acidity, and extreme high temperature at seedling stage and during flowering.

Pearl Millet Breeding

Basics

Fig. 5 A pearl millet breeding program at ICRISAT in Hyderabad, India. Licensed under CC BY-SA 3.0 via Wikimedia Commons.

Pearl millet (chromosome number of 2n=2x=14) is a diploid hermaphrodite with a protogynous type of flower development. Protandry (the stigma is receptive before the anthers are ready to shed pollen) in the hermaphroditic flowers of pearl millet enhances a high rate of cross pollination (> 85% outcrossing). Breeding of pearl millet began in Asia, particularly in India, in the early 1930s with an emphasis on high yield production and productivity, while in the USA the focus was on forage and biomass yield production. In West Africa, early breeding for pearl millet started in the 1950s emphasizing increases in grain yield. The discovery of A1 cytoplasmic-nuclear male sterility (CMS) system in Tifton, Georgia, USA and initiation of breeding of a commercially viable male-sterile line (A-line) is a breakthrough for hybrid cultivar development in pearl millet and this led to the release of the first F_1 grain hybrid for production in India.

Male Sterility

Cytoplasmic-nuclear male sterility also provides control over outcrossing, enabling the application of testcross method where a large number of inbred lines could be crossed with few, better and high general- and specific-combining ability CMS inbred lines.

Breeding objectives of pearl millet at ICRISAT include:

1. high grain yield with compact head, more tillers, earliness and reduced plant height;
2. high forage yield with high biomass and good digestibility;
3. resistance to diseases, insect pests and striga;
4. tolerance to drought, heat and acid soils.

Pearl millet breeding programs employ both hybrid and population improvement approaches at ICRISAT and West Africa and these methods help breeders to develop open pollinated cultivars (mainly in Africa) and hybrid cultivars (India and China).

Breeding Methods

Mass selection: This is the most common type of cultivar development method being used in several African and Asian countries. In this method, a group of pearl millet plants are selected from an open-pollinated population and the seeds from selected plants are mixed and planted to begin the next cycle of selection. Mass selection in pearl millet has helped to improve traits with high heritability. The main criteria that have been taken into consideration to improve grain yield in pearl millet are head characteristics such as compactness, length of ear, the weight of grain, and uniform maturity.

Synthetic cultivar development: Synthetic varieties are developed in open pollinated crops by mixing several hundred elite genetic stocks/germplasm with one or more important traits in common. The synthetic cultivar developed in the first generation or cycle exhibits considerable heterosis.

Hybrid breeding: The hybrid breeding program at ICRISAT and West Africa includes the development of inbred lines and pure line selection and the use of cytoplasmic male sterility. Cytoplasmic male sterility in pearl millet has been used to produce a hybrid for grain production in India and for forage production in the USA. Several sources of male-inducing cytoplasm have been discovered in pearl millet including A1, A2, A3, A4, and A5. A1 is the most commonly

used male sterile line for hybrid grain production in India. The CMS system involves the development of three-line systems (A, B, and R) in order to produce hybrid seeds. Line A is male sterile and serves as the seed parent, line B has the recessive form of the fertility restorer gene in the nucleus and does not have the capacity to restore fertility in A system; it maintains sterility. The R line has the dominant form of the fertility restorer genes, and so reverses the effects of the CMS cytoplasm of the A-line, therefore resulting in fertile hybrid seeds when used as a male parent. B and R lines should be multiplied in separate and isolated fields to maintain purity. For details, refer to Crop Improvement modules 5 (Steps in Cultivar Development) and 6 (Breeding Methods).

References

Bruken, J., J.M.J. de Wet, and J.R. Harlan. 1977. The morphology and domestication of pearl millet. Eco. Bot. 31:163-174.

Harlan, J. R. 1971. Agricultural origins: centers and non-centers. Science, 14: 468474.

Harlan, J. R., J. M. J. de Wet, and A. B. L. Stemler. 1975. Plant domestication and indigenous African Agriculture. Pages 1-9. In: Origins of African domestication. (J.R. Harlan, J.M.J. de Wet and A.B.L. Stemler, eds.) Mouton Publishers, The Hague, The Netherlands.

Khairwal, I.S., C. Ram, and A.K. Chhabra. 1990. Pearl Millet Seed Production and Technology. Manohar Publications, New Delhi, India.

Sabgm, D., H. Upadhyaya, S. Senthilvel, and C. Hash. 2012. Millets: Genetic and Genomic resources. Plant Breeding Reviews. Vol 35. Pp 246-374.

Tostain, S., M. F. Raindey, and L. Marchias. 1987. Enzyme diversity in pearl millet (Pennisetum glaucum). 1 West Africa. Theoretical and applied genetics 74:188-193.

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Chapter 11: Rice Breeding

Arti Singh; Asheesh Singh; and Anthony A. Mahama

Rice is grown on all six continents in the world except Antarctica. The two cultivated rice species are *Oryza glaberrima*, commonly known as African rice, and *Oryza sativa* L., commonly known as Asian rice, which has two major subspecies (japonica and indica). Cultivated rice is a diploid species (2n=2x=24) with basic chromosome number of 12. Twenty-one different wild varieties exist. Rice is one of the most important food crops in the world and is the staple food in numerous countries, particularly in Asia.

Learning Objectives

- Become familiar with the rice crop
- Demonstrate knowledge about the crop's biology and classification system
- Know the origin and domestication of the crop
- Outline the classification of the different production systems
- List breeding institutions working on the crop
- Discuss the breeding methods used to develop pureline and hybrid rice cultivars

Origin

Rice has three distinct cultivated species and 21 different wild varieties. Information on the origin of glaberrima and the two oryza subspecies is as below:

- Oryza glabberima – domesticated in West Africa between about 1500 and 800 BC or about 2,000-3,000 years ago (Linares, 2002).
- Oryza sativa japonica – domesticated in central China about 7000 BC or about 8,200-13,500 years ago (Molina et al 2011; Huang et. al., 2012; Harris, D. R., 1996; Vaughan et al, 2008)
- Oryza sativa indica – domesticated in the Indian subcontinent about 2500 BC (Londo et al, 2006)

Major Categories

Rice is grouped into four major categories worldwide:

- indica is the long-grain type and is non sticky when cooked
- japonica is the short-grain type which becomes sticky when cooked
- aromatic is the medium to long-grain type which when cooked has nut-like aroma and taste.
- glutinous is the type that is especially sticky and glue-like when cooked

Domestication and Diversity

Wild Ancestor of Rice

The wild ancestor of cultivated rice (*Oryza rufipogon*) existed over a broad range of geographic regions across Asia (Fig. 1). Domestication of *O. rufipogon* in response to human selection resulted in complete transformation of morphological and physiological traits of the plant. Consequently, cultivated rice (*O. sativa*) displays reduced dormancy, grain shattering and outcrossing, and reduced loss of pigmentation in the hull and seed coat.

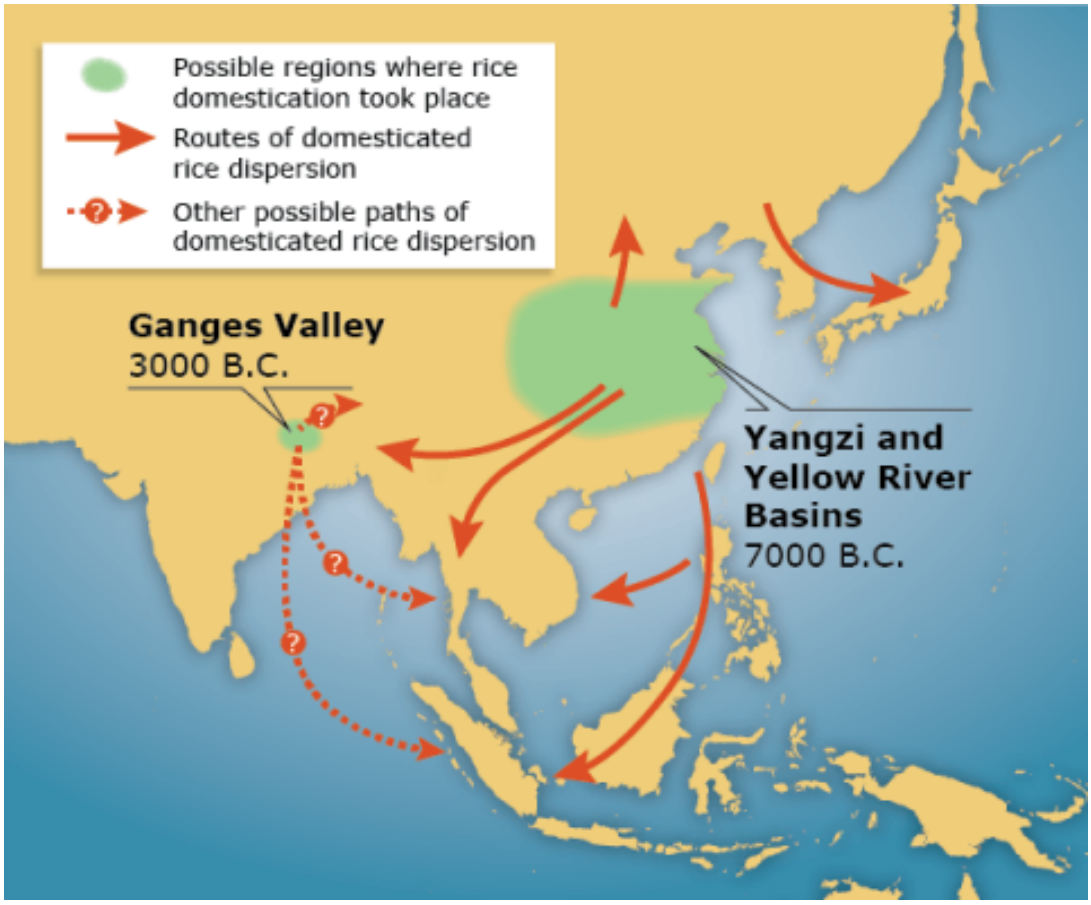


Fig. 1 The origin and dispersal of domesticated rice. Adapted from Kovach et al. 2007.

Transformation to Cultivated Rice

Fig. 2 Transformation of wild O. rufipogon to cultivated O. sativa due to domestication of rice. a) Panicle – wild O. rufipogon; b) Seeds – wild O. rufipogon; c) Panicle – cultivated O. sativa; d) Seeds – cultivated O. sativa. Photos by Kovack et al., 2007.

In addition, there is a better synchronization of tiller development and panicle formation in modern rice cultivars along with an increased number of secondary panicle branches (Fig. 2), higher grain yield and weight, and improved photoperiodic response.

NERICA

NERICA stands for "New Rice for Africa" developed using interspecific hybridization of *O. glaberrima* (African rice) and *O. sativa* (Indian rice) at the Africa Rice Center (WARDA) (Fig. 3). NERICA was developed for the purpose of raising the yield of African rice cultivars.

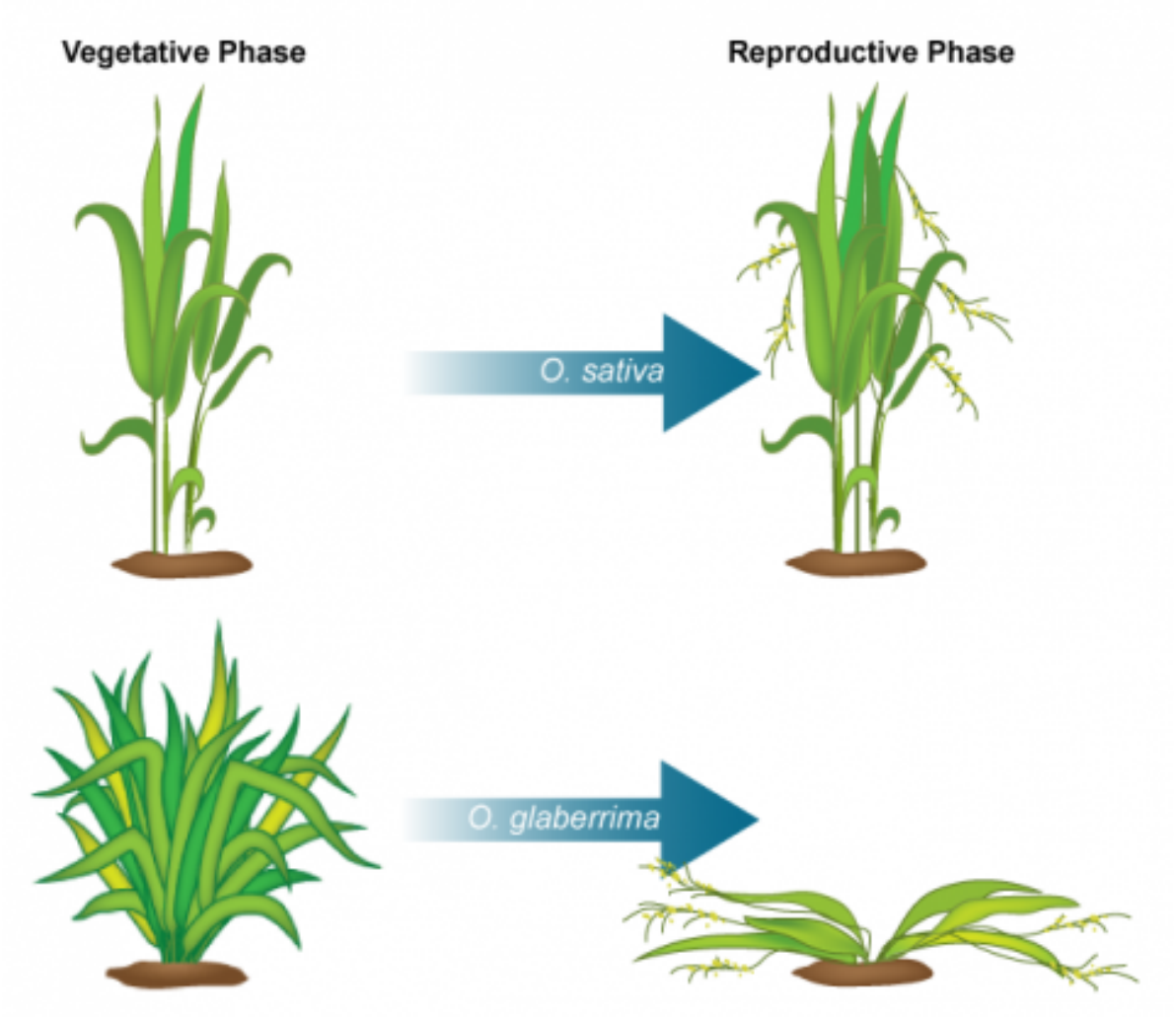


Fig. 3 The vegetative and reproductive growth habit of O. sativa (top) and O. glaberrima (bottom) upland rice species which grow on dry soil compared to lowland rice species which grow in rainfed or irrigated flooded fields. Adapted from Dingkuhn et al., 2004.

Since inter-specific crosses do not result in viable seed, embryo-rescue technique was used for the production of NERICA rice. The resulting hybrid rice cultivar has a higher yield due to increased grain size, better growth and also resistance to biotic (diseases and pest) and abiotic (drought) stresses. Dr. Monty Jones won the 2004 World Food Prize for creating a rice cultivar specifically bred for the ecological and agricultural conditions in Africa. The new rice cultivar was suitable to African drylands including and is grown in Guinea, Nigeria, Côte d'Ivoire, and Uganda.

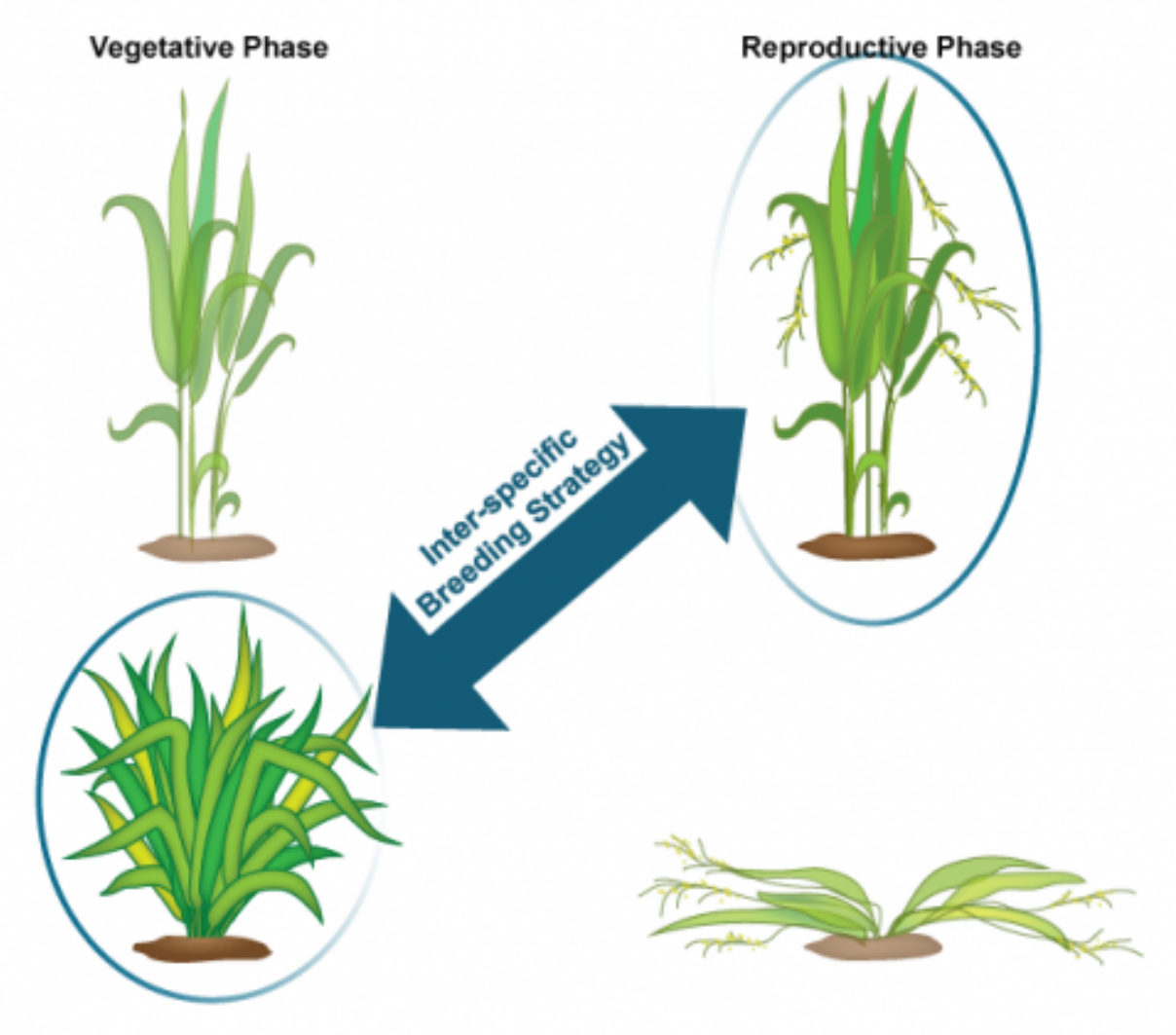


Fig. 4 The vegetative and reproductive growth habit of O. sativa (top) and O. glaberrima (bottom) upland rice species which grow on dry soil compared to lowland rice species which grow in rainfed or irrigated flooded fields. Adapted from Dingkuhn et al., 2004.

Biology of the Crop

General Characteristics of the Development of the Rice Plant

The growth of rice plant can be divided into three developmental stages (Fig. 6):

1. vegetative (germination to panicle initiation)
2. reproductive (panicle initiation to heading);

3. grain filling and ripening or maturation (milky stage to maturity)

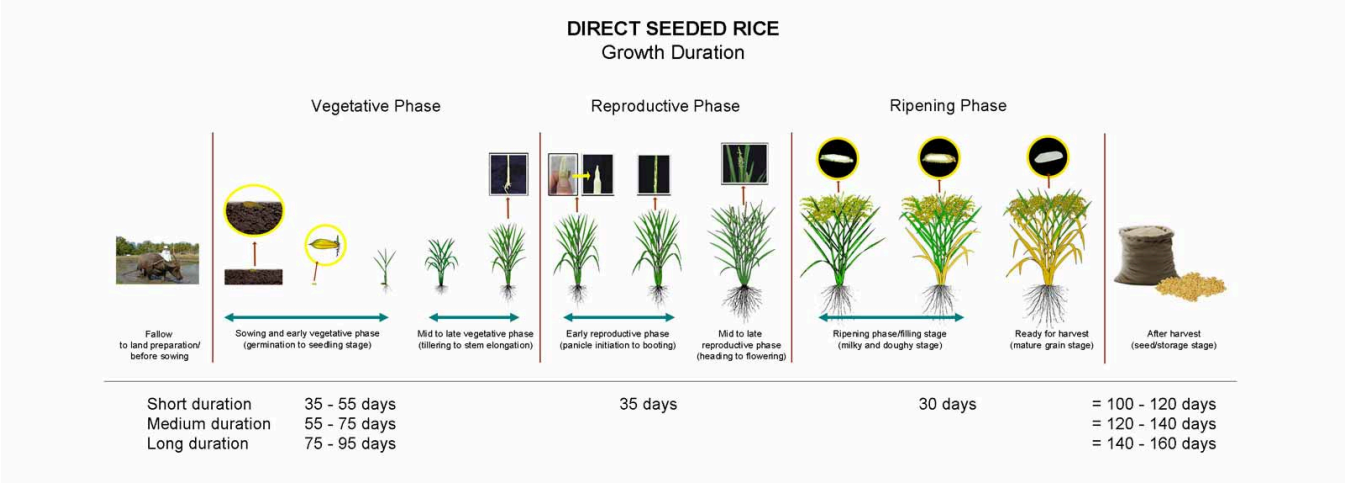


Fig. 6 Growth and developmental stages of transplanted rice. Illustration by Rice Knowledge Bank, licensed under CC-BY-NC-SA 3.0.

Development Stages

The **vegetative phase** is subdivided into three stages

1. Germination
2. Early seedling growth and
3. Tillering

The **reproductive phase** is subdivided into four stages

1. Stem elongation
2. Panicle initiation
3. Panicle development
4. Flowering

The **ripening phase** is subdivided into four stages

1. Milk grain
2. Dough grain
3. Mature grain

In a tropical environment, approximately half of the days of growth (from seeding to harvest) are in vegetative phase, and one-quarter each in the vegetative phase and the ripening phase.

Seed Development

In 2000, Counce et al., proposed a rice developmental staging system divided into three main phases of development:

1. Seedling
2. Vegetative
3. Reproductive and Ripening

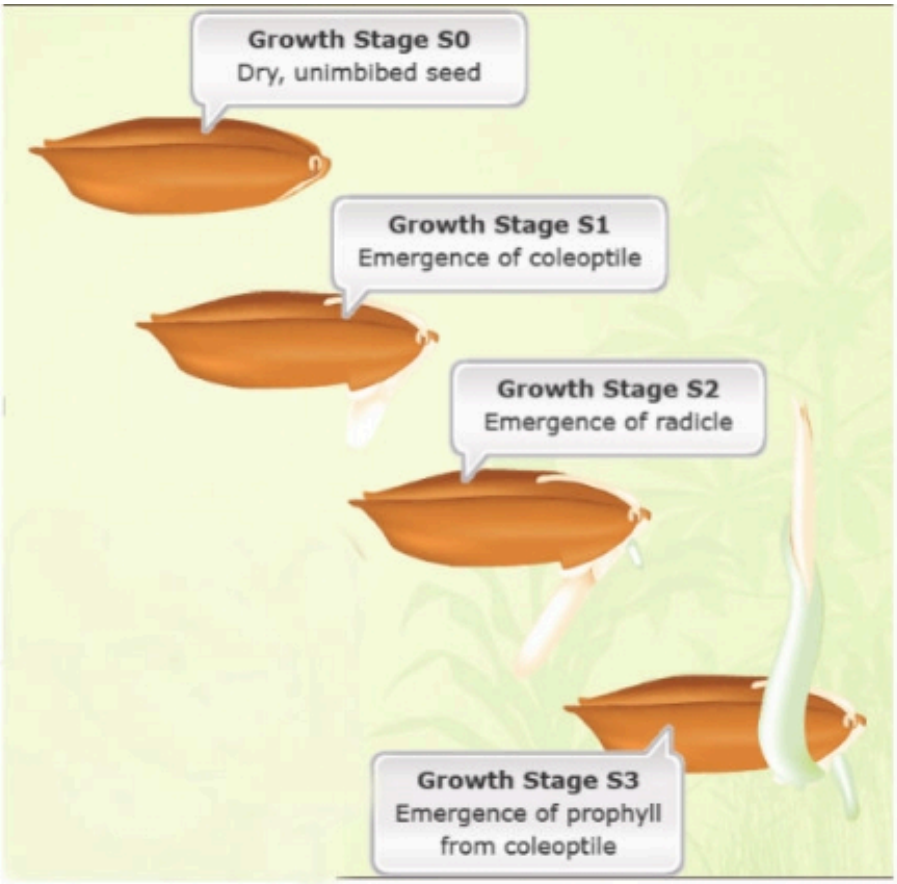


Fig. 7 Rice seedling growth stages. The rice coleoptile may emerge first from seed or the radicle may emerge first from seed. Emergence of either coleoptile or radicle is S1. Stage S2 is the stage at which both coleoptile and radicle emerge. At S3, prophyll (the first leaf to emerge, but lacks leaf blade and collar and consists only of the leaf sheath) emerges from the coleoptile before the radicle emerges from the seed. Adapted from Counce et al., 2000.

The sequence of normally occurring seedling developmental events is presented, noting that there are exceptions to the sequence given.

Seedling development can be further divided into four stages (Fig. 7):

1. Unimbibed seed (S0)
2. Coleoptile emergence (S1)
3. Radicle emergence (S2)
4. Prophyll emergence from the coleoptile (S3)

Vegetative Development

Vegetative development consists of V1, V2..... VN; where N is equal to the final number of leaves with collars on the main stem (Fig. 8).

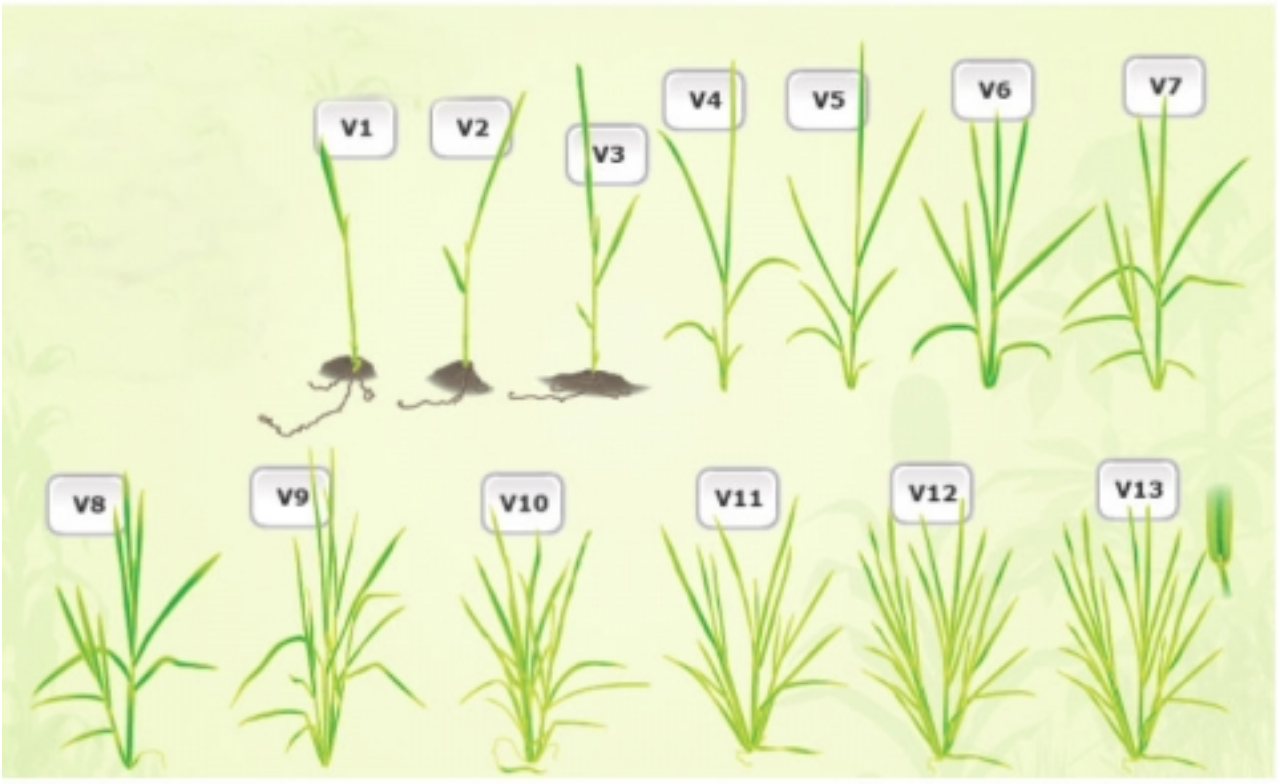
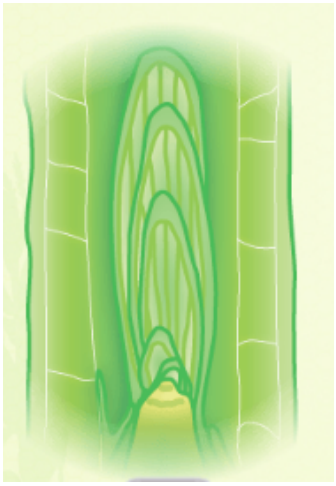
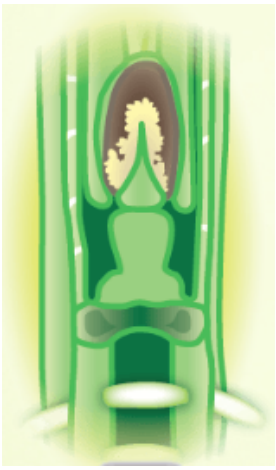
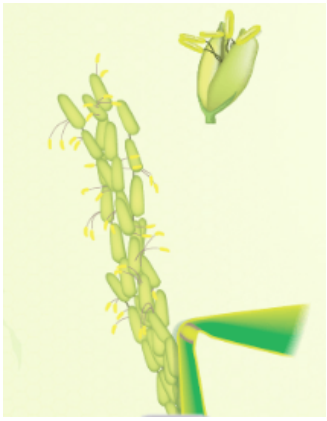
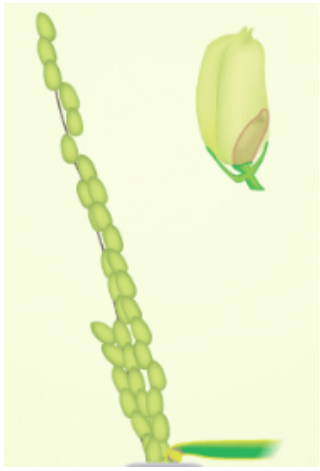
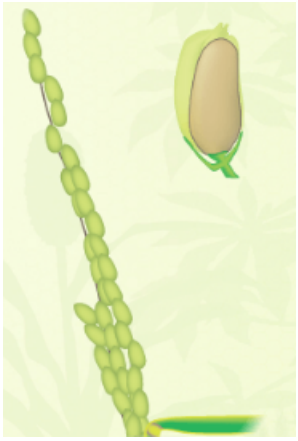


Fig. 8 Rice vegetative growth stages. Rice vegetative growth stages for a rice cultivar with 13 true leaves on the main stem. VF denotes flag leaf and VF-n denotes the nth node before the flag leaf (stages V9-V13). Adapted from Counce et al., 2000.

Reproductive Development

Reproductive development consists of 10 growth stages based on distinct morphological measures as shown in Table 9.

Table 9. Rice reproductive growth stages.

R0 Panicle development has initiated	**R1** Panicle branches have formed	**R2** Flag leaf collar formation	**R3** Panicle exertion from boot, tip of panicle above collar of flag leaf
			
R4	**R5**	**R6**	**R7**
One or more florets on the main stem panicle has reached anthesis	At least one caryopsis on the main stem panicle is elongating to the end of the hull	At least one caryopsis on the main stem panicle has elongated to the end of the hull	At least one grain on the main stem panicle has a yellow hull
			
R8	**R9**		

At least one grain on the main stem panicle has a brown hull. The brown hull indicates the grain has begun to dry.	All grains which reached R6 have brown hulls
	

Further Characteristics

The grain yield of rice is comprised of the following four components:

- Number of panicles/m^2
- Number of grains / panicle
- Percentage of ripened grains
- 1000 gm weight.

However, plot yield weight remains the best way to determine the yield of lines or genotypes.

Photosynthesis

Rice is a C3 plant. C3 photosynthetic pathway is not very efficient at transforming inputs to grain, in comparison to the C4 pathway. In order to increase rice yield there are ongoing efforts on development of C4 rice to create a new type of rice with enhanced photosynthetic capacity (Susanne von Caemmerer et al, 2012). The effort to develop C4 rice is worth it as rice is the staple

food source in many Asian countries like India, China and Japan, and also it is grown in places where maize cannot be grown.

Photoperiod and Temperature

Oryza sativa is classified as a short-day plant (i.e., requires long nights to flower). This means that heading date (the number of days it takes for the panicle to begin to exert from the boot, that is, the flag leaf sheath) is accelerated under short-day conditions, while heading date is delays when long-day conditions exist (Garner and Allard, 1920).

General Classification of Rice Production Systems

According to the International Rice Research Institute (IRRI), rice can be classified into four major production ecosystems (Fig. 10):

i. **Irrigated rice** – Rice is grown in well watered condition and is flooded throughout the rice growing season.
ii. **Rainfed lowland rice** – Rice grown under this condition is dependent on rainfall only, and land is prepared such that it preserves the rain water.
iii. **Upland rice** – Rice grown without irrigation water and relies completely on rainfall.
iv. **Flood-prone rice** – Rice grown in river areas is deep water rice, with no inbuilt water control system.

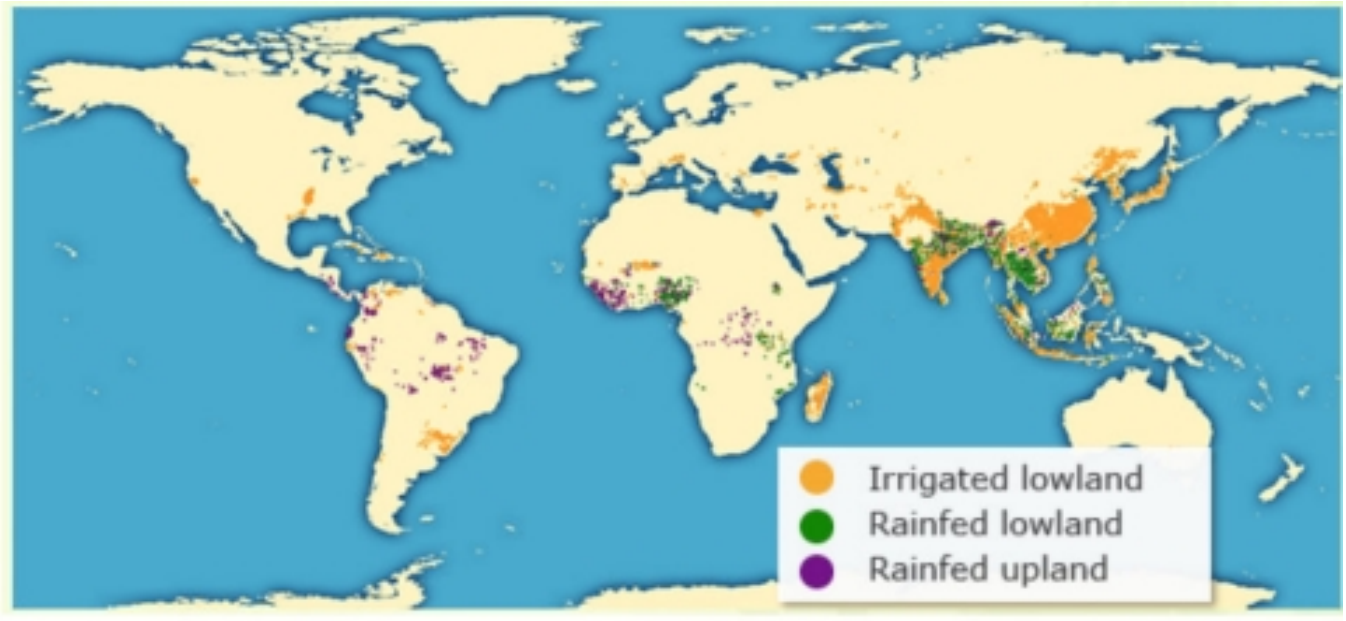


Fig. 10 Rice production ecosystems. Data from IRRI, 2009.

Table 1 Total production area and total rice production (%) in three Ecosystems, 2009.

Rice Ecosystem	**Total Production area (%)**	**Total rice production (%)**
Irrigated lowland	55-60	≈ 75
Rainfed lowland	≈ 30	≈ 20
Rainfed upland	≈ 10	< 5

Diversity

There is huge diversity in Oryza species for shape, color, size as can be seen in Fig. 11. Rice production totals in different regions of the world is shown in Fig 12.

Fig. 11 Rice grains of different specimens. Photo by IRRI Images; licensed under CC BY 2.0 via Wikimedia Commons.

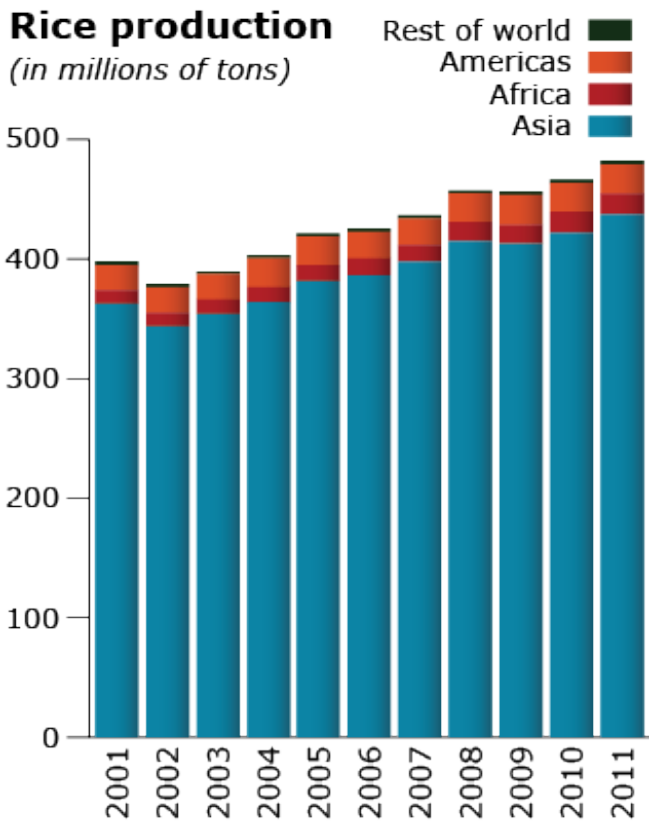


Fig. 12 Rice production by geographic region and year. Data from IRRI World Rice Statistics Online Query Facility.

Adaptation, Economic Importance and Uses

Rice in the Human Diet and Nutrition

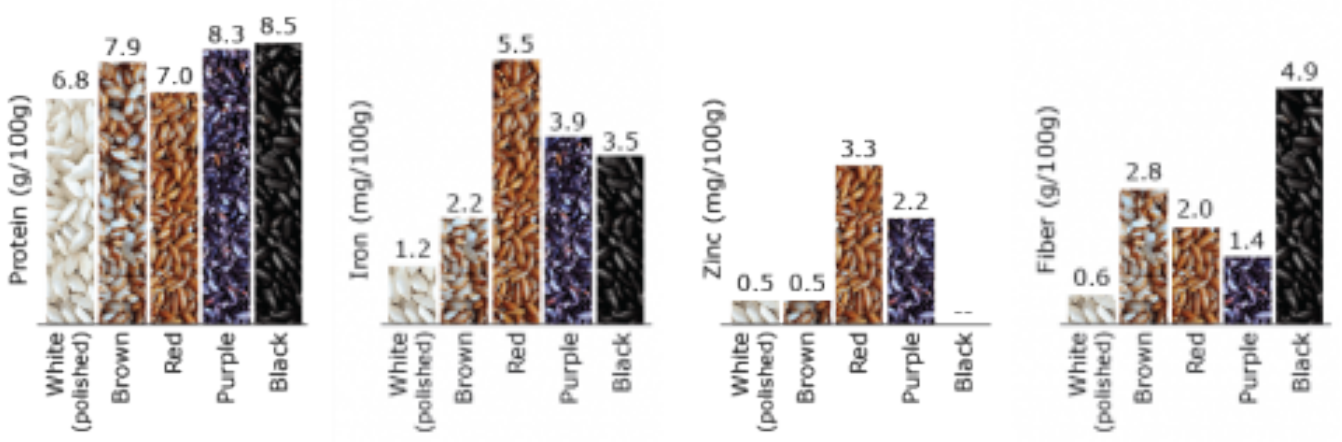


Fig. 12 Nutrient content of different kinds of rice. Data from Food and Agriculture Organization of the United Nations, 2004.

Processing

The procedure of milling and consequently polishing rice, results in the highly valued white rice which removes nearly all the outer layers and germ and leaves a product deficient in thiamine. Through fortification and parboiling, adequate quantities of thiamine and other B vitamins can be retained in rice. Parboiling is usually done in the mill where unhusked rice is generally steamed, so that water is absorbed by the whole grain providing an even distribution of vitamins in the whole grain. However, in conventional methods, the paddy is dried and dehusked prior to milling.

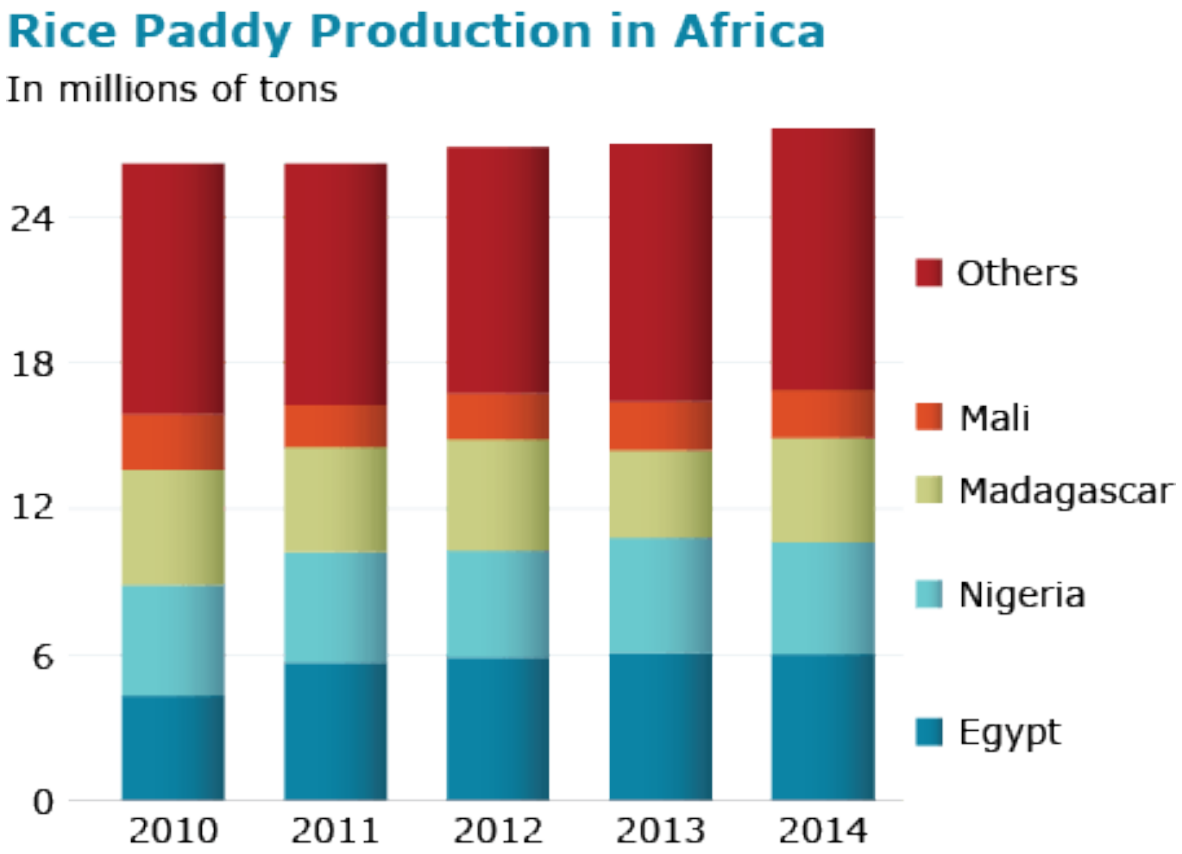


Fig. 13 Predicted rice paddy production in Africa. Adapted from FAO Rice Market Monitor, April 2014.

International Breeding Centers

The Consultative Group on International Agricultural Research (CGIAR) has three centers:

- The International Rice Research Institute (IRRI) has a global mandate to work on rice and its headquarters is in Los Baños, Laguna in the Philippines.
- The West Africa Rice Development Association (WARDA) has mandate to work on rice in West Africa.
- The International Centre for Tropical Agriculture (CIAT) has the regional mandate to work on rice in Latin America.

Breeding Methods

Heterosis

Heterosis refers to the superiority of the F_1 hybrids resulting from a cross of diverse parents, over their parents in performance of desired traits, for example, vigor, yield, number of productive tillers, panicle size, number of spikelets per panicle.

The crosses (×) between rice subspecies showing heterosis in decreasing order are as follows (Fig. 14):

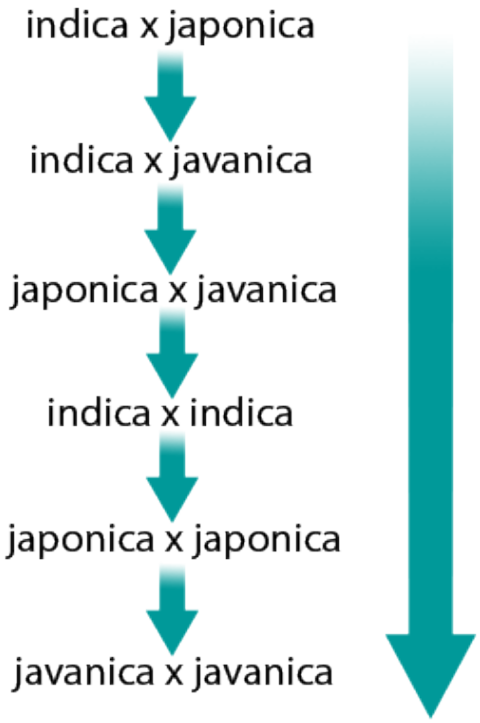


Fig. 14 Breeding protocol of combination of inter- and intra-subspecies crosses of indica, jacanica, and japonica.

Types of Heterosis

Intrasubspecific heterosis: It is the most commonly used heterosis which provides around 15% to 20% more yield than the best check grown under similar conditions. For example

1. *indica* × *indica*
2. *japonica* × *japonica*

Intersubspecific heterosis:*Oryza sativa* ssp. *indica* and ssp. *japonica* are two of the most common subspecies of cultivated rice. The cross between these two subspecies shows maximum heterosis in the F_1 hybrid. Major limitation in intersubspecific heterosis is the high spikelet sterility and long growth duration. The discovery of wide compatibility (WC) genes has provided a solution to overcome these problems allowing the utilization of these type of crosses.

Interspecific heterosis: The crosses in cultivated species refer to only *O. sativa* and *O. glaberrima*. However, heterosis of yield is very high and plant stature remains a problem.

In rice, the interspecific F_1 hybrids cannot be used commercially. Examples of interspecific hybrids as a result of wide hybridization, generates genetic variability and bring together several biotic and abiotic stress resistance genes.

1. *O. sativa* × *O. longistaminata*
2. *O. sativa* × *O. rufipogon*
3. *O. sativa* × *O. perennis*

Hybrid Rice

For details on hybrid rice research at public and private commercial sectors, refer to the following links:

- IRRI
- Pioneer
- DuPont

The commercial rice crop grown as a hybrid crop is an F_1 hybrid developed from the cross of two genetically diverse pureline parents. Good rice hybrids have the potential to yield 15-20%

higher than the best pureline cultivar when these two (hybrid and pureline parents) are grown under similar conditions.

Since rice is a self-pollinated crop, the male sterility system has been used to develop commercial rice hybrids. Commercial companies are more interested in developing hybrid cultivars because of the profits accrued from farmers returning each year to buy new seed. The higher cost of hybrid seed is partly due to the increased cost of development of the parents used to make the hybrids.

Male Sterility in Rice

Male sterility is defined as the inability of a plant to produce functional pollen grains. The use of male sterility in hybrid seed production has a great importance as it eliminates the process of mechanical emasculation. Three forms of male sterility that can be used are:

1. Cytoplasmic genetic male sterility (CGMS)
2. Environment-sensitive genic male sterility (EGMS)
   EGMS is classified in the following categories
   1. TGMS: temperature-sensitive genetic male sterility
   2. rTGMS: reverse temperature-sensitive genetic male sterility
   3. PGMS: photoperiod-sensitive genetic male sterility
   4. rPGMS: reverse photoperiod-sensitive genetic male sterility
   5. PTGMS: photothermosensitive genetic male sterility
3. Chemically induced male sterility (CIMS)

Cytoplasmic Genetic Male Sterility

In CGMS, three lines are involved in hybrid rice development and the process flow is as follows (Fig. 15):

1. **Cytoplasmic male sterile line** (**A line**) – The male sterility is controlled by the interaction of sterile cytoplasm (S) and fertility-restoring genes (rf) present in the recessive form in the nucleus.
2. **Cytoplasmic male fertile line also known as maintainer** (**B line**) – is iso-cytoplasmic to the CMS A-line since it is similar to it for nuclear genes but differs as it has normal cytoplasmic factor (N). A-lines are developed from B-lines using backcross breeding to

transfer the CMS gene. The N gene makes the B-line self-fertile, and is used in crossing with the sterile A-line to maintain A-line seed production.

3. **Restorer line** (**R line**) – R line possesses dominant fertility-restoring genes (Rf), and so is different from the A line and B-line; the restorer line is developed separately to maintain genetic dissimilarity from A-line for expression of heterosis. The restorer gene in the dominant homozygous (RfRf) or heterozygous (Rfrf) state has the ability to restore the fertility in the F_1 hybrid.

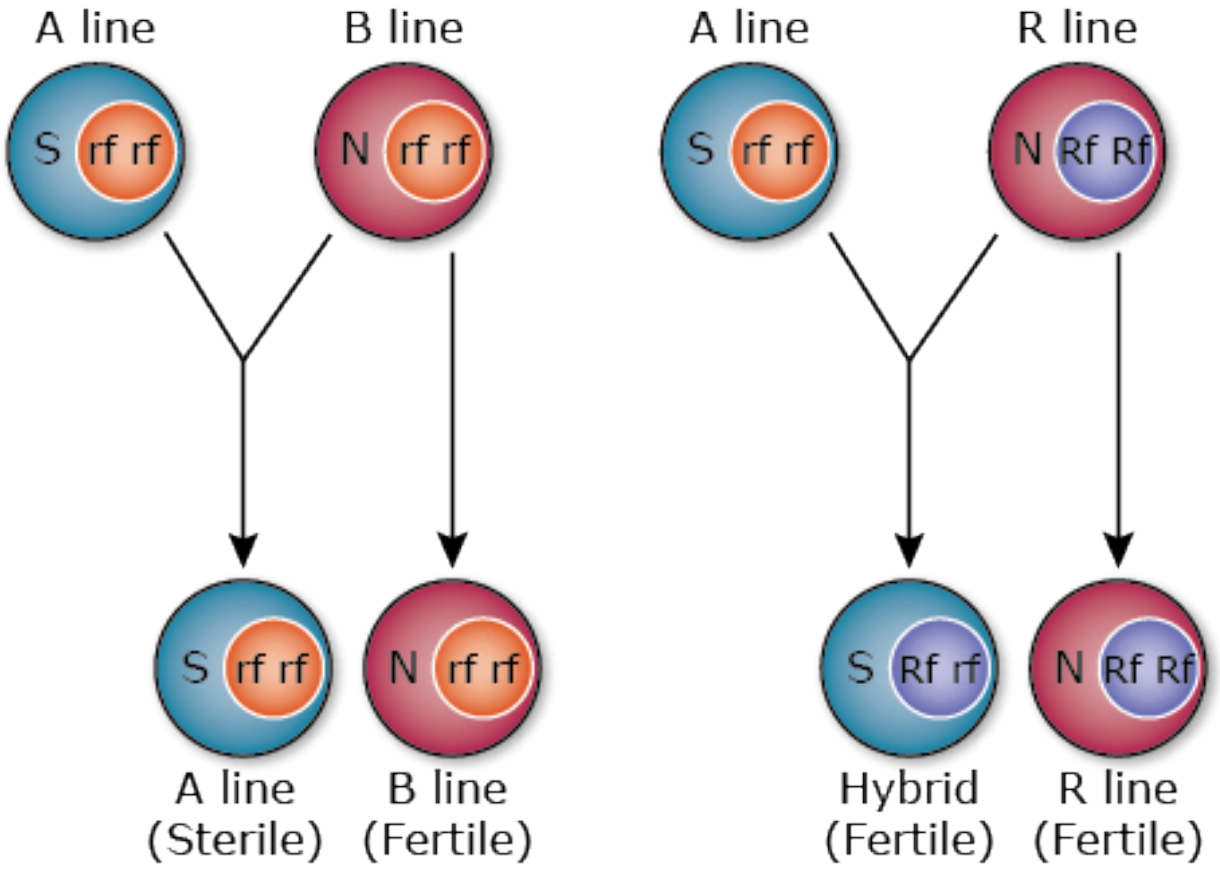


Fig. 15 Breeding strategy using cytoplasmic genetic male sterility. Adapted from Khush, 2001.

Hybrid Seed Production Using CGMS

Hybrid seed production involves two steps:

1. CMS line (**A line**) **multiplication** (**AxB**)**:** The seed of the CMS line (A line) is multiplied by crossing with the maintainer line (B line) either by hand (in plant breeding programs where a small quantity of seed is required) or in the field under isolation by space or time (to produce

breeder seed for commercial seed production). Remember that the A-line is only a parent in hybrid production and it is not for commercial seed sale for farmer production. Seed companies may be interested to obtain marketing rights of A-line for their hybrid breeding program). Generally, A-line seed production field consists of 6 or 8 rows of A-lines alternating with 2 rows of B-lines. This pattern is repeated throughout the field as depicted below (6 rows of A-lines, alternating with two rows of B-lines and repeating pattern in field).

… B B A A A A A A B B A A A A A A B B A A A A A A B B …

2. Hybrid seed production (**AxR):** Hybrid seed is produced by crossing the A line with the R line in isolation. The hybrid seed is sold to farmers for commercial production so a large increase of parent seed and hence hybrid seed is necessary. More than one location may be planted to make the hybrid seed to minimize the impact of loss of field due to an environmental event or other causes. In the field, 8-10 rows of A-lines are grown interspersed with two rows of R-line to produce hybrid seed.

… R R A A A A A A A A R R A A A A A A A A R R A A A A A A A A R R …

Additional Information For Hybrid Seed Production

- The planting dates may need to be staggered to achieve synchronized flowering of the two parents.
- For better pollen dispersal (from male parent) and seed set (on female parent), ropes or sticks are often used.
- Hormone treatment, such as Gibberellic acid (GA), can increase the receptivity of female to accept pollen. This happens due to better emergence of female panicles from the sheath, exposing the ovary to male pollen.

Hybrid Rice Production Flowchart

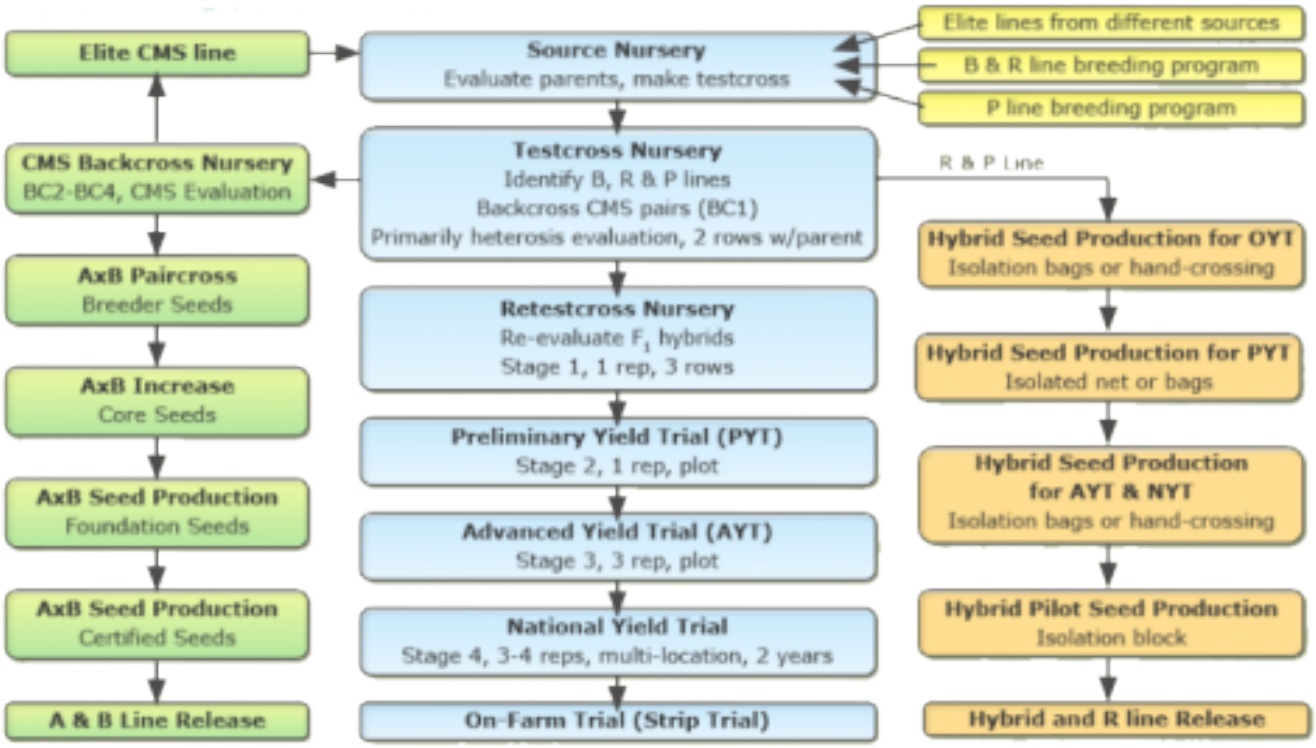


Fig. 16 Flow of hybrid rice evaluation and seed production in a 3-line system. Adapted from FangMing Xie, IRRI, Hybrid Rice Breeding & Seed Production. Used with permission.

Hybrid Rice Breeding using EGMS

In the **two-line method**, the male sterile line is (male sterility controlled by a recessive gene) crossed to a pureline that is male fertile (i.e. it possesses the dominant gene for sterility). Male sterility in the female line is genetically controlled by recessive genes and sterility expression is influenced by environment (temperature, photoperiod, or both), and the male parent is selected to be good pureline pollen producer.

Classification of the EGMS System

Types of EGMS Systems Used in Two-Line Hybrid Rice Breeding

1. **Thermo-sensitive genetic male sterility** (**TGMS**) – In TGMS lines, the sterility or fertility expression is controlled by temperature. Regardless of photoperiod, TGMS lines are

usually highly sterile under high temperature and highly fertile under low temperature. The first TGMS line was reported by Japanese Scientist in the rice variety Remei where gamma ray induced mutation resulted in sterility (31-24ºC) to partial fertility (28-21ºC) and complete fertility (25-15ºC).

2. **Photoperiod-sensitive genetic male sterility** (**PGMS**) – In PGMS lines, the sterility or fertility expression is controlled by daylength. Under long-day conditions, most PGMS lines remain male sterile. Under short-day conditions, they revert back to being fertile. The first spontaneous PGMS mutant, Nongken 58S (NK58S), was reported in 1973 from the japonica (O. sativa ssp. japonica) cultivar Nongken 58 (NK58). NK58S retained male sterility under long day length (longer than 13.75 h) during anther development, while under short day length (less than 13.5 h), partial or complete male fertility was observed. Temperature response was also observed for this line. Under long-day conditions at high temperatures (~29°C) slightly more male sterility was observed.
3. **Reverse photoperiod-sensitive genic male sterility** (**rPGMS**) – PGMS lines express sterility under short day length and under long day length revert to being fertile. This system is known as reverse PGMS (rPGMS).
4. **Photo-thermosensitive genetic male sterility** (**PTGMS**) – PTGMS lines are sensitive to both photoperiod and temperature. Temperature is the important factor since PTGMS lines become completely male sterile or fertile beyond (over or under) a threshold temperature range, without any influence of photoperiod. In this system the effect of temperature and photoperiod is difficult to separate and under natural conditions both factors interact to determine sterility or fertility.

Advantages of EGMS Systems

1. There is no requirement for seed multiplication of a maintainer line, therefore making the seed production system cheaper.
2. No need for backcross breeding to develop a CMS A-line from B-lines.
3. Hybrid breeding efficiency is higher in two-line breeding than three-line breeding since it allows use of any fertile line as a pollen source parent.
4. Undesirable effects of sterility-inducing cytoplasm do not occur.
5. It is ideal for developing indica by japonica hybrids as there is no requirement for restorer lines.

Disadvantages of the EGMS Systems

1. The sterility trait is under the control of environmental factors, and any variation such as temperature fluctuation because of a storm, rain etc., will impact the sterility of EGMS lines.
2. Seed production can be done in the latitudes with optimal photoperiod length, therefore limiting options in some cases for which locations can be used. The seed multiplication (lines and hybrids) are constrained by space and season.

Two-Line System Production Flowchart (Fig. 17)

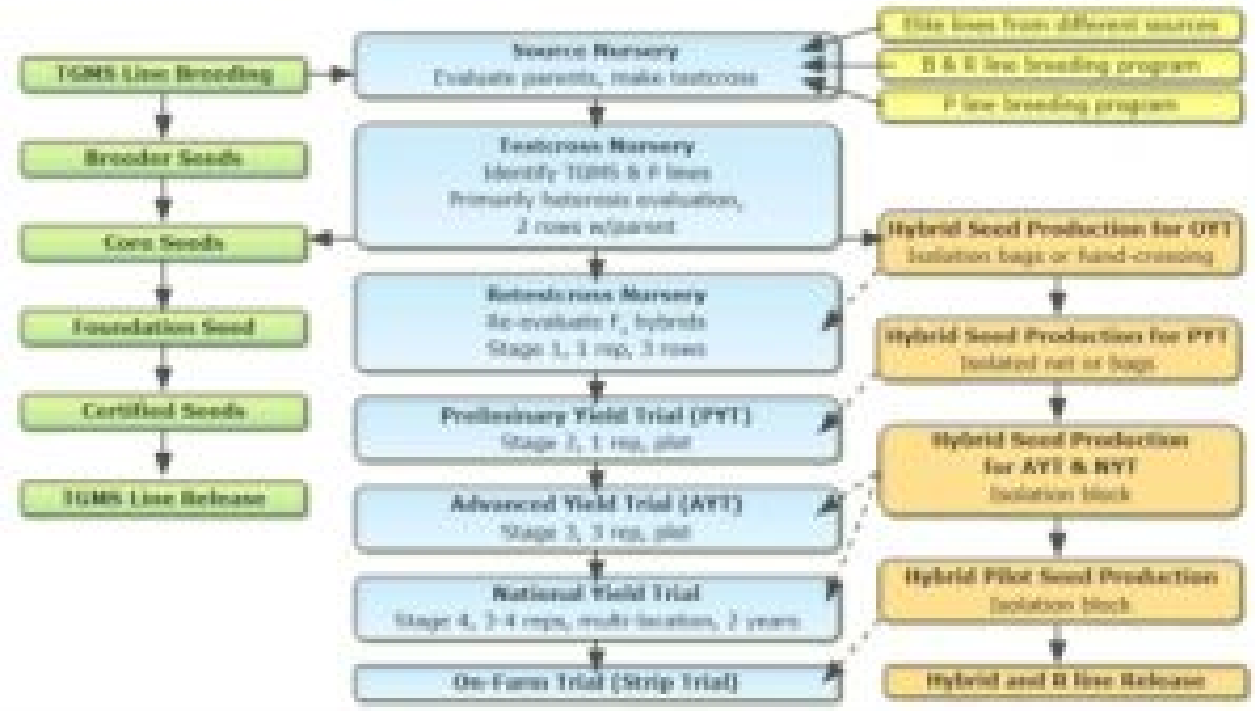


Fig. 17 Flow of hybrid rice evaluation and seed production in a 2-line system. Adapted from FangMing Xie, IRRI, Hybrid Rice Breeding & Seed Production. Used with permission.

Conventional Rice Breeding Program

In conventional breeding, and for rice programs as well, two parents are crossed and segregating generations are screened for the trait of interest, for example, disease resistance, maturity, height and protein. Uniform lines are tested for yield and along with resistance, desirable

varieties are selected and released. The development process from making the initial cross to variety release is shown in Fig. 18.

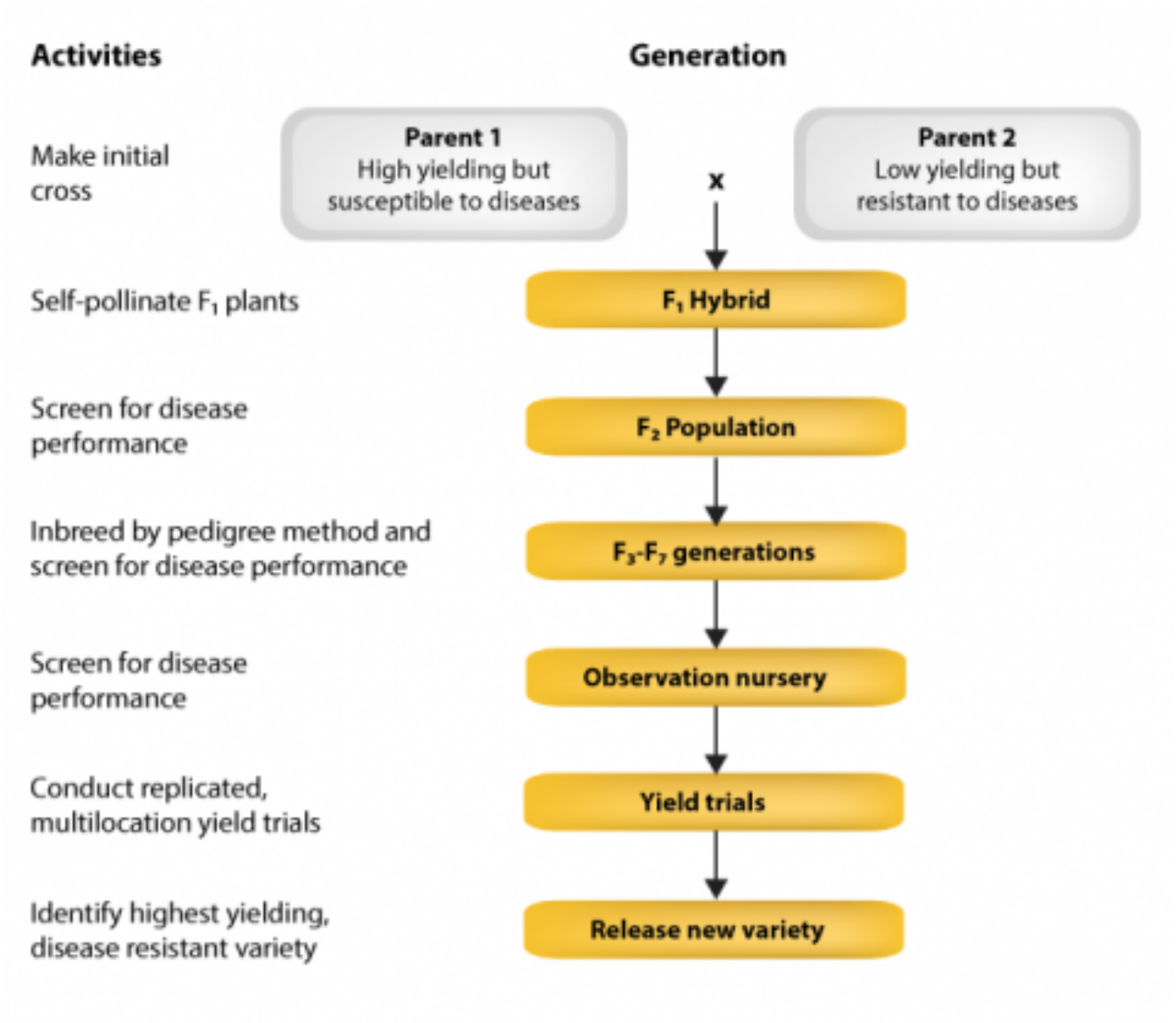


Fig. 18 Conventional Breeding for new variety development and release. Adapted from Khush, 2001.

References

Counce, P. A., T. C Keisling, and A. J. Mitchell. 2000. A Uniform, Objective, and Adaptive System for Expressing Rice Development. Crop Sci. 40: 436-443.

Dingkuhn, M., B.B. Singh, B. Clerget, J. Chantereau, and B. Sultan. 2004. Past, Present and

Future Criteria to Breed Crops for Water-Limited Environments in West Africa. In Fischer T et al. New directions for a diverse planet: Proceedings for the 4th International Crop Science Congress, Brisbane, Australia, 26 September – 1 October 2004.

FAO 2014. Rice Market Production Monitor, April 2014.

Garner, W. W., and H. A. Allard. 1920. Effect of the Relative Length Of Day And Night And Other Factors Of The Environment On Growth And Reproduction In Plants. Mon. Wea. Rev., 48, 415–415.

Harris, D. R. 1996. The Origins and Spread of Agriculture and Pastoralism in Eurasia. Psychology Press. p. 565.

Huang, X., N. Kurata, X. Wei, Zi-Xuan Wang, A. Wang, Q. Zhao, Y. Zhao, K. Liu, et al. 2012. "A map of rice genome variation reveals the origin of cultivated rice". Nature 490 (7421): 497–501.

IRRI. 2009. Rice growing environments, Lesson 1: Where is rice grown? http://www.knowledgebank.irri.org/submergedsoils/index.php/rice-growing-....

IRRI. World Rice Statistics Online Query Facility. http://ricestat.irri.org:8080/wrs2/entrypoint.htm.

Khush, G. S. 2001. Green revolution: the way forward. Nature Reviews Genetics 2, 815-822. Kovach MJ, Sweeney MT, McCouch SR. 2007. New insights into the history of rice domestication. Trends in Genetics 23:578-587.

Linares, O. F. 2002, African rice (Oryza glaberrima): History and Future Potential. PNAS 99 (25):16360-16365.

Londo J. P., Y.C. Chiang, K. H. Hung, T. Y. Chiang, and B. A. Schaal. 2006. "Phylogeography of Asian wild rice, Oryza rufipogon, reveals multiple independent domestications of cultivated rice, Oryza sativa". Proc. Natl. Acad. Sci. U.S.A. 103 (25): 9578–83.

Molina, J., M. Sikora, N. Garud, J. M. Flowers, S. Rubinstein, A. Reynolds, P. Huang, S. Jackson, B. A. Schaal, C. D. Bustamante, A. R. Boyko, and M. D. Purugganan. 2011. "Molecular evidence for a single evolutionary origin of domesticated rice". Proceedings of the National Academy of Sciences 108 (20): 8351.

von Caemmerer S., W. Paul Quick, and R. T. Furbank. 2012. The Development of C4 Rice: Current Progress and Future Challenges. Science 336, 1671-1672.

Vaughan, D.A., B. Lu, and N. Tomooka. 2008. “The evolving story of rice evolution”. Plant Science 174 (4): 394–408.

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Chapter 12: Sorghum Breeding

Teshale Mamo; Asheesh Singh; and Anthony A. Mahama

Sorghum (*Sorghum bicolor* L. Moench) has historically been a major staple food source globally, and is currently ranked the fifth most important cereal. Recently, it has become a multipurpose crop produced not only for food but for feed, fuel and forage, and being bred for use as a cover crop in pastures, through varieties with compacted internodes. Sorghum is serving as a vital model for tropical grass species for functional genetics and genomic studies, made possible by the availability of genomes of three sorghum lines and numerous genetic stocks and populations. It is therefore a crop of immense importance in tackling current global food security challenges.

Learning Objectives

- Students become familiar with the Sorghum crop
- Know crop biology and classification system
- Describe adaptation and usage
- Outline production constraints
- List breeding institutions working on the crop
- Discuss breeding methods used to develop sorghum cultivars

Origin, Domestication, and Diversification

Sorghum (*Sorghum bicolor* (L.) Moench) is an ancient crop that originated in North Eastern Africa. These places are also areas where greatest diversity of wild and cultivated species of sorghum are found to this day (Fig. 1). Domestication of sorghum probably took place in Ethiopia and some parts of Congo by selecting wild sorghum, approximately 5,000 years ago. India, Sudan and Nigeria are considered as secondary centers of origin. From these centers of origin, sorghum was probably distributed to other parts of the world (Acquaah, 2007). This early distribution and introduction of the crop helped generate further genetic diversity in other continents, such as Asia. The genus Sorghum has greatest genetic diversity ranging

from 20 to 30 species. Cultivated sorghum along with the two perennial species [*Sorghum halepense* L (2n=40-forage sorghum) and *Sorghum propinquum* (Kunth)] are included in the genus sorghum. Based on morphological classification, all cultivated sorghums (*Sorghum bicolor* spp.) are grouped in five races along with ten intermediate races. The five races are:

- durra,
- kafire,
- guinea,
- bicolor, and
- caudatum.

Most of these races differ mainly in their panicle morphology, grain size and yield potential. Durra type of sorghum originated primarily in Ethiopia and the horn of Africa, and then spread to Nigeria and other parts of West Africa where it became popular. Kafir types of sorghum developed in the eastern and southern parts of Africa where they grow well. Guinea types developed in West and Central Africa and grow well in that region, while the bicolor type originated in East Africa but is less important to African production.

Fig. 1 Sorghum field showing genetic diversity in grain color. Photo by Teshale Mamo, Iowa State University.

Biology of the Crop

General Characteristics of the Development of the Sorghum Plant

Sorghum is an annual grass, and belongs to the graminae family. It reaches up to 5 m in height with one to several tillers, and these tillers emerge first from the base of the plant and sometimes later from the stem nodes. The tillers on stem notes form when growing conditions are favorable. These tillers form on upper or lower nodes and are undesirable because they form later and produce a small amount of grain that is unripe by harvest with higher moisture content. This can cause delayed harvest, as well as problems in storage, delivery and sales.

Lower plant density (i.e. sparse planting) causes more tillering and higher plant density in field planting suppresses tillering. Tillering is suppressed when growing conditions are unfavorable.

Optimum temperature for germination ranges from 27-35^{o}C, and after germination the plant goes through root and leaves development rapidly. Sorghum has a fibrous root system which is mostly concentrated in the top 90 cm of the soil, but root growth can extend twice that depth under dry environments. Sorghum leaves are alternate with the leaf sheath and ranges from 15-35 cm in length. Total number of leaves on the plant varies between 7 to 24 depending on the variety and environmental conditions. Sorghum leaves have rows of motor cells along the midrib on the upper surface of the leaf which is unique characteristic of sorghum leaves as these cells can help the leaves to roll up rapidly during drought stress to minimize water loss from the leaves. In addition, morphological and physiological characteristics of sorghum such as extensive root system, wax on the leaves (minimize water loss) and the ability to stop growth during moisture stress and resume growth when moisture levels increase (from rain) are inherent characteristics of sorghum to adapt to drought conditions.

Growth Stages

The inflorescence or head of sorghum is called panicle that may be loose or dense. Under favorable conditions, initiation of panicle takes place after one third of the growth cycle. Each fully developed panicle can contain 800 to 3000 grains, each one usually enclosed by glumes. The color of the seed is variable. Sorghum flowers usually open during the night or early in the morning with those flowers at the top of the panicle opening first, and it takes 6 to 9 days for the whole panicle to flower. Sorghum is a self pollinated crop due to its flower structure but cross pollination (approximately 2-25 %) occurs naturally.

In general, once the sorghum seedling emerges, the plant goes through three distinct growth stages represented as growth stage I, II and III. The first growth stage (GS I) is recognized as vegetative growth. During this stage, the plant develops leaves, internodes and tillers. This stage helps the plant to prepare for grain formation and growth. At this stage the plant can tolerate drought stress, heat and freezing temperatures. The second growth stage (GS II) is the reproductive phase in which the panicle is developed and maximum number of seeds per plant are set. This growth stage starts with panicle initiation and it continues to flowering. It is reported that it is the most critical period that determines the level of grain production. This is the stage when the crop's water requirement is high. Hence if severe moisture stress occurs at this stage, panicle initiation is hindered or delayed, leading to incomplete flowering, seed set

and loss in grain yield. The third growth (GS III) is the grain filling period which starts with flowering and continues until the grain is filled with dry matter.

Photosynthesis, Photoperiod, and Temperature

Sorghum is one of the C4 grasses with high photosynthetic efficiency. It is a short day plant requiring long nights before flower initiation (start of reproductive stage) (Craufurd et al., 1999). The optimum photoperiod for flower initiation ranges from 10 to 11 hours and a photoperiod beyond 12 hours can stimulate vegetative growth. Tropical cultivars are more photoperiod sensitive than short-season sorghum cultivars (quick mature).

Sorghum is a dry land crop requiring high temperature ranges from 27 to 30^0C for its growth and development (Craufurd et al., 1999). Increased day and night temperature beyond plant requirements can delay flower initiation and development of flower primordia, and this reduces yield. The sorghum plant can tolerate a temperature as low as 21^oC without significant effect on growth and yield.

General Classification

Classification by Utilization or Mode of Consumption

Grain sorghum is the most widely cultivated type of sorghum in the world, and it is the main staple food in dry land (semi-arid tropical region) areas of Africa and Asia. It is an important part of diet that is prepared in the form of boiled porridge, unleavened bread (pancake), popped (like maize), dumplings, beers and non-alcoholic fermented beverages. Sorghum grain is also used as animal feed, and the stems and leaves are used as green chopped animal feed, hay and pasture feed. It is grown as grain and fodder crop in the USA, Europe and Australia (Berenji and Dahlberg, 2004).

Human Food

In Africa and Asia, many people consume sorghum grain in unfermented and fermented pancake (breads), porridges, dumplings, snacks, and malted alcoholic and nonalcoholic beverages. White grain sorghum is mostly preferred for cooking while red and brown grain

sorghum are preferred for beer making. In some parts of Africa, e.g., around Lake Victoria, where bird pressure is high, farmers may grow red and brown grain sorghum instead of white grain types, because these types of sorghum are rich in tannin and are bitter tasting thus preventing bird feeding and associated losses.

In the USA, sorghum is primarily grown as a fuel crop (for ethanol production) and there are few food products available to consumers; however several researchers have developed and introduced products from sorghum into the food market. In addition, several researchers have been working on health benefits associated with sorghum grain that might increase its use in the health food industry. For example, food products made from sorghum grain did not show toxicity to celiac patients (Ciacci et al., 2007), and several gluten-free sorghum products have been developed and are being popularized (Schober et al., 2005).

Animal Feed

In the United State, Central and Southern America, Europe, Australia and China, sorghum grain is mainly used as cattle, pig and chicken feeds. Similar to the use of silage corn, the sweet sorghum type is also used as cattle feed in Europe. The problem with sorghum as cattle feed is the presence of prussic acid (HCN) which causes death in cattle if the animal consumes fresh sweet sorghum. This problem is eliminated through cultivar choice and proper agronomic practices.

Renewable Energy

Sorghum is one of the crops that can be used for production of renewable fuels in temperate regions. It is unique among grasses in being used as feed stocks for renewable energy because it can be used in various forms for biofuel production. Starch and sugar are converted to ethanol, and lignocellulose (composed mainly of cellulose, hemicellulose and lignin – inedible parts of the plant) is converted to biogas, making sweet sorghum a unique biofuel crop that is also used as food and fodder.

Classification of Grain Sorghum by Intended Purpose

Sorghum is classified into four major groups based on the applications.

1. *Grain sorghum:* this group is used as staple food in the tropical areas of Africa and Asia and is often used as raw materials for making alcoholic beverages, sweets and glucose.
2. *Sweet sorghum:* this group is mainly produced for sugar production. This sugar is used as material to produce sweet syrup, which is similar to molasses.
3. *Broom sorghum:* this is recognized by long panicles with fine, elastic branches called fibers with the seed on their tip which is used as material to making brooms.
4. *Grass sorghum:* this is mainly grown for green feed and forage purpose.

Classification of Grain Sorghum by Agronomic Groups

Commercial grain sorghum is classified into seven groups.

1. *Kafir sorghum:* this group of sorghum is originally from South Africa. In this group, the stalk is thick and juicy, have large leaves, and the panicles are cylindrical in shape without awn. The seed are medium in size, color could be white, pink or red.
2. *Milo sorghum:* came from east Africa, has short, compact and oval panicles, with less juicy stems than kafir, and has light green leaves. The seed size is relatively large with either yellow or white seed color. The plant in this group has more tillers than kafir. The varieties in this group are more heat and drought tolerant than kafir.
3. *Hegari sorghum:* came from Sudan and is similar to kafir but has more nearly oval panicles. Plants in this group have more leaves than kafir, and the grain produces a sweeter juice which is desirable for forage. Seeds are chalky white in color.
4. *Feterita sorghum:* is originally from Sudan. Plants have few leaves and dry stems. Panicles are oval compact shape. Seeds are large in size and chalky white in color.
5. *Dura sorghum:* came from Mediterranean and Middle East regions. Plants have dry stems, flat seeds with pubescent glumes. The panicles in this group are erect but compact or loose. The varieties in this group are mainly grown in North Africa, India and the Near East.
6. *Shallu sorghum:* This group is from India, and plants have tall, slender and dry stalks. Panicles are loose, and seed color is pearly white. The varieties in this group are late maturing, thus requiring a relatively long growing period.
7. *Kaoliang sorghum:* This group is mainly grown in China and Japan. The varieties have slender, dry and woody stalks with sparse leaves. They have an open semi compact panicle. Seeds are small in size and white or brown in color and are bitter in taste.

Adaption and Economic Importance and Uses

Adaption

Sorghum is a small cereal crop adapted to wide range of environmental conditions, but is particularly adapted to a warm weather. Sorghum is mainly grown between 40^{o}N and 40^{o}S in arid, semi-arid tropics and subtropics, and it can also be grown at an altitude of up to 2300 m above sea level in the tropics with annual rainfall ranges from 300 -1200 mm. It is also widely grown in temperate regions mainly in South China and USA and some parts of Europe. Cold tolerant sorghums are also grown successfully in Central America.

Sorghum is a short day plant requiring 90 to 140 days to mature depending on climate and type of cultivar. Its genetic variation in response to photoperiod and temperature contribute to its adaptation to the wide range of growing environments. Sorghum's most outstanding characteristics are its heat and drought tolerance and it can also be grown on a wide range of soil types from vertisol (clay soil) in the tropics to light sandy soil. The soil pH requirement ranges from neutral to high pH (5.0 to 8.5) and it is tolerant to salinity compared with corn. Sorghum can be grown in poor soil and can produce grain where other crops fail to produce fruit.

Cropping System

Intercropping is a cropping system involving the growing of two or more crops in the same space and at the same time. It is a common practice among small scale farmers in the semi-arid areas of Africa and Asia in order to increase productivity per unit area of the land (Kidane et al., 1989). Sorghum is one of the important cereal crops being used for intercropping. It is commonly intercropped with a legume crop such as sorghum-chickpea, sorghum-common bean, sorghum-pigeon pea, sorghum-cowpea and sorghum-mung bean. It is also intercropped with other cereals such as sorghum-millet and maize-sorghum. Several researchers have reported that a significant yield was obtained from intercropping compared to pure stands. Sorghum yield was increased 8-34% in a sorghum-legume intercropping system compared to sole sorghum crop stand (Singh, 1977). In Ethiopia, sorghum-mung bean intercropping gave extra yield of 495 kg/ha of sorghum compared to sole sorghum crop (Kidane et al., 1989). Striga infestation on sorghum was reduced when mung bean was intercropped with sorghum. In general, intercropping of sorghum with legumes has a benefit in that the legume crop

allows efficient use of both space and time to optimize effects (increased land productivity). Intercropping promotes diversification of crops so that the farmers can harvest two or more different crops from the same piece of land, and it provides better weed control and reduces diseases and pests incidence.

Production Constraints

Biotic Constraints

Diseases and pests are the main causes of significant yield losses in sorghum. The fungal disease **Smut**, caused by *Sphacelotheca* spp., may cause more yield losses than other fungal diseases and is widely important in the eastern, central and southern parts of Africa where sorghum is used as major staple food. The different types of smut are: loose smut, kernel smut, head smut and long smut. They are controlled by seed treatment with fungicides. Through breeding, use of resistant cultivars provide protection against this disease. **Rust**, which is caused by *Puccunia purpurea,* is another important fungal disease and is widely distributed in many parts of sorghum producing regions particularly in Africa. **Grain mold** is caused by several fungi, *Curvularia lunata*, *Fusariumspp*, and anthracnose (*Colletotrichum graminicola*) are the most important sorghum diseases that infect the grain during grain development and can cause severe discoloration of grain and loss of seed quality. Continued rainfall throughout grain maturity period increases the occurrence of grain mold and causes delayed harvest. Grain mold control measures include the use of resistant cultivars and adjustment of planting time to avoid long maturation period during prolonged rainy season. Downy mildew, ergot and bacterial streak are occasional important constraints.

Sorghum insect pests important in tropical Africa are stem borer (particularly, *Busseola fusca* and *Chilo partellus*) and shoot fly (*Antherigona soccata*). Yield losses due to these insect pests are significantly high and the problem is widespread in major sorghum producing African countries.

Other biotic constraints such as Striga, weeds, and quelea are also considered as major production contains. Striga is rated as causing high yield losses in all regions in Africa. In some countries, the yield losses were estimated to be more than 50%, particularly in Rwanda and Kenya (Wortman et al. 2009).

Abiotic Constraints

Abiotic stresses such as extreme drought (in all sorghum growing regions), saline soil (some parts of India and Middle East countries) and acidic soil (mostly in Latin America) are major production constraints.

International Breeding Center

Collections Diversity

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), a member of the Consultative Group on International Agricultural Research (CGIAR), based at Patancheru, India was established in 1972 with sorghum as one of its five mandate crops.

A total of 36,774 accessions have been collected from 90 countries (Reddy et al., 2008) and maintained in a gene bank, and these collections exhibit 80% of the diversity present in the crop.

ICRISAT coordinates all sorghum research programs in the semi-arid (dry land) tropics of the world. It has strong collaboration and active breeding programs covering over 55 countries in Asia and sub-Saharan Africa with a mission to reduce poverty, hunger, and environmental degradation in the dry land areas of the tropics. ICRISAT has been addressing national, regional and global concerns for sorghum improvement through developing sorghum cultivars with genetic resistance to major diseases and insect pests. ICRISAT is also developing intermediate breeding products such as a wide range of male sterile lines that are widely used by public and private breeding centers for hybrid cultivar development. ICRISAT is more involved in diversification of sorghum breeding populations through incorporation of major abiotic and biotic resistance traits that have not previously been used in sorghum improvement programs. The traits that are currently being given emphasis at the global level include tolerance to drought, heat, Aluminum toxicity, salt, head and stem pests, and grain molds. Earliness with high grain and biomass yield, and tillering capacity are also emphasized.

Additional Collections Locations

In addition to ICRISAT, large sorghum collections are held in temperate regions including USA

(National seed storage lab) and China. Similarly, in tropical Africa, large sorghum germplasm collections are held in Zimbabwe (SADC/ICRISAT sorghum and millet improvement program, Matopos), Ethiopia (Institute of Biodiversity Conservation, Addis Ababa), Kenya (National gene bank, Crop Plant Genetic Center, KARI) and Uganda (Serere Agricultural and Animal Production Research Institute). All these accessions are a valuable genetic resource for further germplasm development efforts.

Breeding Methods and Strategies

Breeding Opportunities and Objectives

Sorghum (2n=2x=20) is predominantly a self-pollinated crop with out-crossing ranging from 2 to 25 %. It has a small genome size (730 Mbp) compared to maize or sugar cane. Sorghum genome is a fully sequenced and provides many useful opportunities to plant breeders and genomics researchers.

Breeding objectives of sorghum include: high grain and fodder yield potential, resistance to diseases (smut, rust, grain mold, bacterial blight, anthracnose, and downy mildew etc.), resistance to insect pests (stalk borer, shoot fly, and midge), resistance to drought and extremely acidic soil, wide adaptation and improved quality (for use in bread, porridge, snacks, and beverages).

In sorghum breeding programs, breeders are developing two kinds of cultivars: 1) open pollinated (OP) or pure line cultivars (mainly for developing countries), and 2) hybrid cultivars (mainly for industrialized countries where the seed system are well developed).

The breeding methods for open pollinated variety (OPV) is different from pure-line or hybrid cultivar development. Recurrent selection schemes are used for OPV, while breeding methods that we learned for self-pollinating crops are used to develop pure-lines. Hybrid development programs will also use pure-lines, however, they use three different kinds of pure-lines: A-line (cms line), B line (maintainer line) and R-line (restorer line) details presented in Breeding Methods module.

Open Pollinated and Pure Line Cultivars – Development Methods

1. **Population improvement:** This is the most common type of breeding method being used in developing countries (Africa), and it includes a group of sorghum plants sharing a common gene pool. Sorghum population improvement program is mainly used for developing broad-based gene pools through recurrent selection methods. In population improvement, the recurrent selection methods are the most useful methods for improving quantitatively inherited traits by increasing frequency of genes that effect trait/traits under selection and to maintain genetic variability by recombining superior genotypes for further and continuous improvement. The method of population improvement is grouped into Intra-population improvement (practiced within specific population for its improvement), and Inter-population improvement methods (selection is based on the intercross performance between two populations). The most convenient population improvement methods in sorghum are mass selection, S1, and S2 progeny testing (ICRISAT annual report, 2010). Details of these methods are covered in the chapter on Breeding Methods.
2. **Pedigree method (or another method applicable to self-pollinated crops):** In this method, sorghum breeders are hybridizing between desirable complementary parental lines (Fig. 2), followed by selection of desirable plants from segregating populations until homozygosity is achieved. It is applicable for improving specific trait such as disease and insect pest resistance, plant height, early maturity, etc. These methods will lead to the development of pureline cultivars.

Note: This method is used to develop B-line and R-lines for Hybrid development and production programs.

3. **Backcross breeding:** Backcross breeding in sorghum is used to transfer favorable single or few genes including resistance to diseases (grain mold, rust and smut), and resistance to insect pests (stalk borer and shoot fly) from donor genotype, which generally has poor agronomic performance, into elite genotype (recipient).

Note: This method is used to develop A-line version of B-lines for Hybrid development and production programs.

Fig. 2 A crossing block. Photo by Teshale Mamo, Iowa State University.

Hybrid Cultivar Development (Hybrid Breeding)

This method of hybrid cultivar development in sorghum closely resembles that of hybrid corn breeding. The two major differences are that (a) heterotic groups are not well defined in sorghum as in maize, so groups are based on fertility restorer genes, however more recently, reproductive groups are emerging with the differentiation and use of nuclear fertility genes. The other difference (b) is that sorghum utilizes cytoplasmic male sterility system (3-line system) to facilitate hybrid seed production unlike maize where manual detasseling, which works very well is employed (detassel female inbred line and allow male inbred line pollen to pollinate) to create hybrid seed on female ears.

Briefly, a plant breeder will develop B-line (maintainer) and R-line (restorer) under two separate reproductive groups (to maximize heterosis) using pedigree or Single Seed Descent approach

or any other method suitable for a self-pollinating crop. Once new and superior B-lines are developed, backcross breeding is used to convert them to A-lines (CMS lines). As the backcross breeding program continues, general combining ability (GCA) or specific combining ability (SCA) may be assessed to decide which B-line conversion to continue and also to generate information on suitable R line parent in combination. The A-line is cytoplasmic male sterile and serves as the seed bearing parent. The B-line has recessive form of fertility restorer gene and is used as a maintainer for the A-line. The R line has the dominant form of fertility restorer gene in the nucleus and has the capacity to restore fertility in the A system and it is used as the pollen parent. For details, see the Breeding Methods e-module for diagram on fertility and restorer genes in cytoplasm and nucleus, respectively.

References

Ehlers J. D., T. Hall, P.N. Roberts, A.N. Eckard, and W.C. Mattews. 1994. Blackeye Varietal Improvement – 1994 Progress Report.

Ehlers, J. D., A.E. Hall, P.A. Roberts, W.C. Mathews, A.M. Ismail, and A.N. Eckard. 1995. Blackeye Varietal Improvement – 1995 Progress Report.

Ehlers, J. D., A.E. Hall, P.A. Roberts, W.C. Mathews, A.M. Ismail, B.L. Sanden, C.A. Frate, and A.N. Eckard. 1996. Blackeye Varietal Improvement – 1996 Progress Report.

Ehlers, J. D., A.E. Hall, B.L. Sanden, P.A. Robert, W.C. Mathews, C.A. Frate, A.M. Ismail, and A.N. Eckard. 1997. Blackeye Varietal Improvement – 1997 Progress Report.

Ehlers, J. D. , A.E. Hall, A. M. Ismail, P.A. Robert, W.C. Mathews, B.L. Sanden, C.A. Frate, and S.C. Mueller. 1998. Blackeye Varietal Improvement – 1998 Progress Report.

Ehlers, J. D., A.E. Hall, P.A. Roberts, W.C. Mathews, and B.L. Sanden. 1999. Blackeye Varietal Improvement.

Hall A.E. 1989. Blackeye Varietal Improvement – 1989 Progress Report.

Hall, A.E., P.N. Patel, and A.N. Eckard. 1990. Blackeye Varietal Improvement – 1990 Progress Report.

Hall, A.E., P.N. Patel, A.N. Eckard, and M. Abdalla. 1991. Blackeye Varietal Improvement – 1991 Progress Report.

Hall, A.E, J.D. Ehlers, and A.N. Eckard. 1992. Blackeye Varietal Improvement – 1992 Progress Report.

Roberts, P.A., W.C. Mathews, A.E. Hall, J.D. Ehlers, S.R. Temple, and D. Helms. 1993. Blackeye Bean Tolerance and Resistance to Root-Knot Nematodes, Progress Report for 1993.

Timko, M.P., J.D. Ehlers, and P.A. Roberts. 2007. Cowpea. In: Genome Mapping and Molecular Breeding in Plants, Pulses, Sugar and Tuber Crops. Volume 3. Edited by Kole C. Berlin: Springer-Verlag; 2007: 49-68.

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Chapter 13: Sweetpotato Breeding

Jessica Barb and Anthony A. Mahama

Sweetpotato (*Ipomoea batatas* (L.) Lam., Convovulaceae) is a hexaploid species (2n = 6x = 90) that originated in both Central and South America. Recent evidence (Roullier et al. 2013) suggests that sweetpotato evolved from at least two autopolyploid events involving distinct populations of *I. trifida* or a now extinct species that was an ancestor to both *I. batatas* and *I. trifida*, which is similar to extant populations of wild tetraploid Ipomoea. The primary center of diversity for sweetpotato includes Central and South America (Huang and Sun, 2000; Zhang et al. 2000). Uganda in East Africa (Austin, 1987) (Yada et al 2010) and the region including Papua New Guinea (Fajardo et al. 2002) are both considered secondary centers of diversity.

Learning Objectives

- Become familiar with the basic biology and importance of sweetpotato
- Provide examples of breeding schemes used to develop cultivars of sweetpotato

Importance

Sweetpotato is a tremendously important low-input crop in Sub-Saharan Africa (SSA) that grows well without fertilizers and limited water and requires little care other than occasional hand weeding. In 2013 sweetpotato was the sixth most important food crop-based on total food crop production after cassava, maize, yams, rice, and sorghum, and 10th based on harvested land area (FAOSTAT).

Usage

Sweetpotato is grown for its enlarged storage roots and leaves that are harvested for human consumption (i.e., table stock), for animal feed, as a source of starch, and for industrial purposes. It is an important food security crop in African countries, especially in rural areas. Sweetpotato

is a hardy crop that can provide reasonable yield in a variety of different environments and requires less external inputs (i.e., fertilizers, water) than most grain crops. Sweetpotato is a useful crop for subsistence purposes because it can be planted and harvested throughout the year in many locations, and a farmer can "store" roots in the ground and harvest the roots as needed. Roots are boiled, baked, fried, and included in a variety of dishes. Roots can also be dried and pulverized to make flour.

Sweetpotatoes are extremely variable in size, shape, color, moisture content, and carbohydrate content. Most consumers in SSA prefer the white-fleshed high dry matter types, but efforts are underway to develop orange-fleshed sweetpotato (OFSP) varieties that are both high in bioavailable beta-carotene and dry matter content to alleviate Vitamin A deficiency, especially in children. Sweetpotato leaves and petioles are good sources of protein, fiber, and minerals, especially K, P, Ca, Mg, Fe, Mn, and Cu, and when consumed could prevent malnutrition in developing countries. Purple fleshed types are also available that are usually high in dry matter and have a low level of sweetness.

Because sweetpotato can be planted throughout the year and there is a large range in maturity dates, farmers can manage the supply period and ensure continual yield, both for home consumption and for the local market. In most countries in SSA roots are available 4-8 months out of a year and in countries with two rainy seasons (i.e., Rwanda, Burundi, and Uganda) roots are available 11 months of the year. In most SSA countries except South Africa sweetpotato is grown primarily by smallholder producers who often plant a mix of different varieties in the same field, which is typically rainfed.

Important Diseases and Pests

Infected by Several Different Viruses

Sweetpotato is clonally propagated via vines, root slips (i.e., sprouts), or storage roots therefore, it is often infected by several different viruses. Durable resistance to viruses typically requires a combination of different traits that reduce the severity of symptoms, increase the tolerance of the variety in the field (i.e., decent yield despite the presence of the virus and visual symptoms), and allow the plant to recover from symptoms and revert to a virus free condition after being infected (Mwanga et al. 2013). Unfortunately degeneration of clean material often occurs very rapidly (i.e., within weeks to a year) (Gibson and Kreuze 2014) and yield losses are common.

Sweetpotato growing in humid, tropical, low- and mid-elevation regions of Eastern and Central Africa are mostly affected by Sweet potato chlorotic stunt virus (SPCSV) and Sweet potato virus disease (SPVD) which is caused by co-infection of Sweet potato chlorotic stunt virus (SPCSV) and Sweet potato feathery mottle virus (SPFMV). SPCSV is more detrimental than SPFMV and typically results in permanent symptoms and yield losses. SPCSV is transmitted by white flies. SPFMV is of less concern because infection of just this single virus only produces transient symptoms and very little loss in yield. This virus is transmitted by aphids. Resistance to SPCSV appears to be conferred by a recessive allele, which occurs in low frequency in the sweetpotato gene pool. However, this resistance still needs to be proven in regions where virus pressure is highest. SPVD (SPCSV + SPFMV) causes devastating yield losses in regions of high humidity though some varieties (e.g., NAPSPOT 11 and Tanzania) are reported to possess some levels of resistance. Other viruses that are less prevalent include: Sweet potato mild mottle virus (SPMMV), Sweet potato latent virus (SPLV), and Sweet potato virus G (SPVG).

Diseases and Pests in Africa

Sweetpotato growing in the humid, tropical highland regions of Eastern and Central Africa are affected by SPVD but are more affected by Alternaria stem blight which is the dominant disease of sweetpotato in this region. Other diseases that cause problems to a lesser extent throughout Africa include: scurf (caused by *Monilochaetes infuscans*), foot rot (caused by *Plenodomus destruens*), chlorotic leaf distortion (caused by *Fusarium denticulatum*), and Rhizopus soft rot (caused by Rhizopus stolonifer and R. arrhizus)

Two of the major pest groups that cause considerable damage to sweetpotato are plant-parasitic nematodes and weevils (*Cylas* spp.). Weevils are a major problem in drought-prone regions of Southern and Eastern Africa. Currently, there is no resistance to sweetpotato weevils.

Breeding Goals

Sweetpotato is grown for human consumption (i.e., table stock), processed starch, bioethanol, colorants/dyes, and for foliage for human and animal consumption. Examples of breeding goals include: resistance to sweetpotato virus disease (SPVD) and Alternaria stem blight, weevil resistance, improved yield, improved size, shape, and uniformity of roots, yield stability, high dry matter content, orange-flesh varieties with high nutritional value [i.e., beta-carotene content for combating vitamin A deficiency, improved chemical composition (i.e., starch, cellulose,

sugars, protein content, carotenes, anthocyanins), improved micronutrient content (e.g., Zn and Fe)], extended harvest for subsistence cropping, drought tolerance, dense foliage with high protein content, improved palatability, and digestibility, vine survival and vigor after planting (especially during periods of drought), improved storage, resistance to skinning, and lower acrylamide potential.

Breeding Centers in Africa and Elsewhere

International Potato Center in Peru (CIP), Crops Research Institute (CSIR) in Ghana, Mozambique Institute of Agricultural Research (IIAM), National Crops Resources Research Institute (NaCRRI) in Uganda, North Carolina State University (NCSU) in the USA, Louisiana State University (LSU) in the USA, Agricultural Research Council (ARC) in South Africa, the Kenya Agricultural Research Institute (KARI), the Agricultural Research Institute (ARI) in Tanzania, the Zambia Agricultural Research Institute (ZARI), the Department for Agricultural Research Services (DARS) in Malawi, the Rwanda Agriculture Board (RAB), the National Root Crops Research Institute (NRCRI) in Nigeria, the Ethiopian Institute of Agricultural Research (EIAR) and the Environment and Agricultural Research Institute (INERA) in Burkina Faso.

Status of Sweetpotato in Africa (ca. 2015)

Constraints on Yield

Although acreage in Africa planted with sweetpotatoes has steadily increased from the 1960's, the average yield per hectare of sweetpotato has remained basically unchanged (Fig. 1) (FAOSTAT, 2014) due to five main constraints (Low et al. 2009):

- Farmers' inability to acquire virus and disease free planting materials
- The lack of improved varieties
- The damage due to sweetpotato weevils
- Poor agronomic practices
- The lack of easily accessible markets

Average yield of sweetpotato in Africa is 5.6 tons ha^{-1}, which is less than the average yield observed in the USA and China (24.5 and 22.4 tons ha^{-1}, respectively), and far below the maximum achievable yield of 40-50 tons ha^{-1}. Experiments conducted in East Africa suggest that

yield could double if farmers had access to clean planting material of improved varieties, and the addition of improved crop and soil fertility management practices could more than triple the yield potential of sweetpotato in Africa (Gruneburg et al. 2004).

Yield and Acreage Over Time

The lack of improved varieties of sweetpotatoes is mostly the result of limited investment in sweetpotato breeding programs in Africa, though this trend is changing as government and NGO's are now focusing more efforts towards the training and support of plant breeders working on secondary crops including sweetpotato. Additional effort is also now being focused on linking farmers with breeders to ensure that varieties produced by breeding programs are meeting the needs of farmers and consumers of sweetpotato.

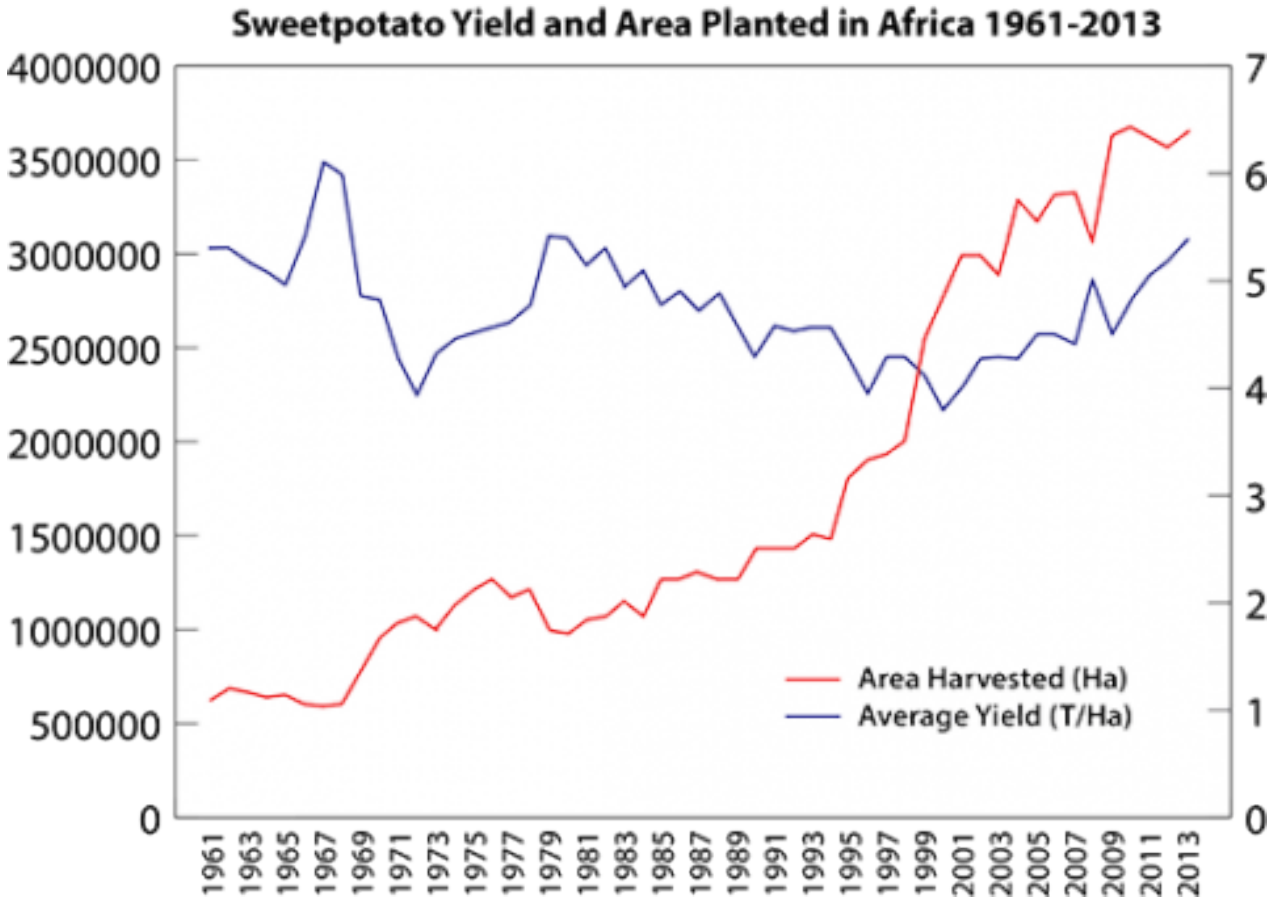

Fig. 1 Yield and acreage of sweetpotato in SSA over time. FAOSTAT.

Breeding Sweetpotato

Breeding Factors

The rate of progress that is achievable for a breeding program is dependent on the gene frequencies in the base population, the effectiveness of the breeding methods that are used, and the access the breeder has to field sites, greenhouses, lab equipment, and trained personnel needed to conduct a breeding program.

A breeder should consider the following when deciding on which breeding method to use:

- The germplasm that is available
- The inheritance of the target traits (if known)
- Biological constraints of the species (i.e., low seed set per plant, self-incompatibility, etc.)

Genetic improvement of sweetpotato is complicated by a number of factors:

- Self- and cross-incompatibility
- The highly heterozygous nature of individual clones
- The large number of chromosomes (2n = 6x = 90)

These factors contribute to a low correlation between parent performance and offspring performance. In general the success of a sweetpotato breeding program relies mostly on the ability to grow and evaluate a large number of clones/hybrid progeny in a selective environment that closely resembles the target environment. Thus the development of rapid and reliable screening methods is critical.

Hexaploid Nature of Sweetpotato

Cultivars of sweetpotato are phenotypically homogenous (because they are clonally reproduced) and genetically heterozygous (because they are self-incompatible outcrossers). Sweetpotato is a hexaploid with 2 non-homologous genomes (B1B1B2B2B2B2) with tetradisomic inheritance (Lebot 2010), so the genetics of simple traits is more complex with up to six alleles per locus. Furthermore, because sweetpotato is a hexaploid, heterozygous genotypes occur in much larger

frequencies (Fig. 2) making heterosis more important for quantitatively inherited traits (e.g., yield, yield stability, vigor after planting, etc.).

The hexaploid nature of sweetpotato and the fact that this species is self-incompatible makes it extremely difficult to fix recessively inherited traits like resistance to certain viruses and some quality traits e.g., orange flesh) (Fig. 2) even if the frequency of these recessive alleles is greater than 70%.

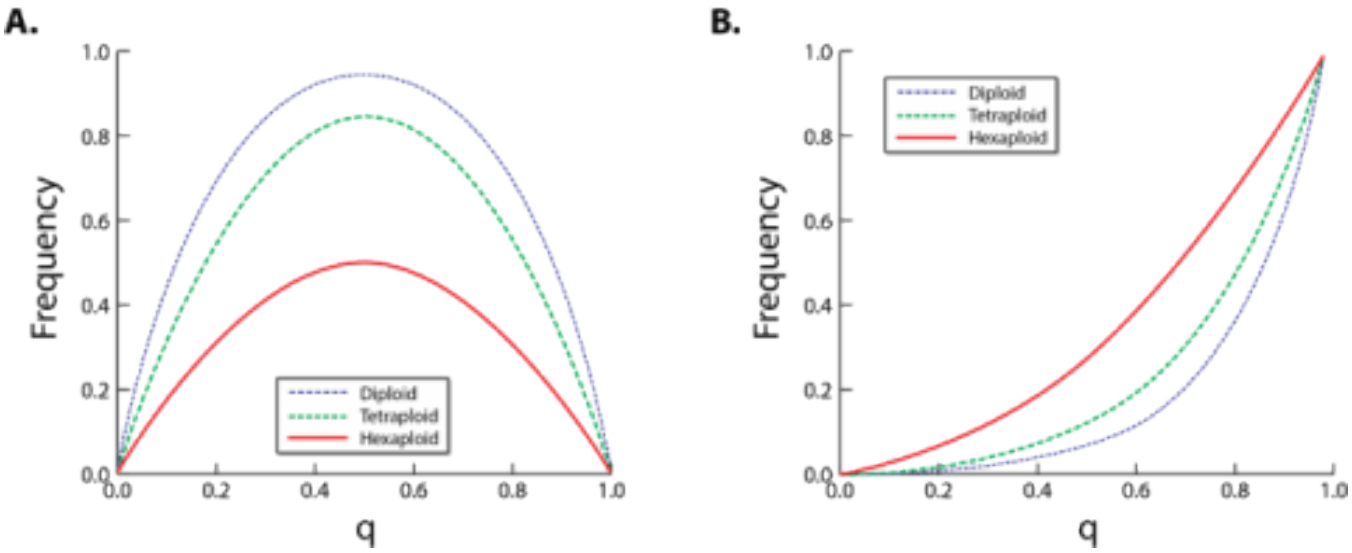

Fig. 2 Effect of ploidy on frequency of genotypic (A) and phenotypic (B) expression of traits (W.J. Grüneberg et. al (2015).

Characteristics of Clonally Propagated Crops

Sweetpotato is an open-pollinated clonally propagated crop. Characteristics common to clonally propagated crops include:

- A strong positive relationship between productivity/vigor and level of heterozygosity
- Selfing reduces vigor due to inbreeding depression
- Vigor/heterozygosity can be fixed and maintained for the life of the clone
- Polyploids/aneuploids can be maintained via clonal propagation
- Often difficult to create a large quantity of clones from one plant, whereas it is relatively easy to produce a large amount of seed from most sexually propagated crops
- Clonal propagules are typically bulky and difficult to store

A basic breeding procedure for a clonal crop like sweetpotato includes the following steps:

1. Define breeding objectives (i.e., yield stability, adaptation, taste, and pest and disease resistance)
2. Assemble germplasm (i.e., local varieties, wild species, cultivars developed in other parts of the world, etc.) and establish a breeding nursery
3. Develop segregating populations via hybridization (i.e., biparental cross, polycross nursery, or diallel crosses) and/or induced mutagenesis
4. Evaluate and select superior clones
    - Plot size, the number of replications, and the number of locations where clones are evaluated is increased after each round of selection in an attempt to reduce the variability due to the environment
    - Select early for traits with high heritability, select later for traits with low heritability
5. Name and release a cultivar and multiply and distribute clones

Basic Breeding Scheme for Sweetpotato

A basic breeding scheme for sweetpotato (Fig. 3) usually involves one (or more) cycles of hybridization to generate genetically variable progeny (i.e., true seeds) that are first evaluated in a seedling nursery. Superior genotypes are then clonally propagated and selection is conducted in replicated trials in multiple environments over multiple years (i.e., observational, preliminary, and advanced yield trials). Superior genotypes that are selected throughout the process are often recycled back into the system and used as parents for the next cycle which is the basis for recurrent selection.

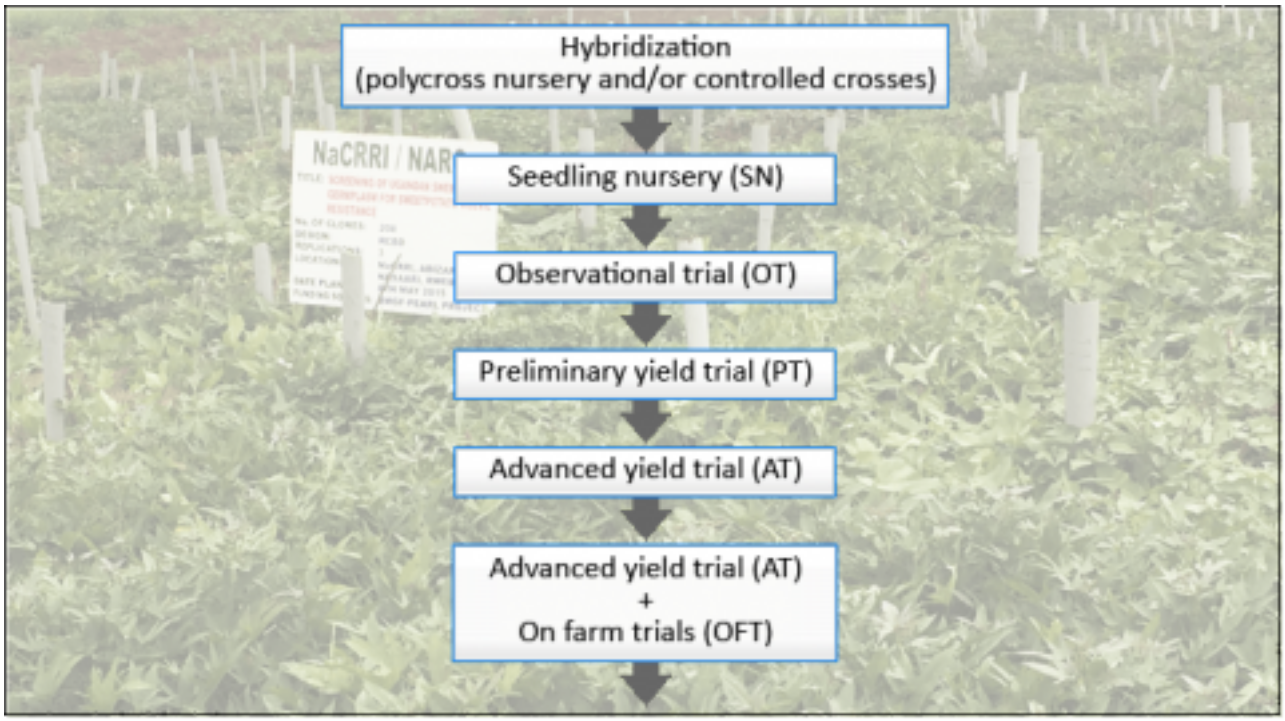

Fig. 3 Basic breeding scheme for sweetpotato. Photo by Iowa State University.

Recurrent Selection

Recurrent selection is a common method for improving a breeding population that captures additive variance and is most effective for traits with moderate to high heritability. The basic scheme consists of three phases:

1. Selection of genotypes and their hybridization in an insect-pollinated polycross nursery or using controlled crosses
2. Evaluation of progeny
3. Selection of superior progeny and the creation of a new polycross nursery or controlled crosses with or without the best parents from the initial polycross nursery in Step 1.

Recurrent selection is a proven method for increasing the frequency of desired alleles and for creating a genetically broad-based population especially when new germplasm (i.e., new parents/genotypes and/or superior progeny from previous cycles) is added to the polycross nursery/controlled crossing block after each cycle.

Recurrent selection allows for the rapid increase in the percentage of minor and recessive alleles, but it requires accurate screening techniques to be successful.

Accelerated Breeding Scheme (ABS)

Population Improvement and Variety Development

Many sweetpotato breeders are now using the Accelerated Breeding Scheme (ABS) for population improvement and for variety development (Table 1, below) (Gruneberg et al. 2009a, Gruneberg et al. 2015). The ABS reduces the total time needed to develop a new variety from 7-8 years to 4 years by compressing the early breeding stages into two years (i.e., 1 year of crosses/seedling multiplication + 3 to 4 years of evaluation in a single location vs. 1 year of crosses/seedling multiplication + only 1 year of evaluation in multiple locations). This method evaluates as many genotypes as possible in the first season of year 2 in 3-4 locations without replication. This allows the breeder to simultaneously select traits in multiple environments in a single year vs. growing clones in one location per year and sequentially selecting clones over several years. This shortens the time needed to develop a variety and conserves resources while still allowing the breeder to estimate the stability of the clones being tested. In sweetpotato, genotype x year variation (i.e., temporal variation) is typically less than genotype x location variation (i.e., spatial variation) (Gruneberg et al. 2004) so the focus is shifted to making selections in more environments in fewer years. For the second season of Year 2 a second replication and more locations are added.

G×E Interactions

High yield and stability in different environments is not well correlated in sweetpotato, suggesting that GXE interactions are important and yield needs to be evaluated under different conditions. For sweetpotato GXE interactions are often larger than or equal to the genetic variation for some traits (Gruneberg et al. 2009b) (Table 2, below); therefore, reducing replication to increase the number of locations can be beneficial. Because replication is eliminated in Season 1 of Year 2 an accurate understanding of the variability in the field is critical to ensure that the early evaluation stages are adequate for distinguishing superior genotypes from poor-performing genotypes. The inclusion of check varieties throughout the field (i.e., alpha lattice

design) is suggested to account for microenvironments in a field that may bias the performance of individual clones.

The efficiency of the ABS can be increased by using a low input or high stress (i.e., drought) environment during Season 1 of Year 2 in addition to 2-3 normal input locations. Selection is conducted sequentially during Season 1 such that genotypes that don't perform well in the low input/high-stress location are eliminated from the normal input locations before selections are made. This procedure is called "independent culling) (Gruneberg et al., 2009a) (Lebot 2010). This allows the breeder to select only genotypes that perform well both under normal and stressful environments in a single season.

In general breeding programs allocate >60% of the budget for replicated trials during the later stages when a few clones are evaluated in advanced yield trials in multiple locations. The accelerated breeding scheme shifts the emphasis of the budget to Year 2 to maximize the number of clones evaluated in multiple environments.

Accelerated Breeding Scheme

Table 1 Accelerated Breeding Scheme for Sweetpotato.

Year	Season	Name of trial	# of clones/ genotypes	Plot type	# of clones/ genotypes	# of locations	# of reps/ location	traits	Notes
1	1	Crossing block	n/a	n/a	n/a	n/a	n/a	n/a	n/a
1	2	Seeding nursery (SN)	2000	single hills (50cm x 50cm)	1	1	1	only highly heritable traits (i.e., disease-resistant, storage root color)	n/a
2	1	Observational trial (OT)	2000	single row plot (1m x 1m)	3-4	3-4	1	harvest index, storage root characteristics (i.e., dry matter content, protein, starch, sugars, provitamin A concentrations)	superior clones are often selected from OTs and used as parents for controlled crosses where the progeny are added to the next seeding nursery
2	2	Preliminary yield trial (PT)	150-300	2 row plot	30 plants (15 plants/ row)	minimum of 3	2	all traits	RCBD, 1 location can be used to create multiple environments if a treatment (i.e., fertilizer, irrigation) is applied

3	1	Advanced yield trait (AT)	40	5 row plot	75 plants (15 plants/ row)	minimum of 4	2	all traits	n/a
3	2	Advanced yield trial (AT) + On-farm trials (OFT)	5-8	5 row plot	75 plants (15 plants/ row)	minimum of 4 plus 10 more on-farm locations (OFT)	n/a	all traits	field design/ plot size determined by the farmer for OFTs
4	1	Multiplication	1-3	1-3 ha fields	n/a	n/a	n/a	n/a	n/a

Estimation of Variance Component

Table 2 Estimation of variance component due to genotypes (σ^2_G). Genotype by environment interactions ($\sigma^2_{G\times E}$) and the plot error σ^2_E from 1146 CIP genebank clones evaluated at three locations: (i) arid irrigated, (ii) humid tropic lowland, and (iii) mineral stress humid tropic lowland with two plot replications per site (95% confidence limits of parameter estimates in brackets).

n/a	**Genotype** σ^2_G	**Genotype by environment** $\sigma^2_{G\times E}$	**Error** σ^2_E	**Ratio** $\sigma^2_G : \sigma^2_{G\times E} : \sigma^2_E$
Yield (t^2/ha)	36.2 (20.6 – 43.6)	39.4 (33.8 – 46.9)	64.2 (60.0 – 68.9)	1 : 1.1 : 1.8
Dry matter ($\%^2$)	14.8 (13.3 – 16.6)	5.7 (5.0 – 6.5)	5.7 (5.3 – 6.1)	1 : 0.4 : 0.4
Protein ($\%^2$ DM^+)	0.21 (0.16 – 0.30)	0.67 (0.59 – 0.78)	0.73 (0.68 – 0.79)	1 : 3.2 : 3.5
Starch ($\%^2$ DM^+)	21.5 (19.3 – 24.2)	3.2 (2.3 – 4.9)	16.3 (15.2 – 17.5)	1 : 0.2: 0.8
Sucrose ($\%^2$ DM^+)	5.6 (4.9-6.5)	2.1 (1.6 – 2.8)	7.4 (6.9 – 7.9)	1 : 0.4 : 1.3
Total sugar (%DM^+)	17.0 (15.2 – 19.2)	6.0 (5.2 – 7.1)	9.0 (8.4-9.7)	1 : 0.4 : 0.5
Carotene (ppm^2 DM^+)	6327 (5681-7091)	2462 (2224 – 2740)	1421 (1323 – 1529)	1 : 0.4 : 0.2
Calcium (ppm^2 DM^+)	74001 (61485-90791)	95.990 (82657 – 112849)	157303 (147021-168708)	1 : 1.3 : 2.1
Magnesium (ppm^2 DM^+)	143005 (12351 – 16764)	9880 (8360 – 11858)	17638 (16451 – 18960)	1 : 0.05 : 0.1
Iron (ppm^2 DM^+)	2.33 (1.92 -2.87)	3.46 (3.0 – 3.97)	3.85 (3.59 – 3.97)	1 : 1.7 : 2.2
Zinc (ppm^2 DM^+)	0.8 (0.62- 0.97)	1.37 (1.20 – 1.59)	1.72 (1.60- 1.85)	1 : 1.7 : 2.2

Establish a Crossing Block

YEAR 1 (SEASON 1) – ESTABLISH A CROSSING BLOCK AND/OR MAKE CONTROLLED CROSSES AND HARVEST TRUE (HYBRID) SEED

Sweetpotato breeding has traditionally involved making crosses between complementary parents using controlled crosses or a polycross nursery with multiple parents.

The major goal of a crossing block or controlled crosses is the improvement of the overall population mean from one cycle to the next.

The ability to generate genetic variation is easy in sweetpotato due to the high heterozygosity of individual clones/parents.

The creation of genetic variation via hybridization is easy in sweetpotato because this species is generally self-incompatible so seeds do not develop from self-fertilization.

If a breeder has limited information about the value/usefulness of individual crosses (i.e., from prior test crosses or data from previous years) then the goal should be to maximize the number of parental combinations.

A minimum of 15 parents should be used in a polycross nursery or for controlled crosses. Most breeding programs use 20-30 parents – though larger breeding programs (e.g., CIP) are now using 150 or more different parents that are separated into different gene pools (i.e., heterotic groups).

YEAR 1 (SEASON 2) – SEEDLING NURSERY (SN)

Hybrid seed from the crossing block or controlled crosses is harvested and planted in 0.5 x 0.5 m plots (depends on quantity of seed available and the size of the breeding program). Selection during this step is usually limited to natural selection for tolerance and resistance to pathogens and pests. Artificial selection by breeders is typically avoided during the "true seed plant stage" because seed plants often grow differently than plants grown from vegetative cuttings and because this stage is often grown in an artificial environment (e.g., shade or glass house in pots) that is not representative of the field environment where the clones will eventually be grown. Stem cuttings are harvested after 10 weeks.

YEAR 2 (SEASON 1) – OBSERVATIONAL TRIAL (OT)/HILL TRIAL (HT)

Hybrid seed from the crossing block or controlled crosses is harvested and planted in 0.5 x 0.5 m plots (depends on quantity of seed available, size of breeding program). Selection during this step is usually limited to natural selection for tolerance and resistance to pathogens and pests. Artificial selection by breeders is typically avoided during the "true seed plant stage" because seed plants often grow differently than plants grown from vegetative cuttings and because this stage is often grown in an artificial environment (e.g., shade or glass house in pots) that is not representative of the field environment where the clones will eventually be grown. Stem cuttings are harvested after 10 weeks.

YEAR 2 (SEASON 2) – PRELIMINARY YIELD TRIAL (PT)

Larger 2 row plots (30 plants, 15 plants/row) are planted at a minimum of 3 locations. A second rep is added at each location. Selection for lower heritability traits (e.g., yield, biomass) begins at this stage. A breeder can begin to assess yield stability at this step but this requires 3 or more environments with the best results achieved when more than 6 environments are included (Gruneberg et al. 2009a).

YEAR 3 (SEASON 1) – ADVANCED YIELD TRIAL (AT)

Larger 5 row plots (75 plants, 15 plants/row) are planted at a minimum of 4 locations. Selection for lower heritability traits (e.g., yield, biomass) continues.

YEAR 3 (SEASON 2) – ADVANCED YIELD TRIAL (AT) + ON FARM TRIALS (OFT)

On farm trials are added where the field design, plot size, and inputs are determined by the individual farmers. Farmers are asked to rate the clones on a common set 5-6 traits plus any additional traits that are important to them.

YEAR 4 (SEASON 1 AND 2) – MULTIPLICATION AND DISTRIBUTION OF VARIETIES

Superior genotypes are multiplied and distributed to farmers

Trial Conditions

A breeder is strongly encouraged to plant check varieties, guard rows, and spreader/susceptible rows and to use an appropriate field design for each location. It is best to harvest cuttings from plants that are ten weeks old, so it is helpful to plant a multiplication plot for each clone about 2.5 months before a trial is planted. These plots should be maintained separately and managed (e.g., weeded, fertilized, irrigated) to promote vegetative growth. A single vine cutting usually produces five vine cuttings. Storage root sprouts can also be used (i.e., an average-sized storage root yields about 20 vine cuttings). A breeder should use tip cuttings of the same length and vigor when possible. If uniform cuttings are not available divide the cuttings up by replication (i.e., best in rep 1, second-best in rep 2, etc.). All field management (e.g., weeding, fertilizer, irrigation, etc.) should be applied uniformly across all reps. If possible each task should be divided so each worker completes an entire rep. For example, if planting an entire field takes 3 people, 1 worker should plant rep 1, a second worker would plant rep 2, and a third worker would plant rep 3, so differences in planting technique can be partitioned out as a rep effect when the data is analyzed. The same procedure should be applied for data/note-taking. If multiple people are making evaluations each individual should be assigned to evaluate specific reps and workers should not collect data for others.

It is nearly impossible to simultaneously select the top genotypes for every trait without growing out an impracticably large population because the number of genotypes to be screened increases exponentially as the number of traits increases. For example, if a breeder wanted to select the top 10 clones from among 100 genotypes for 10 traits then 100^10 genotypes need to be grown and evaluated. Thus a breeder usually has to compromise and select 3-5 quality traits at a time while simultaneously maintaining sufficient genetic variation for yield, yield stability, and adaptability.

ABS – Mulitple Concurrent Cycles

Breeders will usually have 3-5 different cycles of an ABS at various stages (SN, HT/OT, PT, AT)

running at the same time (see Fig. 4). This allows a breeder to use advanced clones from later stages of one cycle to create new polycross nurseries/controlled crosses.

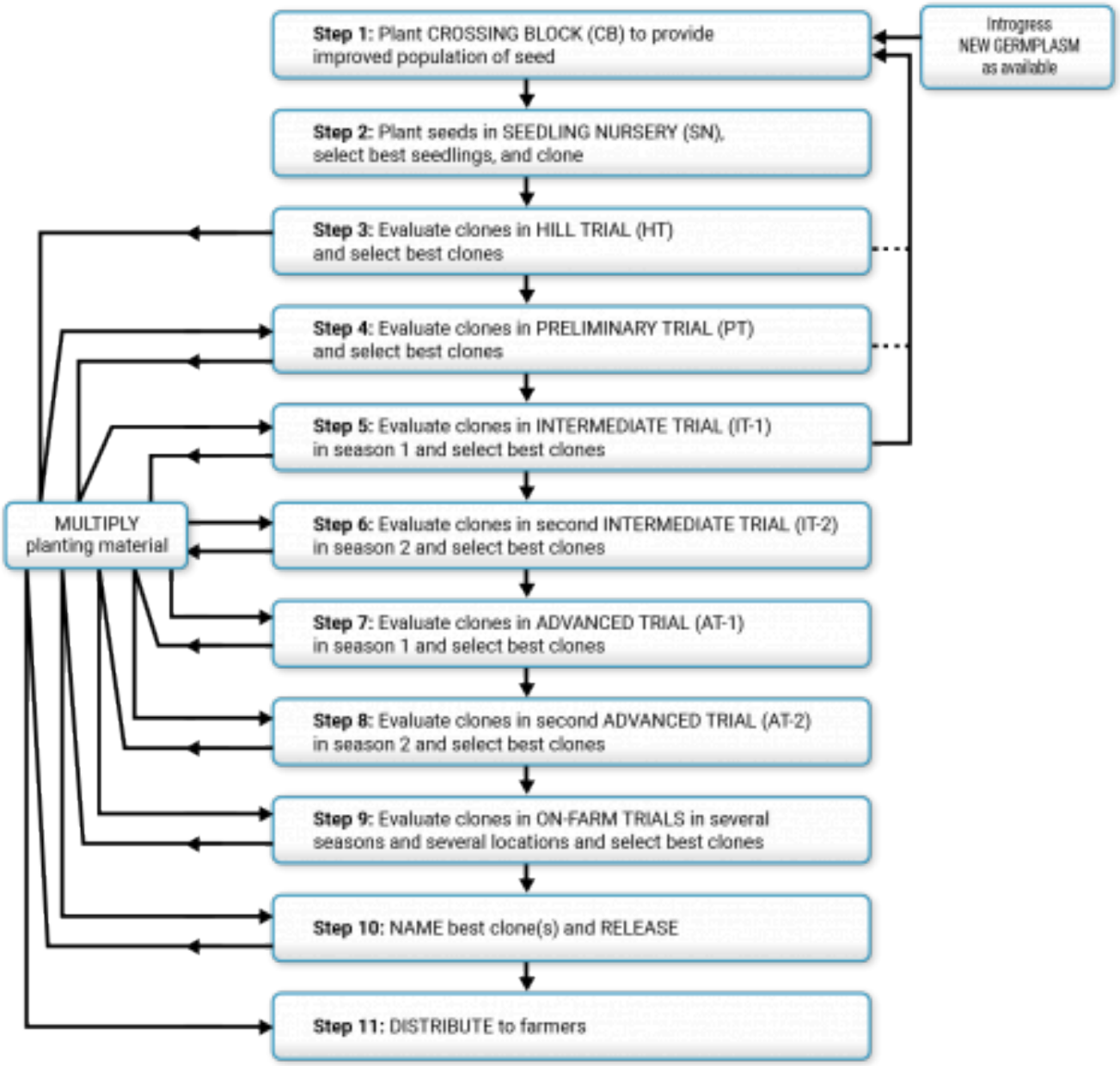

Fig. 4 Multiple concurrently run cycles of recurrent selection. Click the image to see a larger version.

Sources of Parents/Germplasm

Institutions that maintain and distribute Ipomoea germplasm include: CIP (Centro Internacional de la Papa) (> 4,200 accessions), USDA/ARS National Genetic Resources

Information Network (>900 accessions), NaCRRI National Crops Resources Research Namulonge/Uganda, and IITA (International Institute of Tropical Agriculture), in Nigeria.

Though some interspecific hybrid combinations can be made especially using species within the *Batatas* section, wild species are typically not used in most breeding programs.

Polycross Nursery vs. Controlled Crosses

Ensuring Specific Cross Combinations

Often a combination of open-pollination (i.e., polycross nursery) and hand-pollination (i.e., controlled crosses) is used to ensure that specific cross combinations are made. Some of the advantages and disadvantages of using a polycross nursery vs. controlled crosses are shown in Table 3.

Table 3 Advantages and disadvantages of using an insect-pollinated polycross nursery vs. controlled crosses to generate progeny.

Polycross nursery		Controlled crosses	
Advantages	**Disadvantages**	**Advantages**	**Disadvantages**
Requires less labor	Only the female parent is known so genetic advancement is based on only ½ of the genetic variation that is available	Both parents are known so genetic advancement is based on all of the genetic variation that is available	Requires more labor to make crosses by hand
Makes more seed/more cross combinations	Unbalanced contribution from some clones (poor seed set, low pollen production, limited and/or asynchronous flowering)	Superior combinations are tracked and can be recreated	Less seed/less combination are created

Polycross nurseries are theoretically less efficient because genetic advancement is halved because parental control is based only on the female parent while parental control for controlled crosses is based on a control factor of 1 because both parents are known.

$G_S = (c)(i)V_A / \sigma_P$

G_S is the expected gain or predicted genetic advance from selection

c = parental control factor, ½ if only the female parent is known,1 if both parents are known

i = the selection intensity, a constant based on the percent selected and obtained from statistical tables

V_A = additive genetic variance

σ_P= phenotypic standard deviation

Note: the phenotypic values must exhibit a normal, or bell-curve, distribution for G_S to be valid.

Using Different Methods

To better understand the impact of using different methods to generate progeny, CIP breeders and collaborators compared the population means (i.e., unselected and after one cycle of selection) of sweetpotato progeny created by 3 different pollination designs (I, II, and III) to determine which method of generating progeny produced the best results. For this experiment the breeders used the same 22 clones but crossed them in different ways: (I) using an open-pollinated polycross nursery, (II) using a partial diallel design where 4 of the best clones were crossed by hand to each other and to the rest of the clones (4 x 22), and (III) using a factorial design where the best 5 clones were crossed by hand to rest of the remaining clones (5 x 17) ("the best by the rest"). The progeny of each of these designs were planted in unreplicated plots in a single location and the population mean was determined for each = average performance of the unselected population. The breeders then selected the best 100 genotypes from each population and averaged their performance as a measure of the achieved increase in root yield after one cycle of selection. The standardized response to selection (R) was then calculated for each method by comparing the unselected mean to the mean of the 100 selected genotypes. The results for this experiment showed that the average root yield of the progeny created by method (II) (18.4 t/ha) and the average root yield of the 100 selected genotypes (23.5 t/ha) after one round of selection were both higher than the unselected and selected progeny generated by the other methods (I and III) (Table 4). These results indicated that for this scenario, controlled crosses made by a breeder guided by a partial diallel design (II) produced better progeny and more genetic advancement per cycle than an open-pollinated polycross nursery (I). The factorial design (III) was the least successful method though it only differed from the partial diallel design in that it did not include progeny from the intermating of the top 5 clones. This suggests

that much of the success of the partial diallel design may be attributed to the successful performance of the progeny created by crossing the top 5 clones.

Case Study: Comparing the Use of a Polycross Nursery vs. Controlled Crosses

Considering Other Factors

Table 4 Results from an experiment comparing progeny and genetic advancement made from progeny created using 3 different pollination designs.

Design	Pollination design	Population mean of progeny before selection (t/ha)	Population mean of progeny before selection (t/ha)	Standardized R
I	Open pollinated polycross nursery (22)	Open-pollinated polycross nursery (22)	21.2	1.35
II	Partial diallel (4 X 22)	Partial diallel (4 X 22)	23.5	0.904
III	Factorial (5 X 17)	Factorial (5 X 17)	17.4	0.715

Despite the gains made using controlled crosses vs. a polycross nursery in this particular scenario a breeder must also consider other factors as well when deciding which method to use for their program. For example, not all programs have enough staff to make all of the hand pollinations necessary for a partial diallel design so a polycross nursery may be a better option. If a breeder does choose to use an insect-pollinated polycross nursery it is important that all clones be equally represented so experimental design and replication are crucial. If a polycross nursery contains ten or fewer clones a Latin square design is recommended (Table 5). For a large number of clones a randomized complete block design with replication is recommended (Table 5).

Hybridization Using a Polycross Nursery

Table 5 Recommended planting designs for polycross nurseries to ensure equal contribution from all parents.

Latin square – n × n array, each genotype occurs exactly once in each row and exactly once in each column						Randomized complete block design					
A	B	F	C	E	D	A	B	C	C	A	E
B	C	A	D	F	E	D	E	F	B	H	F
C	D	B	E	A	F	G	H	I	D	I	G
D	E	C	F	B	A	F	A	G	I	A	B
E	F	D	A	C	B	B	D	H	G	H	F
F	A	E	B	D	C	E	C	I	D	C	E

- Planted in isolation
- Natural insect cross-pollination & artificial crosses to ensure random pollination
- Plant extra reps of less vigorous clones or clones that don't flower well
- Stagger planting times so that all clones flower at the same time

Crossing Block/Controlled Crosses

- Flowering is best under short day conditions and begins about 1.5 months after the crossing block is planted/established and continues for ~3-5 months
- It is advisable to plant extra replications of clones that do not flower well
- Clones that don't flower well can also be grafted onto *I. setosa*, or *I. nil*, or on a genotype that flowers prolifically to promote flowering
- Clones that flower later than the other clones should be planted earlier to ensure that all clones have an equal chance of contributing to the gene pool of the progeny
- Seed harvest begins about 2 months after the crossing block is established and continues for up to 4 months
- Plant 2 vines per 1×1 m plot, 5 randomized plots of each clone
- Stake/trellis plants and erect a windbreak if needed to protect the crossing block from

high winds
- Label each stake with the clone number to make identification easier
- Avoid using a high N fertilizer to promote flowering
- Monitor plants for insect and disease problems, but avoid using pesticides that may injure bees, and other pollinating insects
- Most clones of sweetpotato are self-incompatible and do not produce selfed seed
- Each flower opens early in the day just after sunrise and lasts for only a few hours before fading around noon
- Each pistil contains 1 superior ovary with two carpels and each carpel has two locules that contain 1 or 2 ovules, so a single capsule can produce 1 to 4 seeds
- Flowers that are hand-pollinated usually produce 1 or 2 seeds and capsules that are open-pollinated produce 2 to 3 seeds.

Seed Harvest

Seeds mature 4 to 6 weeks after pollination. The capsule and pedicel will both turn brown and dry and begin to shrivel when the seed is ready to harvest. Capsules that are left too long will dehisce (split open) so care must be taken to harvest seeds before they are lost.

Seed Scarification

Seeds can be hand-scarified by scratching a small notch in each seed coat with a sharp needle or a small, 3-cornered file or acid scarified with concentrated sulfuric acid for 40 minutes followed by a 5-10 minute rinse under running water.

Polycross Nursery vs. Controlled Crosses

Cross Incompatibility

Self-fertilization is rare because sweetpotato possesses a homomorphic, sporophytic type of self-incompatibility that is not affected by environment, chemical treatment, and cannot be overcome using bud pollination. This system is likely controlled by a single S-locus with

multiple alleles with a dominant-recessive relationship. Heterostyly also occurs in sweetpotato; however, it does not appear to affect fertility.

Cross incompatibility among different varieties can limit recombination and seed production and hinders targeted breeding especially when the parents with desirable traits of interest, such as disease resistance, drought tolerance, enhanced levels of protein, vitamins, macro- and micro-nutrients and dry matter are closely related and belong to the same incompatibility group. As a result breeders must maintain large populations that contain non-related accessions with complementary traits. Three types of cross compatibility (Table 6) exist depending on the success or failure of reciprocal crosses: reciprocal fertility occurs when fertility is present in both directions, reciprocal incompatibility occurs when incompatibility occurs in both directions, and unilateral fertility/incompatibility occurs when fertility occurs only when a genotype is used as the female but not when used as a male and vice versa.

Table 6 Types of cross-compatibilities

Type of cross-compatibility	**Expected outcome; A and B are different genotypes**
Reciprocal fertility	A x B and B x A both produce seed
Reciprocal incompatibility	A x B and B x A both do not produce seeds
Unilateral fertility/incompatibility	A x B produces seed but B x A does not OR B X A produces seed but A x B does not

Incompatibility and Sterility

Incompatibility and sterility are often used interchangeably although this is done incorrectly. Sterility is the failure of reproduction due to the failure of a plant to produce viable gametes and incompatibility is the failure of viable gametes to fertilize one another. Sterility or reduced fertility in sweetpotato is not uncommon as aneuploidy due to multivalent formation among non-homologous chromosome pairs can often lead to an unbalanced number of chromosomes in the gametes. Sterility is also caused by gene action.

Participatory Plant Breeding (PPB)

Women are responsible for most of the labor when it comes to growing sweetpotato. Although

men are usually involved in land preparation, especially when land needs to be cleared or soils are heavy, and in marketing, particularly where sweetpotato is a significant cash crop. Men also significantly contribute to weeding and harvesting, particularly when sweetpotato is intercropped.

Traits that are important to farmers (e.g., piecemeal harvest for subsistence farming and resistance to regionally important abiotic and biotic stresses, etc.) are difficult to select for outside of the target environment(s), therefore, it is helpful to cooperate with local farmers/growers to gain access to additional testing locations and to gain insight into farmer/grower preferences. Participatory plant breeding practices are beneficial especially during the earlier steps when genetic diversity is high and most traits (e.g., quality characteristics, disease resistance, plant architecture, etc.) are visually evaluated. PPB practices are less useful at the later stages of selection because those traits that are usually selected based on visual evaluations are typically highly heritable and are already fixed in the population. The involvement of farmers and consumers also helps facilitate the rapid adoption of new varieties and lessens the chance that superior varieties are rejected because they fail to meet the expectations of the farmers. Regional and local preferences for flesh color, dry matter content, and texture, as well as adaptability to the local environment are critical traits in sweetpotato and should be considered during the early stages of selection.

CIP's Convergent-Divergent Scheme

CIP breeders are currently using a convergent-divergent approach that is designed to meet the regional needs of growers by developing widely adapted cultivars and promoting collaboration among breeders and consumers (Lebot 2010). Due to the difficulty of breeding for adaptation across multiple agro-ecological zones in Africa a program must be conducted in a decentralized way. Therefore programs in different countries can develop cultivars that work best for their region. This method starts with a diverse base population composed of genotypes from a wide range of sources. This base population is evaluated in a single centralized location and superior genotypes are intercrossed. Seed from this cycle is collected and then sent to collaborators in different regions where it is evaluated and intercrossed with elite germplasm that is adapted for each individual location. This scheme introduces new germplasm while also including elite germplasm carrying alleles for traits that are specific to a region (e.g., disease and pest resistance endemic to that location, a particular taste profile, plant architecture that fits a cultivation technique used in that region, etc.). By allowing breeders to introduce alleles specific

to their location and then test the progeny locally GXE interactions are better accounted for. Selection in the centralized location is focused on maintaining high genetic diversity while also introducing desired traits, while selection at the local level focuses more on selecting varieties that will grow well in a specific environment.

Exploiting Heterosis

Creating Distinct Germplasm Pools

Exploiting Heterosis Using 2 Heterotic Gene Pools

Breeders are now exploring the idea of exploiting heterosis in sweetpotato by creating two genetically distinct germplasm pools and selecting and recombining within each pool based on the general combining ability (GCA) of clones that are crossed with the other germplasm pool. Work at the international Potato Center (CIP) is focused on creating high yielding orange-fleshed sweetpotato varieties using this approach. For this experiment, two populations were created: the Jewel population and the Zapallo population. The Jewel population consists of orange-fleshed clones released prior to 2004 and includes mostly North American varieties including Jewel and Resisto. The Zapallo population was created using a factorial crossing design using 8 male parents (Jonathan, Zapallo, Huambachero, Tanzania, Yurimaguas, Wagabolige, Xushu18, and Ninshu1) and 200 orange fleshed females. More than 200 families were then produced from cross combinations involving 49 clones from the Jewel population crossed with 31 clones from the Zapallo population (Fig. 5). Mid-parent (MP) heterosis values for dry root yield were generally low to moderate, but some individual cross combinations/families had MP heterosis values. Root yield of the best clone within each family was typically double the average root yield of the parents, with some clones producing 5 times the average yield of the parents, thus indicating success of the approach.

Crossing Scheme and Heterosis Values

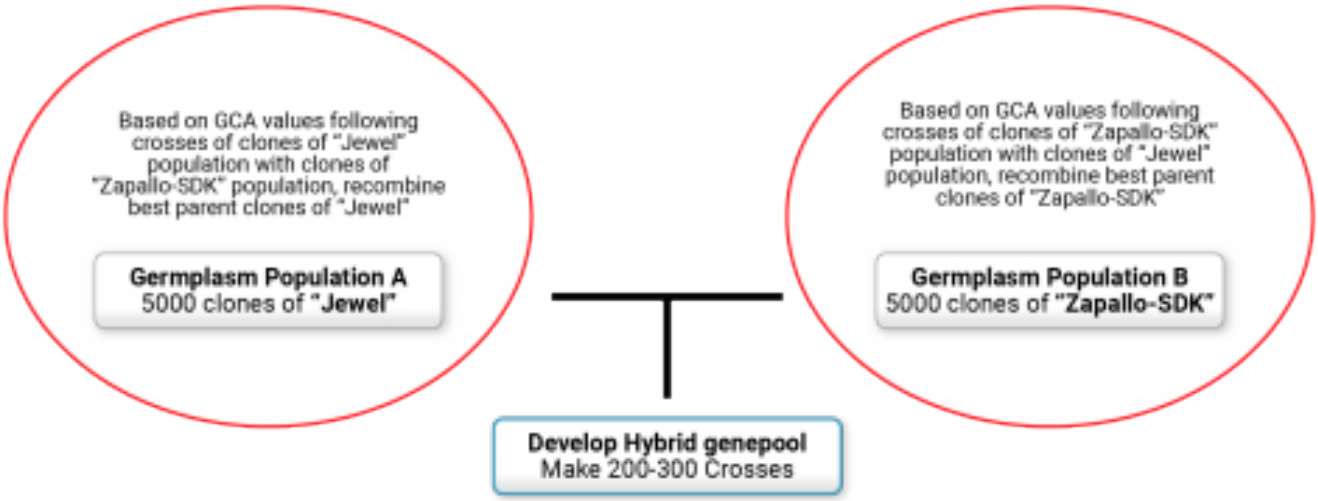

Fig. 5 Crossing scheme used to create families from crosses between the two germplasm pools.

Choosing Parents

Example of Parent Selection

Choosing Parents for a Crossing Block/Controlled Crosses

A breeder will often use morphological data, coancestry/pedigree information, and molecular marker data to choose parental combinations. By using a combination of analyses a breeder can select parents that are phenotypically superior, and genetically distant from one another to limit inbreeding depression and possible cross-compatibility issues. Molecular markers are also useful for identifying duplicate clones or instances when genetically different clones are distributed under the same name.

Example of Parent Selection Based on Molecular Markers vs. Morphological Markers

Clones were characterized using both molecular markers and agronomic characters to identify phenotypically superior yet distantly related clones (Dai-fu et al. 2009). Fifteen clones (Table 7)

and 60 open-pollinated offspring per clone were planted in a common garden and phenotyped for 22 traits. The 15 parental clones were genotyped with ISSR and RAPD markers. The dendrograms (Figs. 6 and 7) based on the morphological traits and the molecular markers are shown below along with Table 8, which summarizes the different groupings based on the morphological and molecular marker data.

Trial Materials and Parental Dendrograms

Choosing Parents for a Crossing Block/Controlled Crosses

Table 7 The list of the trial materials.

Code	Variety	Female[1)]	Male	Source or distribution location
1	Centennial	Unit 1 Porto Rico	Pelican Processor	Louisiana State University, USA
2	Beijing 553	Okinawa100 OP	The Experimental Station of Huabei, China	n/a
3	Beauregard	L78-21 OP	Louisianna State University, USA	n/a
4	Chaun 8129-4	Jiangjin Wujianshao	Neiyuan	The Crop Institute, Sichuan Academy of Agricultural Sciences, China
5	Guangshu 88-70	Zhan75-57 OP	The Crop Institute, Guangdong Academy of Agricultural Sciences, China	n/a
6	Jianshui Huangxin	Landrace	Jianshui County, Yunnan Province, China	n/a
7	Longshju 1	Yanfen 1	Longyan 7-3	Agricultural Science Institute of Longyan, Fuijian, China
8	Sushu 6	Peng S1-12-48	AIS 0122-2	Xuzhou Sweetpotato Research Centre, Jiangsu
9	Tainung 69	Group crossing	Jiayi Agricultural Experimental Station, Taiwan of China	n/a
10	Xiangshu 15	Kyushu 5	Xiangshu 6	The Crop Institute, Hunan Academy of Agricultural Sciences, China

11	Xu 22-5	LO323	AIS 0122-2	Xuzhou Sweetpotato Research Centre, Jiangsu, China
12	Xu 34	Huabei 166	Qunli 2	Xuzhou Sweetpotato Research Centre, Jiangsu, China
13	Xushu 23	P616-23	Yanshu 27	Xuzhou Sweetpotato Research Centre, Jiangsu, China
14	Yanshu 27	77183	Centennial	Agricultural Science Institute of Yantai, Shandong, China
15	Yanshu 5	Yanchi Red	Yan 94-1	Agricultural Science Institute

[1)] OP, open pollination

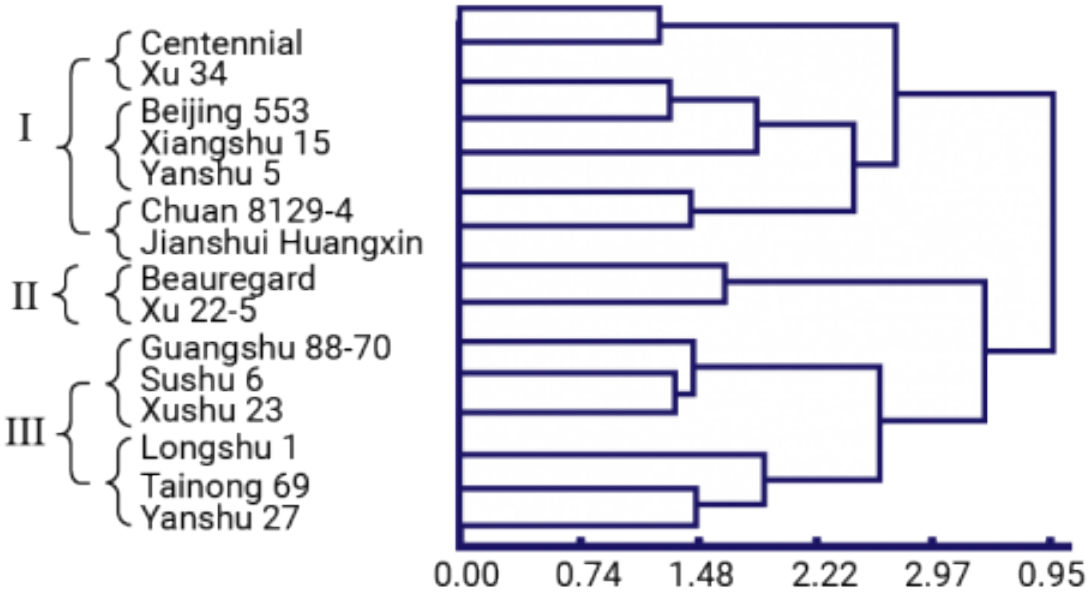

Fig. 6 The dendrogram of the parents based on 22 morphological traits. Dai-fu et al. 2009.

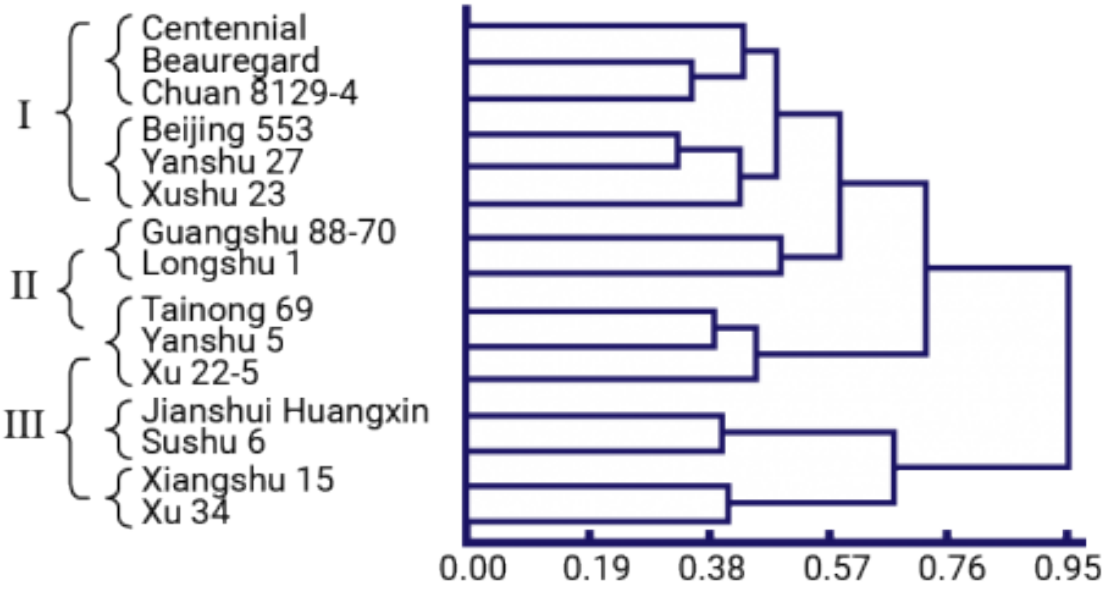

Fig. 7 The dendrogram of the parents based on RAPD and ISSR marker analysis. Dai-fu et al. 2009.

Summary of Groups of Clones

Choosing Parents for a Crossing Block/Controlled Crosses

Table 8 A summary of the groups of clones based on their phenotype and genotype.

Clone	Phenotype Group	Genotype Group	
Centennial	1	1	} Choose 1 from this group of 3
Beijing 553	1	1	
Chuan 8129-4	1	1	
Yanshu 5	1	2	← Choose this one
Xu 34	1	3	} Choose 1 from this group of 3
Xiangshu 15	1	3	
Jianshui Huangxin	1	3	
Baeuregard	2	1	← Choose this 1
Xu 22-5	2	2	← Choose this 1
Xushu 23	3	1	} Choose 1 from this group of 4
Yanshu 27	3	1	
Guangshu 88-70	3	1	
Longshu 1	3	1	
Tainong 69	3	2	← Choose this 1
Sushu 6	3	3	← Choose this 1

A breeder can use this information to select clones that are phenotypically similar (i.e., same phenotype group) but genetically different (i.e., different genotype group) thereby reducing the number of parental clones from 15 to 8 without significantly reducing the genetic variation that is available.

Combining Ability of Individual Clones

Choosing Parents for a Crossing Block/Controlled Crosses

When considering which parents to choose for a polycross nursery or controlled crosses it is also important to consider the combining ability of individual clones. The combining ability of a clone can be estimated by comparing the performance of a clone versus the average performance of open-pollinated progeny harvested from that clone when it is grown in a polycross nursery or other population of mixed genotypes (i.e., seedling nursery, yield trial, etc.), for example Figure 10. If the average performance of the progeny is greater than the performance of the maternal clone then a breeder can assume that a particular clone has good combining ability when crossed with other clones.

Parent Selection

Important to choose parents that possess superior qualities and parents that combine with other clones (superior GCA)

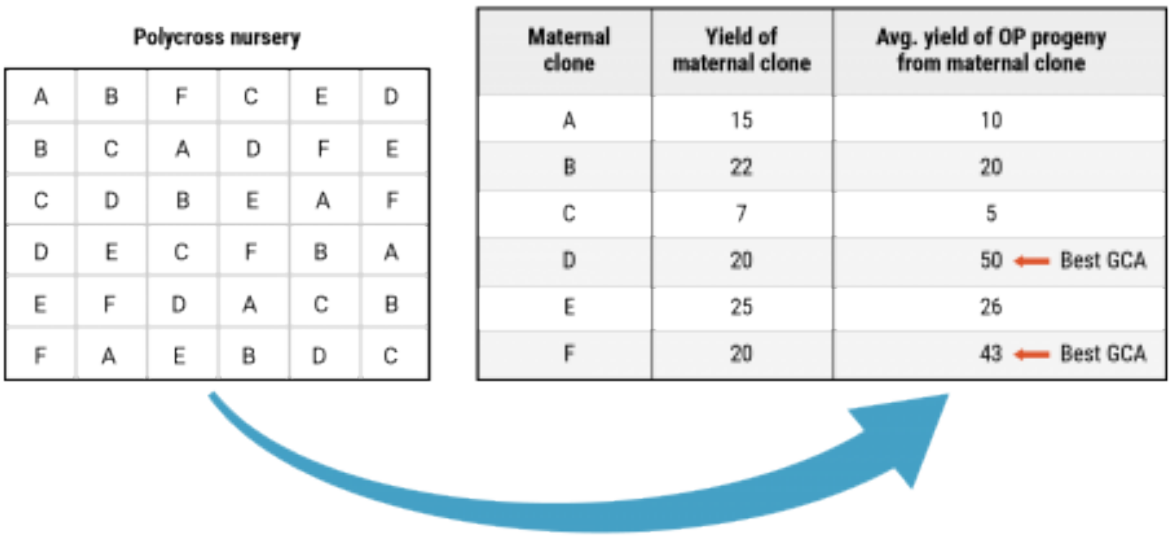

Fig. 8 Maternal clone performance for identification of best GCA parent.

References

Austin, D.F. 1987. The taxonomy, evolution and genetic diversity of sweet potatoes and related wild species. In: Exploration, Maintenance, Utilization of Sweet Potato Genetic Resources. Proceedings of the 1st Planning Conference, Lima, Peru, International Potato Center (CIP), pp. 2759.

Dai-fu et al. 2009. Selection of parents for breeding edible varieties of sweetpotato with high carotene content. Agricultural Sciences in China 2009, 8(10): 1166-1173.

Fajardo D.D., D.R. La Bonte, and R.L. Jarret. 2002. Identifying and selecting genetic diversity in Papua New Guinea sweet potato Ipomoea batatas (L.). Lam. germplasm collected as botanical seed. Genet. Resour. Crop Evol. 49: 463-470.

Gallais, A. 2003. Quantitative genetics and selection theory in autopolyploid plants. France, INRA.

Gibson, R. W., and J. F. Kreuze. "Degeneration in sweetpotato due to viruses, virus-cleaned planting material and reversion: a review." Plant Pathology 64, no. 1 (2015): 1-15.

Grüneberg, W.J., E. Abidin, P. Ndolo, C.A. Pereira, and M. Hermann. 2004. Variance component estimations and allocation of resources for breeding sweet potato under East African conditions. Plant Breeding 123: 311-315.

Grüneberg, W.J., R. Mwanga, M. Andrade, and J. Espinoza. 2009a. Selection methods Part 5: Breeding clonally propagated crops. In: Plant Breeding and Farmer Participation (S. Ceccarelli, E.P. Guimarães, and E. Weltzien, eds.). FAO, Rome, pp. 275-322.

Grüneberg, W.J., Mwanga, R., Andrade, M. and Dapaah, H. 2009b. Sweetpotato breeding. In: Andrade, M.,
Barker, I., Cole, D., Dapaah, H., Elliott, H., Fuentes, S., Grüneberg, W.J., Kapinga, K., Kroschel, J.,
Labarta, R., Lemaga, B., Loechl, C., Low, J., Lynam, J., Mwanga, R., Ortiz, O., Oswald, A. and Thiele, G. Unleashing the Potential of Sweetpotato in Sub-Saharan Africa: Current Challenges and
Way Forward. Working Paper 2009-1. International Potato Center (CIP), Lima, pp. 1–42.

Grüneberg, W.J.; Ma, D.; Mwanga, R.O.M.; Carey, E.E.; Huamani, K.; Diaz, F.; Eyzaguirre,

R.; Guaf, E.; Jusuf, M.; Karuniawan, A.; Tjintokohadi, K.; Song, Y.S.; Anil, S.R.; Hossain, M.; Rahaman, E.; Attaluri, S.I.; Some, K.; Afuape, S.O.; Adofo, K.; Lukonge, E.; Karanja, L.; Ndirigwe, J.; Ssemakula, G.; Agili, S.; Randrianaivoarinovy, J.M.; Chiona, M.; Chipungu, F.; Laurie, S.M.; Ricardo, J.; Andrade, M.; Rausch Fernandes, F.; Mello, A.S.; Khan, M.A.; Labonte, D.R.; Yencho, G.C. 2015. Advances in sweetpotato breeding from 1992 to 2012. *In*: Low, J.; Nyongesa, M.; Quinn, S.; Parker, M. (eds). Potato and sweetpotato in Africa. Transforming the value chains for food and nutrition security. Oxfordshire (UK). CABI International. ISBN 978-1-78064-420-2. pp. 3-68.

Huang, J. C., and Sun, M. 2000. Genetic diversity and relationships of sweetpotato and its wild relatives in Ipomoea series batatas (Convolvulaceae) as revealed by inter-simple sequence repeat (ISSR) and restriction analysis of chloroplast DNA. Theor. Appl. Genet. 100:10501060.

Lebot, V. 2010. Sweetpotato. In J.E. Bradshaw (ed.) Root and tuber crops. Vol 7. Springer Science & Business Media.

Low, J., J. Lynam, B. Lemaga, C. Crissman, I. Barker, G. Thiele, S. Namanda, C. Wheatley, and M. Andrade. "Sweetpotato in Sub-Saharan Africa." In The sweetpotato, pp. 359-390. Springer Netherlands, 2009.

Mwanga, R., G.C. Yencho, R.W. Gibson, and J.W. Moyer. 2013. Methodology for inoculating sweetpotato virus disease: discovery of tip dieback, recovery and reversion in different clones. Plant Disease 97, 306.

Roullier, C, A. Duputie, P. Wennekes, L. Benoit, Fernandez Bringas VM, et al. 2013. Disentangling the Origins of Cultivated Sweet Potato (*Ipomoea batatas* (L.) Lam.). PLoS ONE 8(5): e62707. doi:10.1371/journal.pone.0062707

Yada, Benard, Phinehas Tukamuhabwa, Bramwell Wanjala, Dong-Jin Kim, Robert A. Skilton, Agnes Alajo, and Robert OM Mwanga. "Characterization of Ugandan sweetpotato germplasm using fluorescent labeled simple sequence repeat markers." HortScience 45, no. 2 (2010): 225-230.

Zhang, D., J. Cervantes, E. Huaman, E. Carey, and M. Ghislain. 2000. Assessing genetic diversity of sweet potato (*Ipomoesa batatas* (L.) Lam.) cultivars from tropical America using AFLP. Genet. Resour. Crop Evol. 47:659665.

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Chapter 14: Groundnut Breeding

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Groundnut or peanut (*Arachis hypogaea* L. Millsp) is a self-pollinated species belonging to the Fabaceae family. It is considered as the most important food legume crop in continental Africa because of its multiple purpose uses in food, feed, paints, lubricants and insecticides as the seeds are comprised of 35–56% oil, 25–30% protein, 9.5–19.0% carbohydrates, several minerals like P, Ca, Mg and K, as well as vitamins E, K and B (Gulluoglu et al. 2016).The crop has various industrial uses including products such as food, feed, paints, lubricants and insecticides. Because of its natural ability to fix atmospheric nitrogen in the soil, it is considered an ideal crop in crop rotation systems and in intercropping under small scale subsistence farming systems. Plagued by many biotic and abiotic growth limiting factors, production has been lower than the potential. Breeding to alleviate or overcome these limiting factors is therefore key new cultivars to ensure increased productivity to contribute toward assured food security.

Learning Objectives

- Become familiar with the basic biology and importance of groundnut
- Provide examples of breeding schemes used to develop cultivars of groundnut

Crop Attributes

Crop Biology

Fig. 1 An *Arachis hypogaea* plant. Licensed under Public Domain via Wikimedia Commons.

Groundnut or peanut (*Arachis hypogaea* L. Millsp) is a self-pollinated species belonging to the Fabaceae family. Groundnut is a disomic allotetraploid (2n = 4x = 40). The two sets of chromosomes of *A. hypogaea* are highly diplodized, meaning there is little recombination between the A and B genomes except when the infrequent quadrivalent is formed. Groundnut is found in the *Arachis* section along with A. *monticola*, also a tetraploid, and ~25 diploid species (Dwivedi et al., 2007). Arachis is subdivided into nine taxonomical sections: *Arachis,Erectoides*, *Rhizomatosae*, *Extranervosae*, *Heteranthae*, *Trierectoides*, *Triseminatae*, *Caulorrhizae*, and *Procumbentes* with groundnut classified in the Arachis section (Dwivedi et al., 2007).

Crop Geography

The genus *Arachis* originated in South America and is comprised 68 species (Dwivedi et al., 2007; Krapovickas and Gregory, 1994). The species of Arachis are easily delineated from other closely related genera because they flower above ground but set seed below ground (Holbrook and Stalker, 2003).

There are six centers of diversity for groundnut in South America including geographic regions in Paraguay-Paraná, the upper Amazon, the west coast of Peru, Brazil, and the southwest Amazon region in Bolivia. A secondary center of diversity also exists in Africa (Holbrook and Stalker, 2003; Wynne and Coffelt 1982). *Arachis hypogaea* is believed to have originated in the South American region encompassing southern Bolivia to northern Argentina (Holbrook and Stalker, 2003). *Arachis hypogaea* is thought to have arisen ~4000 years ago from a single hybridization event between two diploid Arachis species (i.e., **A** genome from *A. duranensis*, **B**

genome from *A. ipaensis*) followed by a spontaneous chromosome doubling of the sterile hybrid to form a fertile allotetraploid (i.e., AABB) (Kochert et al., 1996; Young et al. 1996). Though the fertility was restored in the resulting allotetraploid it was reproductively isolated from its progenitor species creating a strong genetic bottleneck, which is partially responsible for the low allelic diversity present in modern cultivated peanut (Dwivedi et al., 2007; Kochert et al., 1996; Stalker et al., 2013; Young et al. 1996).

Crop Inflorescence

Arachis hypogaea or cultivated groundnut has simple or compound (i.e., 1-5 flowers) inflorescences that occur in the leaf axils on both primary and secondary branches. Typically a single flower per inflorescence opens per day (Holbrook and Stalker, 2003). The flower includes a standard, wing, and keel petals. The standard is deep orange to light yellow in color, and in rare cases may be white. The calyx consists of five sepals attached to the elongated hypanthium. Flowers possess an elongated tubular hypanthium or calyx tube so they look like they are borne on stalks but are instead classified as sessile (Holbrook and Stalker, 2003).

Arachis hypogaea is divided into two subspecies (e.g., *hypogaea* and *fastigiata*) and six botanical varieties (e.g., hypogaea, hirsuta, fastigiata, peruviana , aequatoriana, and vulgaris) (Table 1) (Holbrook and Stalker, 2003).

Subspecific and Varietal Classification

Table 1 Data from Holbrook and Stalker, 2003, with additional comments from Dwivedi et al., 2003; Okello et al, 2013; Rami et al., 2014.

Botanical variety	Market type	Location	Traits
hypogeae	Virginia/ Runner	Bolivia, Amazon	No flowers on the central stem, alternating pairs of floral and vegetative axes on lateral branches, short branches, spreading growth habit, relatively few trichomes Virginia type: large seeds, less hairy, consumed in the shell or roasted, longer growing cycle Runner type: small seeds, less hairy, used to make groundnut/peanut butter
hirsuta	Peruvian runner	Peru	more hairy
fastigiata	Valencia	Brazil, Paraguay, Peru, Uruguay	Flowers in the mainstem, sequential/disorganized pairs of floral and vegetative axes on the branches, more erect to procumbent, shorter life cycle, pods with 2+ seeds, smooth pericarp, consumed in the shell, boiled, or canned, primary type in Uganda
peruviana	n/a	Peru, Bolivia	Less hairy, deep pod reticulation
aequatoriana	n/a	Ecuador	Very hairy, deep pod reticulation, purple stems, more branched, upright
vulgaris	Spanish	Brazil, Paraguay, Uruguay	More branched, upright, shorter life cycle, flowers on the central stem, floral and vegetative axes arranged in a disorganized fashion, 2 seeds per pod

Importance

Groundnut is a legume crop adapted to a hot, semi-arid climate, survives and yields under rainfed conditions, fixes nitrogen, and requires few inputs, making it a crop that is suitable for small shareholder farms. It can be grown in a low input environment and provides a source of fat and protein, and provides many phytonutrients including vitamin E and antioxidants.

Usage

Groundnut is a multiuse crop that is harvested as a source of edible oil, vegetable protein, and forage (haulms) for horse and cattle (Dwivedi et al., 2003). Groundnut is a rich source of oil, protein, minerals (Ca, Mg, P, and K), and vitamins (E, K, and B1) (Savage and Keenan 1994). Groundnut contains 40-60% oil, 20-30% protein and, and 1020% carbohydrate (Dwivedi et al., 2003; Pandey et al. 2012; Savage and Keenan 1994)

In the United States, approximately 70 percent of the groundnuts are runners (small-seeded types of var. hypogaea), 20 percent are virginias (large-seeded types of var. hypogaea), 10 percent are spanish (var. vulgaris), and less than 1 percent are valencia (var. fastigiata) market types (Holbrook and Stalker, 2003; Knauft and Gorbet, 1989. Most of the groundnut produced is for human consumption, but low quality lots (blemished seeds, aflatoxin contamination) are crushed for oil.

The quality of groundnut oil is determined by the ratio of oleic (O) to linoleic (L) fatty acids. A higher ratio results in better storage quality of the oil that is less prone to oxidation and the development of undesirable flavors, and has a longer shelf life. For groundnut varieties harvested for oil extraction (i.e., high oil, high O/L ratio) seed size doesn't matter as much as total yield. For groundnut varieties harvested for human consumption (i.e., low oil, high O/L ratio) larger seed size is desired (Dwivedi et al., 2003).

Groundnuts are used in the food and confectionary industries, but is limited by its allergenic properties in both adults and children, and by concerns about aflatoxin contamination caused by *Aspergillus niger* and *A. flavus*), which is carcinogenic and act as an immunosuppressant in both animals and humans (Dwivedi et al., 2007).

Biotic Constraints

Disease Constraints

Biotic stresses include early leaf spot (*Cercospora arachidicola*), late leaf spot (*Phaeoisariopsis personata*), rust (*Puccinia arachidis*), groundnut mottle virus (*Potyviridae*), and groundnut rosette virus (Tombusviridae) (Okello et al., 2013; Pandey et al. 2012). (Table 2)

Table 2 Major disease constraints to groundnut production. Data from Dwivedi, 2003.

Pathogen	Diseases
Fungi	Rust (*Paccinia arachidis* Speg.), early leaf spot (ELS) (*Cercospora arachidicola* Hori), and late leaf spot (LLS) [*Phaeoisariopsis personata* (Berk. and Curtis) Deighton]
Viruses	Groundnut rosette disease (GRD), peanut clump virus (PCV), peanut bud necrosis virus (PBNV), and tomato spotted wild virus (TSWV)
Bacteria	Bacterial wilt [*Burkholderia solanacearum* (E.F. Smoth) Yabuuchi et al.]
Nematodes	Meloidogyne, Scutellonema, Pratylenchus, Helicotylenchus, Aphelenchoides, Telotylenchus, and Paralongidorus species

Pest Constraints

Important insect pests include aphids (*Aphis craccivora*), jassids (*Amrasca devastans*), leafminers (*Aproarema modicella*), termites (Isoptera), army worms (*Spodoptera litura*), and thrips (*Frankliniella* spp.) (Okello et al., 2013; Pandey et al., 2012) (Table 3). Thrips and aphids are more detrimental as vectors of viruses versus causing direct damage to the plants. The groundnut leaf miner (*A. modicella*) causes extensive defoliation in the major groundnut producing regions in Uganda (Mukankusi et al., 2000; Okello et al., 2013).

Because groundnut seed is sensitive to heat and high moisture, seed storage must be carefully managed at all stages of the seed production chain (Okello et al., 2013).

Table 3 Major insect constraints to groundnut production. Data from Dwivedi, 2003.

Environment	Insect
Field	Leaf miner [*Aproaerema modicella* (Deventer)], army worm (*Spedoptera litura* Fab.), corn earworm (*Helicoverpa armigera* Hubner), lesser corn stock borer (*Elasmopalpus lingosellus* Zeller), southern corn rootworm (*Diabrotica undecimpuctata howardi* Barber), thrips (*Frankinella and Scirtothrips* species), jassids (*Empoasca kerri Pruthi*), aphids (Aphis craccivora Koch.), and termites (*Microtermes and Odontotermes* species)
Storage	Bruchid (*Caryedon serratus* Oliver), red flour beetle (*Tribolioum castanem* Herbst, rice moth (*Corcyra caphalionica* Sainton), and pod-sucking bug (*Elasmolomus* (*Aphanus*) *sordidus* Fab.)

Resistance Traits

Wild Arachis species possess high levels of disease resistance and display a wide range of

morphological variation (Dwivedi et al., 2007). e.g., A. *diogoi* has virus resistance genes that are not present in the cultivated gene pool (Table 4).

Table 4 Sources of resistance to rust, leaf spots, sclerotinia blight, groundnut rosette virus, aflatoxin, nematode, defoliator, aphid, and drought reported in cultivated and wild Arachis species. Data from Dwivedi et al., 2007.

Peanut accessions with beneficial traits reported	**Type**	
Trait	**Cultivated Species**	**Wild Arachis species**
Rust	169	29
Late leaf spot	69	27
Early leaf spot	37	11
Groundnut rosette virus	116	12
Nematode	21	–
Seed infection and/or aflatoxin production by Aspergillus flavus	21	4
Sclorotinia blight	51	–
Defoliator (Leaf miner and Spodoptera)	9	28
Aphid	2	Not evaluated
Drought	40	Not Evaluated

Resistance in Arachis Species (5 tables)

Figures 2 to 7 show Arachis species on the vertical axes and pests and diseases on the horizontal axes. Resistance is indicated by the red shaded boxes.

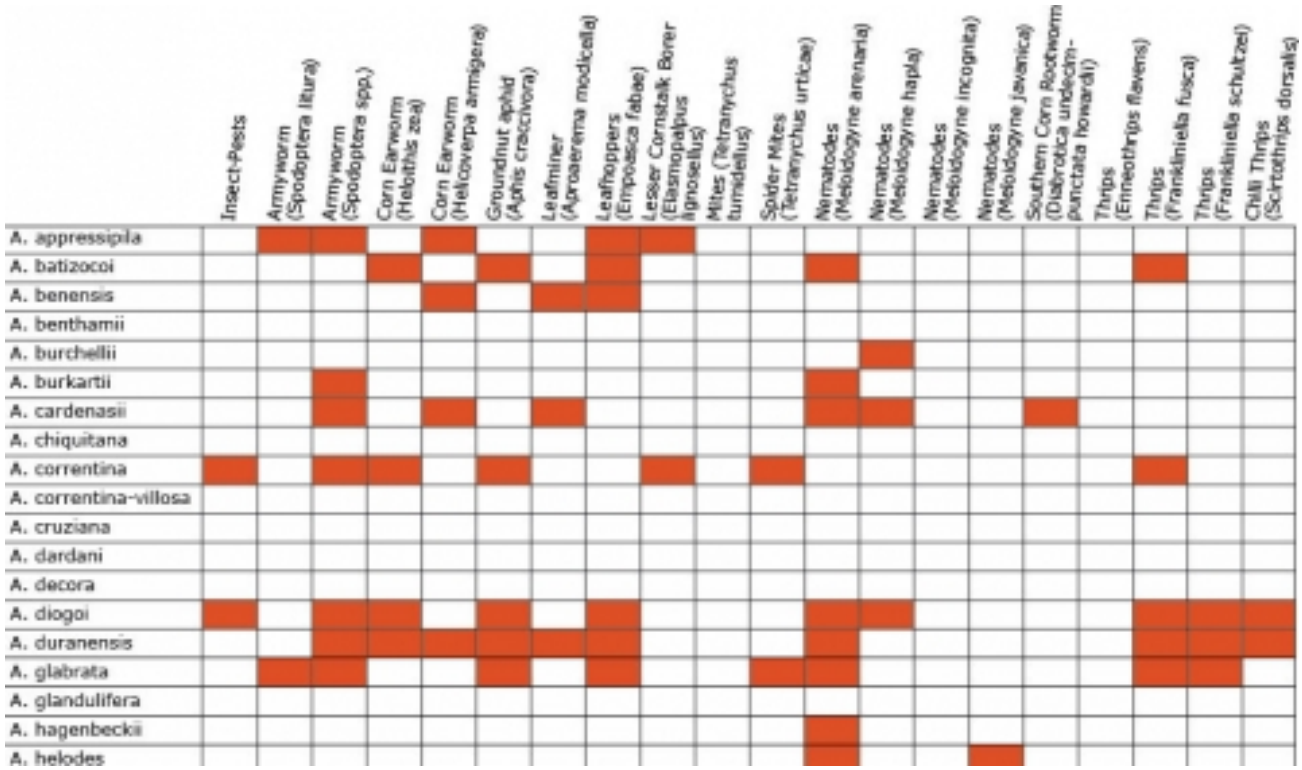

Fig. 2 Arachis species resistant to peanut diseases and pest pressures. Adapted from Stalker et al., 2013.

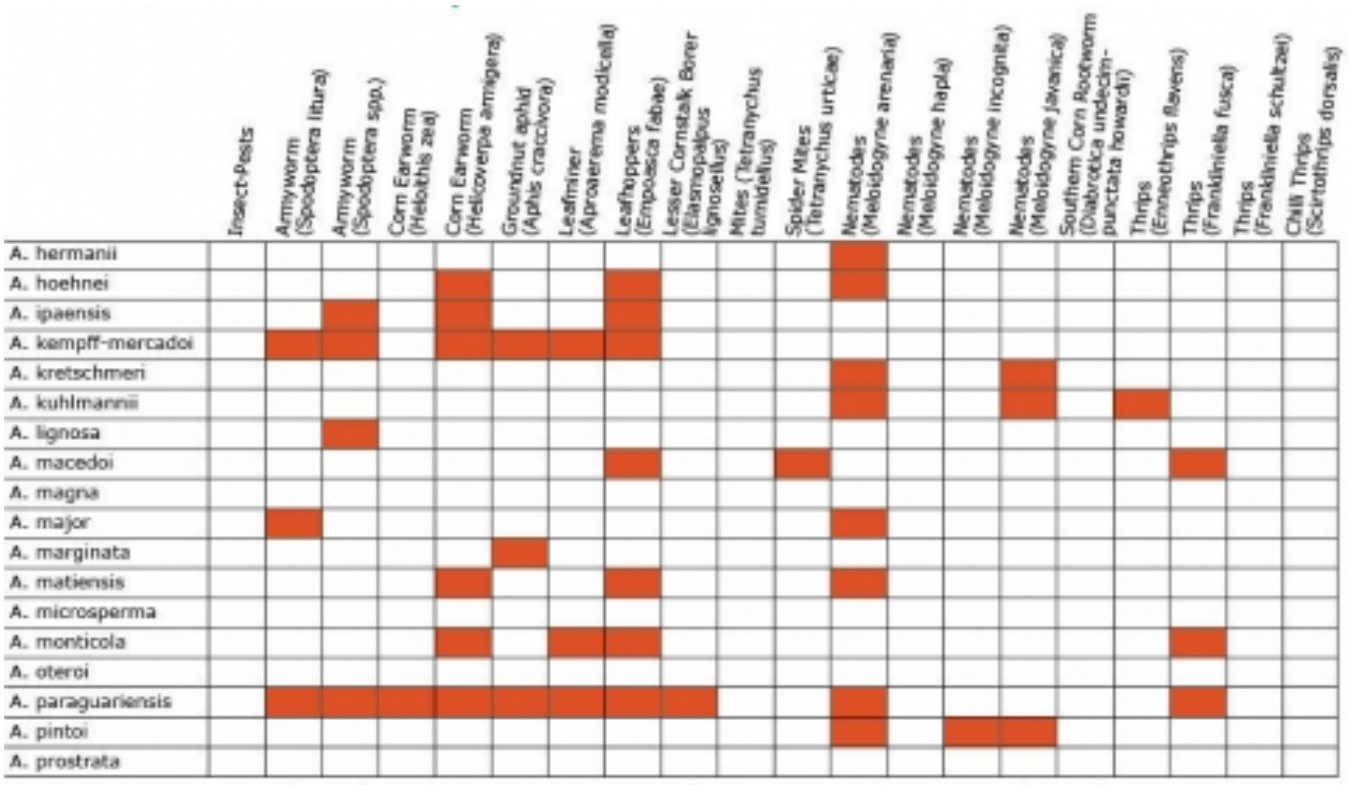

Fig. 3 Arachis species resistant to peanut diseases and pest pressures. Adapted from Stalker et al., 2013.

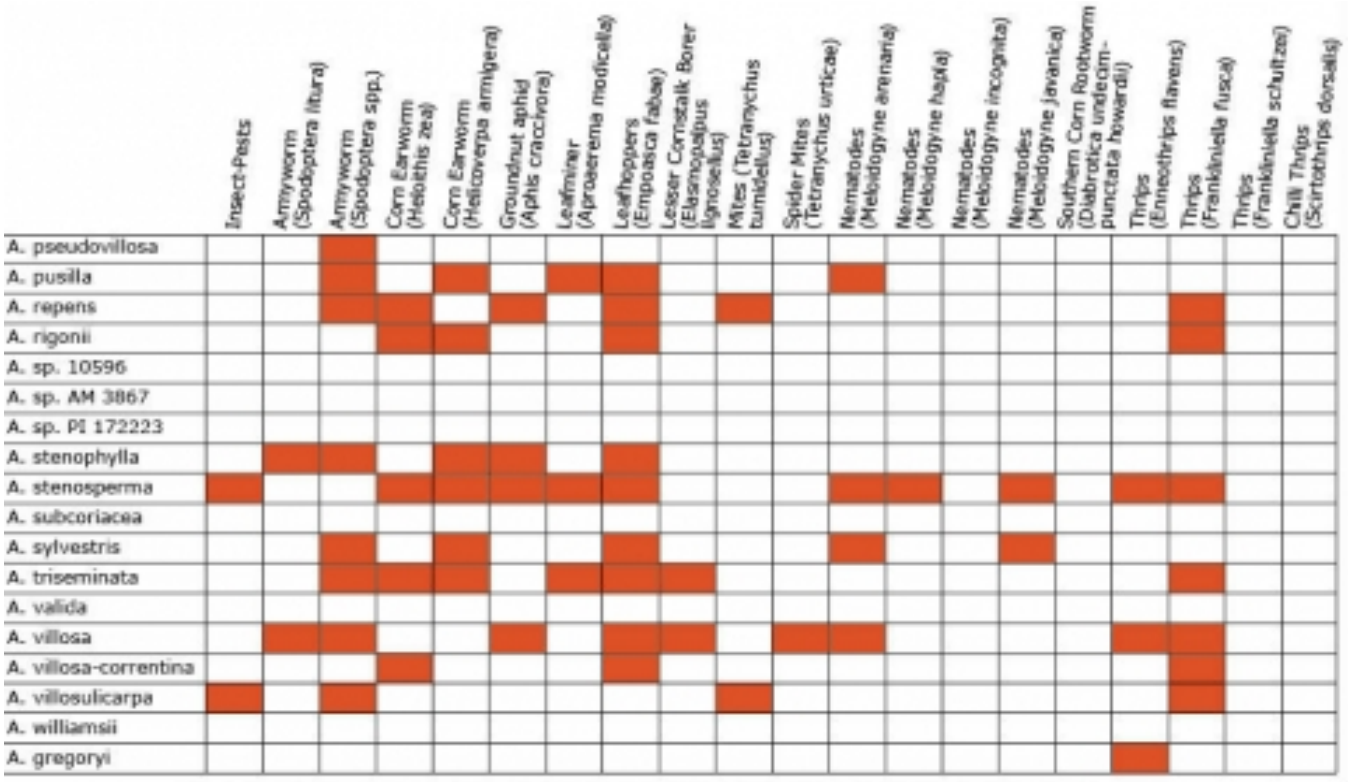

Fig. 4 Arachis species resistant to peanut diseases and pest pressures. Adapted from Stalker et al., 2013.

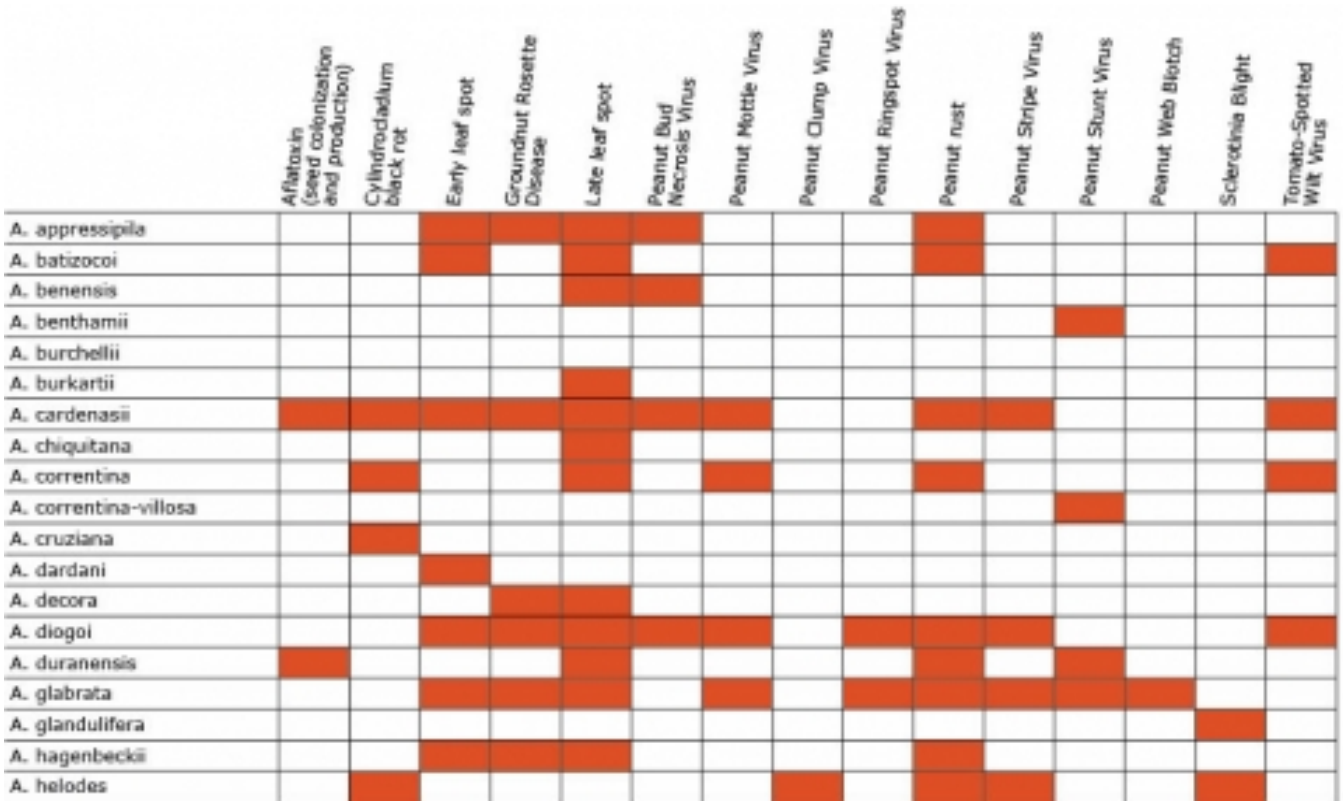

Fig. 5 Arachis species resistant to peanut diseases and pest pressures. Adapted from Stalker et al., 2013.

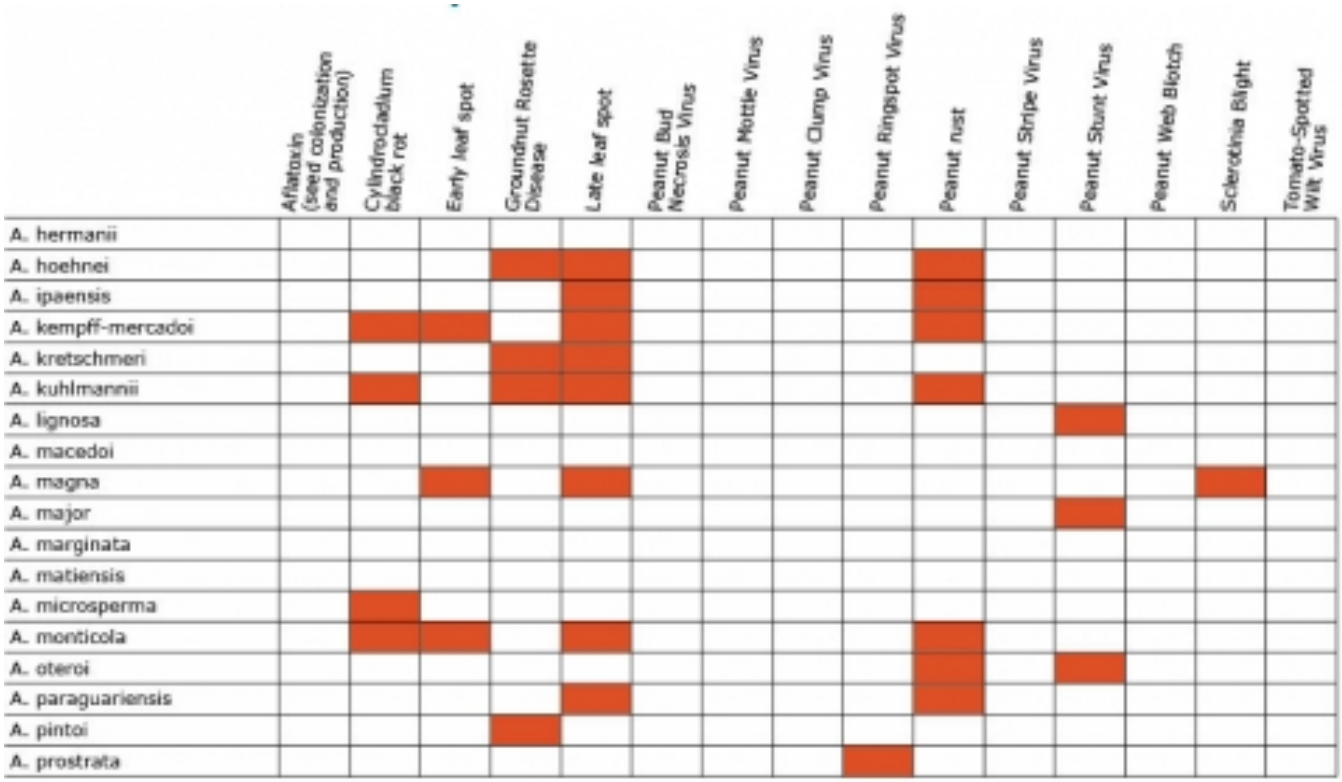

Fig. 6 Arachis species resistant to peanut diseases and pest pressures. Adapted from Stalker et al., 2013.

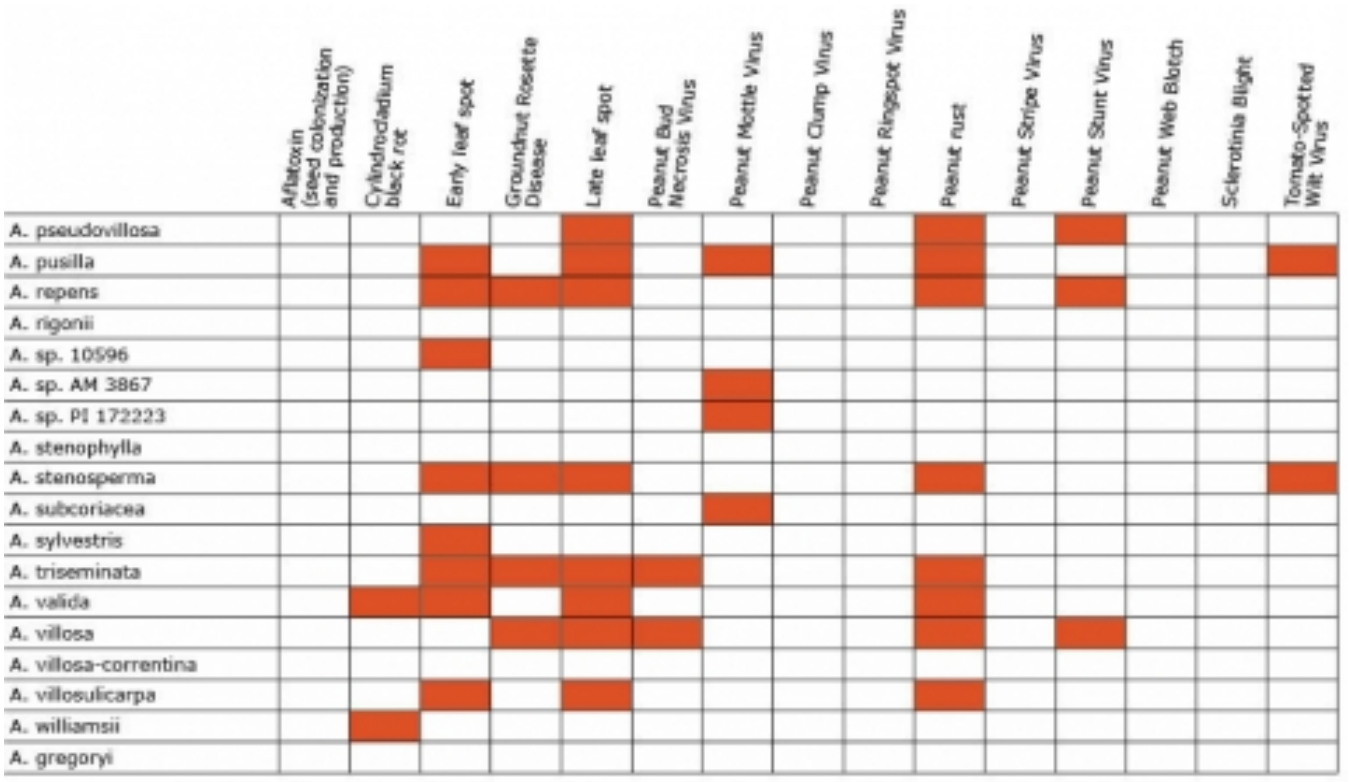

Fig. 7 Arachis species resistant to peanut diseases and pest pressures. Adapted from Stalker et al., 2013.

Breeding Properties

Breeding Goals

Improved yield – less vegetative biomass, shorter main stem, increased flower production (Dwivedi et al., 2003), high yielding potential, high quality, resistance to major pests and diseases (rosette and Cercospora leafspots, peanut bud necrosis, root rot), aflatoxin resistance, short to medium-term maturity periods and tolerance to drought, large seeds for confectionery purposes, high oil content for oil extraction (Okello et al., 2013).

Breeding CentersStalker et al., 2013.

The largest collection of groundnut germplasm is maintained in India at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). This collection includes ~15,000 accessions of cultivated groundnut and ~500 accessions of 48 wild Arachis species representing

all six botanical varieties: var. *hypoagaea* (45.8%), var. *vulgaris* (36.6%), var. *fastigiata* (15.7%), var. *aequitoriana* (0.10%), var. *peruviana* (1.7%), and var. *hirsuta* (0.13%).

Other major collections include the National Research Center for Groundnut in Junagadh, India (~8,000 accessions), the USDA NPGS, Griffin, GA, USA (~9,000 accessions). Large collections of wild Arachis species are also maintained at Texas A&M and North Carolina State University, Raleigh, NC, USA. The National Center of Genetic Resources (CENARGEN) in Brazil maintains over 1200 accessions of 81 species belonging to 9 sections.

Oil Crops Research Institute (OCRI) of the Chinese Academy of Agricultural Sciences (CAAS) (8083 accessions) and the Crops Research Institute of the Guangdong Academy of Agricultural Sciences (4210 accessions) in China

Instituto Nacional de Technologia Agropecuaria (INTA) and the Instituto de Botánica del Nordeste (IBONE) in Argentina

There are also two well-defined core and minicore collections representing the majority of variation present in the cultivated peanut germplasm.

Germplasm Pools

The primary gene pool for groundnut consists of cultivated accessions (*Arachis hypogaea*) and the wild tetraploid species *A.monticola*. The secondary gene pool includes diploid species of the section Arachis that can be successfully crossed with cultivated groundnut. The tertiary gene pool includes species in sections other than *Arachis* that cannot be crossed with A. *hypogaea* using conventional crosses and are limited by both pre- and postzygotic hybridization barriers (Dwivedi et al., 2007) and (Fig. 8).

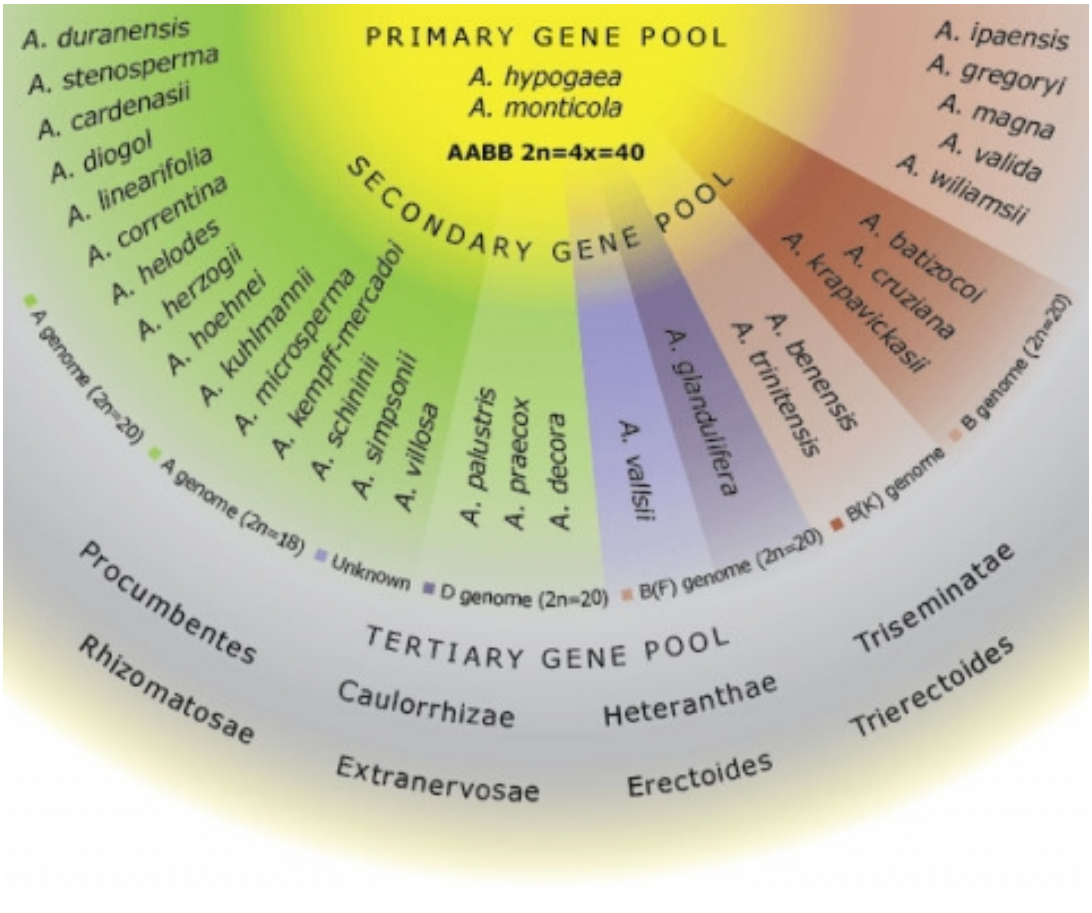

Fig. 8 Primary, secondary and teritary gene pools of the genus Arachis. Adapted from Rami et al., 2014.

Introgressing Diversity from Wild Arachis Species

Wild species of Arachis have been successfully used as sources of resistance to pathogens (i.e., *Sclerotinia* blight, tomato spotted wilt virus, early and late leaf blights) and pests (root knot nematodes).

There are **three main methods** for the strategic incorporation of this diversity from wild species into cultivated groundnut (Dwivedi et al., 2007; Simpson, 2001).

Introgressing Diversity – Method 1

The first method starts with a cross between a diploid wild species and a tetraploid variety of cultivated groundnut (A. hypogaea), which produces a sterile triploid hybrid. The chromosomes

of this sterile F_1 hybrid are then doubled using colchicine to produce a hexaploid plant, which is then backcrossed with cultivated groundnut producing pentaploid progeny. These progeny are then self-pollinated for several generations usually resulting in aneuploid progeny, which over time lose chromosomes due to problems during meiosis (i.e., mispairing, lagging chromosomes) and eventually stabilize at the normal chromosome number of 40 (i.e., tetraploids) (Dwivedi et al., 2007; Holbrook and Stalker, 2003).

Introgressing Diversity – Method 2

For the second method, colchicine is used to double the chromosomes in two wild diploid species (i.e., AA and BB doubled to AAAA and BBBB) (Dwivedi et al., 2007). These autotetraploids are then crossed to produce an allotetraploid hybrid (i.e., AABB), which is then crossed and backcrossed with cultivated groundnut, *A.hypogaea* for multiple generations during which the breeder selects to recover useful agronomic traits from the cultivated parent while simultaneously selecting for exotic traits from the wild species.

For the third method two wild diploid species (i.e., AA and BB) are crossed and the sterile F_1 hybrid (i.e., AB) is treated with colchicine to double the chromosomes to produce a fertile allotetraploid (i.e., synthetic amphidiploid, AABB) (Dwivedi et al., 2007). This allotetraploid is then backcrossed with cultivated groundnut *A. hypogaea* to produce tetraploid hybrids carrying agronomic traits from the cultivated parent and exotic traits from the wild species. Synthetic tetraploid varieties produced via this method are relatively normal and demonstrate normal meiosis, pollen fertility, and genetic recombination.

Introgressing Diversity – Method 3

Method 1 is the most useful for developing new varieties (reviewed by Dwivedi et al. 2003a and Holbrook and Stalker 2003). Method 2 is less useful for developing varieties in a breeding program because of problems with sterility in the autotetraploids (i.e., AAAA, BBBB) (Dwivedi et al., 2007). Method 3 is designed to recreate the events that originally gave rise to cultivated groundnut as described previously [i.e., hybridization of two 2x species (**A** genome from *A. duranensis*, **B** genome from *A. ipaensis*) followed by a spontaneous chromosome doubling of the sterile hybrid to form a fertile allotetraploid (AABB)]. This method has been used to introgress resistance genes from wild species into cultivated groundnut (Simpson and Starr 2001; Simpson et al. 2003). In this example *Arachis cardenasii* and *A. diogoi* were used as the **A** donors crossed

with *A. batizocoi*, which was used as the **B** donor. The resulting allotetraploid was then crossed with cultivated groundnut (reviewed in Dwivedi et al., 2007).

Interspecific crosses are usually more successful when the species with the higher ploidy level (usually *A. hypogaea*) is used as the female parent when crossed with diploid wild Arachis species (i.e., Method 1). Greater success has also been observed when the annual species is used as the female parent and the perennial species (i.e., smaller stigmas) is used as the pollen parent. However, even when a cross successfully produces hybrid progeny, genetic recombination during meiosis in the hybrids is often restricted, and desired genes are not incorporated due to a lack of crossing over between the different genomes (Holbrook and Stalker, 2003). Because of these problems breeders must make multiple crosses using different parents to increase the probability of success.

Summary

Biparental crosses or more complex crosses are used to generate variability then pedigree and bulk-pedigree selection methods are used to identify and select superior genotypes. Single seed descent (with or without concurrent selection) is often used to increase the homozygosity of the breeding population during the early generations prior to selection (Dwivedi et al., 2003).

Typically breeders select for qualitative traits (such as disease/pest resistance) during the early generations F_2-F_5 followed by late generation testing for quantitative traits like yield and/or traits that are influenced by the environment (e.g., oil content, O/L ratio, etc.) (Dwivedi et al., 2003). Most groundnut breeding programs begin preliminary yield trials in the F_5 or F_6 generation, by which time the level of heterozygosity has been minimized through inbreeding and meaningful selection for complex quantitative traits can begin.

Recurrent selection is sometimes used to maintain genetic diversity in breeding populations (Dwivedi et al., 2003) though this method is limited by the presence of negative correlations between disease resistance and yield in some populations (Holbrook and Stalker, 2003).

Backcross breeding is commonly used for introgressing 1 or 2 genes into a superior genotype.

Outcomes

Artificial crosses are most successful when made during the early morning hours after sunrise

when the pollen is mature and viable and the stigmas are receptive (Holbrook and Stalker, 2003). High humidity levels help ensure adhesion of pollen to the stigmatic surface so it is often helpful to spray down the greenhouse floors with water in the morning to increase the humidity levels especially on dry days (Holbrook and Stalker, 2003). Pod development in cultivated groundnut generally begins 16 to 17 days after pollination. In other species pod development is delayed until 23 to 25 days (Halward and Stalker 1987). Pegs of cultivated groundnut are relatively short and robust and do not break easily, but pegs of wild Arachis species may, since they may be 1 m or more in length and are fragile (Holbrook and Stalker, 2003), so it is preferable to use cultivated groundnut as the female parent whenever possible. Because only a small number of progeny (i.e., usually 1-2 seeds per pod) can be made per artificial cross it is important to make as many crosses as possible especially when making backcrosses to introgress traits from a wild Arachis species.

Outcrossing rates in groundnut are typically around 2% though rates near 8% have been identified (Coffelt, 1989; Knauft et al., 1992)

Because photoperiod and temperature greatly affect how growth is partitioned between above and below ground structures it is critical to make selections in an environment that is similar to your target environment. This is especially true in genotypes with large seeds. It is also important to select for resistance to pests and pathogens in an environment that has the same photoperiod conditions as your target environment because late maturing types are often more affected by variation in photoperiod (Dwivedi et al., 2003).

Limitations

Genetic enhancement of groundnut is limited due to the existence of unfavorable linkages between useful disease resistance genes and loci conferring undesirable pod and seed characteristics. A second limitation is due to the fact that most disease resistant accessions are later maturing types that are more sensitive to photoperiod and partition less to below ground pod development. A third limitation is the large genotype by environment interactions for economically important traits. A fourth limitation is the small number of progeny created by artificial crosses (Dwivedi et al., 2003).

Because of these limitations a varied approach to breeding groundnut is recommended (Dwivedi et al., 2003).

Breeding Strategies

Table 5 Traits and breeding strategy suggested for rapid and cost-effective genetic enhancement in groundnut. Data from Dwivedi et al., 2003. X indicates breeding strategy applicable for the trait.

Trait	Conventional breeding	Marker-assisted selection	Wide crosses + marker-assisted backcross	Genetic transformation	Genetic basis
Maturity	X	n/a	n/a	n/a	Polygenic
Pod yield	X	n/a	n/a	n/a	Polygenic
Pod size and shape	X	n/a	n/a	n/a	Polygenic
100-seed weight	X	n/a	n/a	n/a	Polygenic
Shelling outturn	X	n/a	n/a	n/a	Polygenic
Sound Mature seeds	X	n/a	n/a	n/a	Polygenic
Seed dormancy	X	n/a	n/a	n/a	Monogenic
Oleic/Linoleic fatty acid ratio	n/a	X	n/a	n/a	Oligogenic
Aflatoxin	n/a	n/a	n/a	X	Polygenic
Drought	n/a	X	n/a	X	Polygenic
Leaf miner	n/a	n/a	X	n/a	Unknown
Spodoptera	n/a	n/a	X	n/a	Unknown
Rust	X	n/a	n/a	n/a	Oligogenic
Early leaf spot	n/a	n/a	X	n/a	Polygenic
Late leaf spot	n/a	n/a	X	n/a	Polygenic
Bacterial wild	X	n/a	n/a	n/a	Oligogenic
Groundnut rosette disease	X	n/a	n/a	X	Mono- and diagenic
Peanut bud necrosis disease	n/a	n/a	n/a	X	Unknown

Speed Breeding

In temperate regions of the world, groundnut breeders are only able to grow 1 generation per year, thus it usually requires 10-15 years from the first cross to the release of a cultivar. When available the use of a winter nursery for increasing homozygosity levels via single seed descent can increase the total number of generations per year to 2, thereby reducing the time needed to develop a new cultivar. Recent efforts have demonstrated that the use of a controlled environment in which continuous light and optimal temperatures and humidity are maintained can be used to advance lines from the F_2 to the F_4 in a single calendar year, further reducing the time to release a cultivar down to 6-7 years. This concept of "speed breeding" allows the breeder to maximize the efficiency of the breeding program by decreasing the growing period to maturity by ~30% relative to the time needed in the field, thus increasing the number of generations that can be grown per calendar year to 3 (O'Connor et al. 2013).

- Strategy 1 (42 months): pedigree breeding with one summer generation per year
- Strategy 2 (23 months): pedigree breeding with two generations per year (i.e., 1 in the summer, 1 in a winter nursery)
- Strategy 3 (17 months): speed breeding/SSD (i.e., the F_2-F_4 generations grown in a controlled environment; 24 hour lights, optimal temperatures and humidity)

Calendar

In all 3 strategies, the F_5 is grown in the field to maximize $F_{5:6}$ seed numbers to facilitate preliminary yield trials in the F_6.

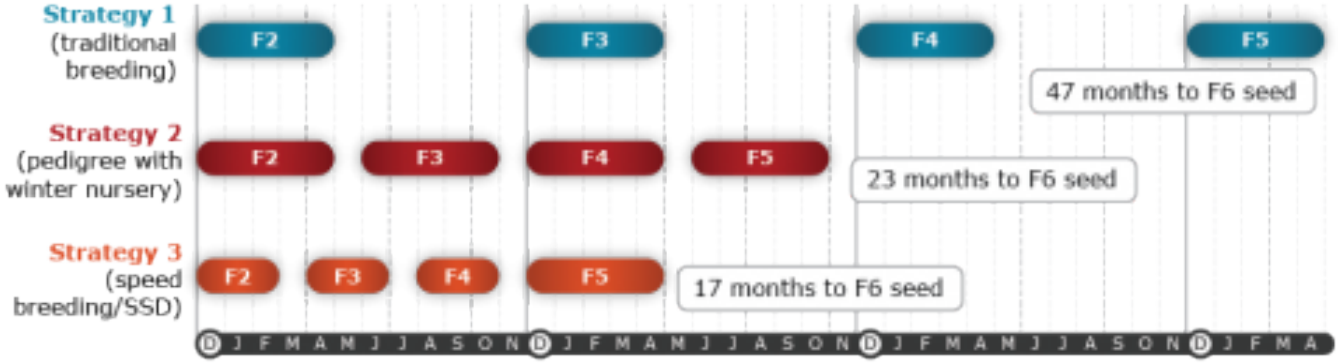

Fig. 9 Comparison of timeline required to develop F6 fixed lines using Strategy 1, 2, and 3. Data from O'Connor et al., 2013.

Strategy 3 of speed breeding/SSD is best suited for programs using a backcrossing breeding strategy focused on incorporating a simply inherited trait controlled by one or two genes into a new variety. This system is also appropriate for the rapid development of RILs which are useful for genetic studies and molecular marker discovery.

Breeding Example

The following is an example of breeders introgressing alleles from *Arachis cardenasii*, a wild diploid species, into tetraploid groundnut.

Breeding objective: develop small-seeded, tetraploid (2n = 4x = 40) runner-type groundnut (*Arachis hypogaea L.* subsp. *hypogaea* var. *hypogaea*) germplasm lines that possess resistance to multiple diseases [e.g., early leaf spot (ELS), Cylindrocladium black rot (CBR), Sclerotinia blight (SB), and tomato spotted wilt (TSW)].

Registration of 'Bailey' Peanut

This is an example of breeders using simultaneous selection for resistance to multiple diseases using both early-generation testing for resistance combined with late-generation selection for improved pod and seed characteristics in superior families.

Breeding objective: develop a large-seeded virginia-type peanut (*Arachis hypogaea* L. subsp. *hypogaea* var. *hypogaea*) with partial resistance to five diseases that occur commonly in the Virginia-Carolina production area: early leaf spot (caused by *Cercospora arachidicola* Hori), late

leaf spot [caused by *Cercosporidium personatum* (Berk. & M.A. Curtis) Deighton], Cylindrocladium black rot [caused by *Cylindrocladium parasiticum* Crous, M.J. Wingf. & Alfenas], Sclerotinia blight (caused by *Sclerotinia minor* Jagger), and tomato spotted wilt (caused by Tomato spotted wilt tospovirus). This variety should possess characters that make it suitable for the confectionary market (i.e., sold to consumers as in-shell groundnut or as a shelled kernel).

The breeders used a modified version of pedigree selection that included several generations of advancement using single seed descent to develop a pureline cultivar named 'Bailey'.

For the initial cross, NC 12C (a cultivar partially resistant to early leaf spot) was crossed with N96076L (a breeding line resistant to early leaf spot, CBR, and TSWV). Multiple F1 plants were then backcrossed as males to NC 12C to increase the chances of recovering an inbred line with suitable agronomic characteristics as NC 12C possessed more desirable traits relative to N96076L.

Extra Questions

Why did the breeder backcross the F_1s to the NC 12C parent? NC 12C was the superior plant.

Groundnut breeding is hampered by the limited genetic diversity in *A. hypogeae* and difficulty introgressing traits from wild Arachis species into cultivated varieties. The narrow genetic base in groundnut is attributed to multiple genetic bottlenecks that occurred as groundnut evolved into the current day species that it is, as well as, during domestication and as breeders work to develop modern-day cultivars. Consumers want a consistent commercial product so successful cultivars are often recycled back into a breeding program and used as the parents to develop the next round of cultivars.

References

Coffelt, T. A. 1989. Natural Crossing of Peanut in Virginia. Peanut Science 16(1): 46-48.

Dwivedi, S. L., J. H. Crouch, S. N. Nigam, M. E. Ferguson, and A. H. Paterson. 2003. Molecular breeding of groundnut for enhanced productivity and food security in the semi-arid tropics: opportunities and challenges. Advances in Agronomy 80:153-221.

Dwivedi, S. L., D. J. Bertioli, J. H. Crouch, J. F. Valls, H. D. Upadhyaya, A. Favero, M. Moretzsohn, and A. H. Paterson. 2007. Peanut. In: Oilseeds. Springer, Berlin Heidelberg, pp. 115-151.

Halward, T. M., and H. T. Stalker. 1987. Comparison of embryo development in wild and cultivated Arachis species. Ann. Bot. 59:9-14.

Holbrook, C. C., and H. T. Stalker. 2003. Peanut breeding and genetic resources. Plant breeding reviews 22:297-356.

Knauft, D. A., A. J. Chiyembekeza, and D. W. Gorbet. 1992. Possible Reproductive Factors Contributing to Outcrossing in Peanut (Arachis hypogaea L.). Peanut Science 19(1):29-31.

Kochert, G., H. T. Stalker, M. Ginenes, L. Galgaro, and K. Moore. 1996. RFLP and cytogenetic evidence for the progenitor species of allotetraploid cultivated peanut, Arachis hypogaea, (Leguminosae). Am. J. Bot. 83:1282-1291.

Krapovickas, A., and W. C. Gregory. 1994. Taxonomy of the genus Arachis (Leguminosae). Bonplandia 8:1-186.

O'Connor, D. J., G. C. Wright, M. J. Dieters, D. L. George, M. N. Hunter, J. R. Tatnell, and D. B. Fleischfresse. 2013. Development and Application of Speed Breeding Technologies in a Commercial Peanut Breeding Program. Peanut Science 40(2):107-114.

Okello, D. K., M. Biruma, and C. M. Deom. 2013. Overview of groundnuts research in Uganda: Past, present and future. African Journal of Biotechnology 9(39):6448-6459.

Pandey, M. K., E. Monyo, P. Ozias-Akins, X. Liang, P. Guimarães, S. N. Nigam, H. D. Upadhyaya, P. Janila, X. Zhang, B. Guo, D. R. Cook, D. J. Bertoli, R. Michelmore, and R.K. Varshney. 2012. Advances in Arachis genomics for peanut improvement. Biotechnology advances 30(3): 639-651.

Rami J-F., S.C.M. Leal-Bertioli, D. Fonceka, M.C. Moretzsohn, and D.J. Bertoli. 2014. Groundnut. In A. Pratap and J. Kumar (eds.), Alien Gene Transfer in Crop Plants, Volume 2:253-279.

Simpson C.E. 2001. Use of wild Arachis species/introgression of genes into A. hypogaea L. Peanut Sci 28:114-116.

Stalker, H. T., S. P. Tallury, P. Ozias-Akins, D. Bertioli, and S.C. Leal Bertioli. 2013. The value of diploid peanut relatives for breeding and genomics. Peanut Science 40(2):70-88.

Wynne, J. C., and W. C. Gregory. 1982. Peanut breeding. Advances in Agronomy 34:39-68.

Young, N.D., Weeden, N.F., and G. Kochert. 1996. Genome mapping in legumes (Fam. Fabaceae) in A.H. Paterson (ed) Genome Mapping in Plants. Landes, Co, USA pp. 211-227.

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Chapter 15: Cassava Breeding

Shui-Zhang Fei and Anthony A. Mahama

Cassava (*Manihot esculenta* Crantz) is a dicot perennial shrub, belonging to the family of Euphorbiaceae. It is also known as tapioca, manioc, mandioca or yuca in different parts of the world. It can reach a height of 1-4 m (Fig. 1). Its tuberous storage roots are rich in starch (20-40%) and are harvested either for direct human consumption, animal feed, or industrial uses.

Learning Objectives

Become familiar with cassava, its biology, center of origin and domestication, utilization, breeding objectives and methods, and the application of molecular tools in cassava improvement.

Cassava Anatomy

Stem

The growth habit of cassava has important implications in cassava breeding as it can affect root yield. There are two growth types:

1. Erect growth type, with or without branching at the top.
2. Spreading type, which is not cultivated.

Branching occurs as a result of flower induction (Ceballos et al 2010), therefore the branchings are often called “reproductive branchings”. The branches can undergo further branching when flowering occurs, resulting in high order branchings (Fig. 2).

Fig. 1 Cassava plants in the Democratic Republic of Congo. Photo by Bob Walker; licensed under CC BY-SA 2.5 via Wikimedia Commons.

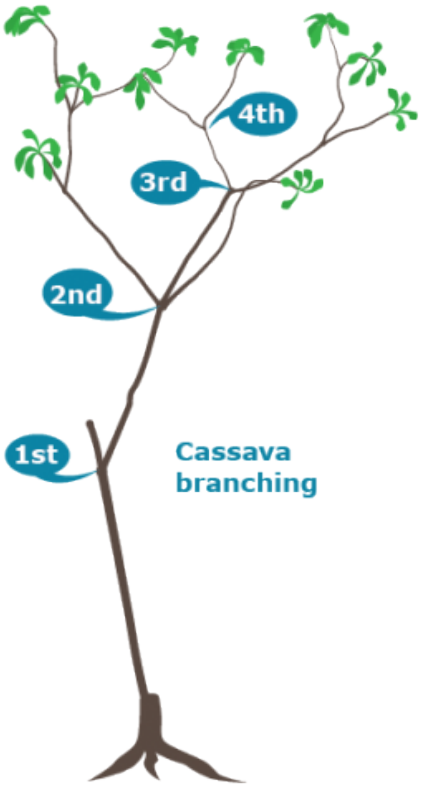

Fig. 2 Cassava branching. Illustration by Iowa State University.

Roots

Cassava roots are true roots, therefore cannot be used for propagation. Cassava root is the most economically important organ of a cassava plant because of the starch-containing cells in the parenchyma tissue, the edible part of the root. Only 3-10 of the fibrous roots of a cassava plant will eventually bulk and become storage roots through secondary growth while the rest of the fibrous roots remain thin and serve to function in water and nutrient absorption (Fig. 3).

Fig. 3 Cassava roots. (Photo by Anthony Mahama; ISU, Ames, Iowa).

Cassava Production

Economic importance

Cassava is the sixth-most important crop in the world following wheat, rice, maize, potato and barley. It is a staple food for over 500 million people, most of whom live in developing countries where food security is a major concern. Cassava is grown successfully between latitudes of 30°N and 30°S. It is drought tolerant and can grow under annual precipitation of 600mm. Cassava can also be grown on marginally fertile soils with a pH ranging from 3-8. Its roots can be left in the ground without harvest, serving as a good food security crop in cases where other crops fail.

Worldwide production

The main cassava production areas in the world are Africa, Asia, the Caribbean and South America. Fig. 4 describes the share of each main production area in 2014. Fig. 5 shows the production in tonnes in ten countries in 2014.

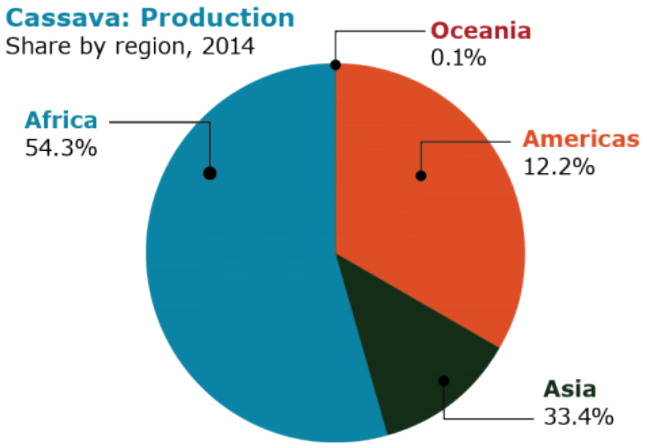

Fig. 4 Share of cassava production in 2014. Source: FAOSTAT.

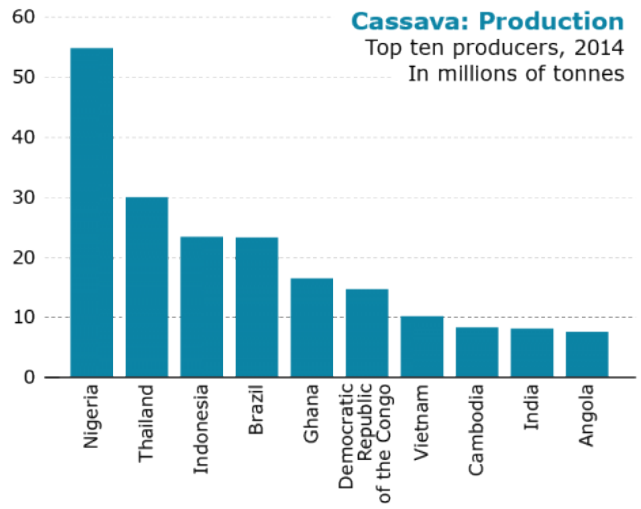

Fig. 5 Top ten nations for cassava production in 2014. Source: FAOSTAT.

Main uses

Cassava roots are either directly consumed by humans as food, used as animal feed or for industrial use.

Food

Fig. 6 Cassava heavy cake. Photo by Joy; licensed CC BY 2.0

Cassava roots can be eaten raw, cooked after removing the skin and rind or even baked and the charred skin removed before consumption. Cassava roots have numerous culinary uses around the world. Cassava leaves can also be consumed as a vegetable.

Animal feed

Fig. 7 Goats feeding on cassava. Photo by Oppong-Apane, K. 2013. *Cassava as animal feed in Ghana*. FAO.

All parts of the cassava plant can be used for animal feeding. In particular, the high energy content of cassava roots makes it an ideal carbohydrate ingredient in animal diet. The majority of the cassava produced in south Asia are used for animal feed in the form of chips and pallets.

Industrial use

Fig. 8 Cassava starch processing in Colombia's south Cauca department. Photo by CIAT; licensed CC BY-SA 2.0

Cassava roots are also used for industrial purposes, primarily for extraction of starch which has a wide variety of uses. Recently bioethanol production from cassava has received great attention due to increased fossil fuel price and concerns over global climate changes caused by burning fossil fuels.

Reproductive Biology of Cassava

Flower morphology and flowering behavior

Cassava is monoecious, producing separate male and female flowers on the same plant. Flowering in cassava is highly genotype and environment dependent. While some early-flowering genotypes can flower one month after planting, others may take 24 months to flower, consequently, synchronization of flowering time can be challenging in cassava breeding programs. Flowering rarely occurs during long dry period, thus irrigation is required for crossing blocks during an extended drought.

Fig. 9 Part of an inflorescence of cassava (*Manihot esculenta*) in Mozambique. Photo by Ton Rulkens; licensed under CC BY-SA 2.0 via Wikimedia Commons.

Fig. 10 Female flower of cassava (*Manihot esculenta*). Cultivar: 'Maria', a non-bitter cassava variety. This picture was taken close to Chimoio, Mozambique. Photo by Ton Rulkens; licensed under CC BY-SA 2.0 via Wikimedia Commons.

Cassava inflorescence is developed from the fork of the branchings (Fig. 9). The female flowers (Fig. 10) which are larger in size but smaller in numbers than the male flowers are situated at the base of the inflorescence while the male flowers, often numerous are located on the upper part of the inflorescence.

Female flowers open 1-2 weeks earlier than the male flowers on the same inflorescence. However, male and female flowers located on different inflorescences of the same plant can still open at the same time. Consequently, selfing can occur.

Pollination behavior

Despite the occurrence of self-pollination, cassava is considered a cross-pollinated species and cross-pollination is done by insects. The average size of cassava pollen ranges from 122-148 µm,

much larger and heavier than pollen of most other species. The longevity of cassava pollen is relatively short, lasting no more than 2 days.

The degree of self-pollination depends on genotype, environmental conditions, and the presence of pollinating insects. Inbreeding depression is severe in cassava, thus seedlings of selfed progeny typically exhibit low vigor and lack competitiveness.

Seed formation, dormancy, and storage

Fig. 11 Cassava fruits in Mozambique. Photo by Ton Rulkens; licensed under CC BY-SA 2.0 via Wikimedia Commons.

Cassava fruit is a trilocular capsule (Fig. 11), with each capsule containing a single seed (Fig. 12). It takes 75-90 days after pollination for a cassava fruit to become physiologically mature. Dehiscing of mature cassava fruits is explosive, therefore bagging must be done before fruits become mature to collect seed in controlled crosses. Freshly harvested seeds are generally dormant and a 3-6 month of dry storage at ambient temperature is necessary to break the dormancy.

Fig. 12 Cassava fruits and seeds. Photo by Roger Culos; licensed under CC BY-SA 3.0 via Wikimedia Commons.

Physiologically active cassava seed can germinate readily in about 15 days. The optimum temperature for germination is between 30-35°C. Cassava seed can be stored at 4-5°C and relative humidity of 60%. A germination percentage of greater than 80% has been reported for seed stored under such conditions for a year.

Propagation Methods

Cassava can be propagated by either seed or stem cuttings (stakes). Because cassava plants are highly heterozygous, seed propagation will result in highly heterogeneous offspring that no longer possess the desirable traits of the seed parent. Consequently, cassava propagation is done mostly through stem cuttings.

Fig. 13 Cassava germplasm multiplication and conservation. Photo taken November 28, 2012 by the International Institute of Tropical Agriculture. Licensed under CC BY-NC 2.0.

Multiplication rate is one of the determining factors that affect whether a new improved cultivar is successfully adopted or not. After one year, a typical mature cassava plant will produce 10-30 stakes sized at about 25cm (Fig. 13). Reducing the stake size to include only two nodes or one per stake will likely produce 100 or 200 stakes from a single plant per year, resulting a multiplication rate of 100 or 200. Such multiplication rates, although high may still not be sufficiently rapid to produce a large quantity of stakes in a short period of time to meet the consumer demand. Even higher multiplication rates have been achieved by growing 2-node stakes in high density in moist chambers and continuously removing sprouting shoots of 15-20cm long for rooting in boiled water. Rooted plantlets are transferred to soil and after a brief period of hardening are transplanted to the field for production. Such a system can produce a multiplication rate of 36,000.

Fig. 14 Meristem excision under aseptic conditions in the laboratories at IITA. Photo by International Institute of Tropical Agriculture; licensed under CC-BY-NC 2.0.

Tissue culture methods have also been developed for rapidly multiplying desirable cassava germplasm. Plantlets can be regenerated from either pre-existing meristem or through somatic embryogenesis (Fig. 14). Virus-indexed plants can be obtained by culturing very small meristems and regenerating plants.

Origin of Cassava and Genetic Diversity

Cassava is widely believed to be originated from the southern rim of Amazonia. It is domesticated about 5,000-7,000 years BC (Allem, 2002) and was introduced to Africa by Portuguese and Spanish explorers, likely in the sixteenth century. Cassava did not become popular in Asia until in the 1960s.

The genus *Manihot* contains more than 100 species, all naturally occurring between 33°N (southwest USA) and 33°S (Argentina). Wild relatives that have been used for interspecific hybridization include *M. catingae, M. dichotama, M. glaziovii, M. melanobasis* and *M. saxicola.* Among these, *M. glaziovii* (ceara rubbertree) is the only species that has made significant contributions in developing cassava germplasm resistant to cassava mosaic disease.

Cassava has 36 chromosomes, forming 18 bivalents at meiosis. However, there are cytological and sequence information supporting the paleotetraploidy nature in cassava.

Breeding Objectives

Yield

Developing high-yielding cassava cultivars remains the highest priority of most, if not all cassava breeding programs. Root yield in cassava is, however, a complex trait and is affected by both genetics, environment, and their interactions. Cassava plants with an intermediate branching height have been shown to be highly correlated with high yield. Similarly, plants with good leaf retention (longer leaf life) are also found to be correlated with high root yield.

Root quality

Root quality is very important as it affects consumer acceptance and successful adoption of a cultivar. Cultivars with highly reduced cyanogenic glucosides and increased dry matter in the roots are desired. Cassava roots are naturally low in protein, therefore cultivars with enhanced protein content are desirable if they are used for animal feed. Cultivars with altered starch content and composition may be developed for specialty use.

Biotic stress

Fig. 15 The inflorescence of cassava (Manihot esculenta, Family Euphorbiaceae), a tropical tuber crop. Muruwere, Manica Province of Mozambique. The leaves show symptoms of cassava mosaic disease, caused by a virus. Photo by Tom Rulkens; licensed under CC BY-SA 2.0 via Wikimedia Commons.

Cassava production is constrained by many biotic stresses including some of the most devastating viral diseases such as cassava mosaic disease (CMD) (Fig. 15) and cassava brown streak disease (CBD) that can cause significant yield loss. Bacterial diseases such as cassava bacterial blight and root rot can also cause damages.

Developing cassava germplasm with resistance to a number of insects including cassava mites, mealybugs, and whiteflies which are responsible for transmitting the devastating CMD is of great importance.

Abiotic stress

The shelf life of cassava roots is notoriously short, often within 2 days after harvest. This post-harvest deterioration of cassava roots is manifested by internal discoloration which causes the immediate loss of marketability (Fig. 16). Developing cassava cultivars that are resistant to this post-harvest physiological deterioration is therefore highly desirable.

Fig. 16 Molding cassava for sale in Nampula market, Mozambique. Photo by International Institute of Tropical Agriculture. Licensed by CC BY-NC 2.0.

Principles of Cassava Cultivar Development: An Overview

Cassava is primarily cross-pollinated, therefore individual plants are highly heterozygous. Because cassava can be easily propagated by stem cuttings, improved cassava cultivars are primarily clonal cultivars that are multiplied by stem cuttings for distribution. Therefore, the principle of developing clonally propagated cultivars for other crops also applies to cassava.

Briefly, large segregating populations are first created from which initial seedling screening is performed. Plants selected from the initial screening are then evaluated in subsequent replicated, multi-location trials that would eventually produce one or more superior clones possessing desirable traits.

The following flowchart describes the general aspects of a cassava breeding procedure:

1. Population development
2. Seedling evaluation and selection
3. Clonal evaluation and selection
4. Preliminary yield trial and selection
5. Advanced yield trials and selection
6. Regional trials

While the size of the initial population is large (for example, 50,000 seedlings), the number of entries is drastically reduced following each step of selection, and at the end of the regional trial, only one or a few clones may endure the rigorous selection process and are released as new cultivars.

Cassava Hybridization Techniques

Fig. 17 Hand pollination in CIAT cassava field. Palmira, Colombia. Photo: Melissa Reichwage. Licensed under CC BY-ND 2.0.

Preparation of female flowers

To determine if a female flower is about to open, Kawano (1980) described a reliable method by which a petal of an unopened female flower is peeled back, if a drop of nectar is observed at the base of the pistil, the flower will open in the afternoon of the same day (Fig. 17). Female flowers ready to be pollinated are then covered with a large (20 x 25 cm) cloth bag to avoid stray pollen. Emasculation is generally not necessary because male flowers on the same inflorescence will not open until 1 to 2 weeks later when the female flowers either developed into fruits or have died.

Preparation of male flowers

Between noon and 2pm in the afternoon, freshly open male flowers are collected by glass bottle or other suitable devices and pollination can be performed immediately. A single male flower can pollinate up to three female flowers. Pollination performed after 5pm will not be as effective.

Pollinated female flowers can be left uncovered to promote seed development with minimal risk of hybridization by stray pollen. However covering of the pollinated flowers with a small cloth bag is needed 1 or 2 weeks after the pollination in order to collect seeds.

Breeding Scheme

Fig. 18 Cassava breeding program in Uganda. Photo by Jessica Barb, Iowa State University.

Population development

To increase the chance of obtaining a superior genotype, distinct genotypes with diverse genetic backgrounds are selected for creating the base segregating population from which evaluation and selection will be performed.

Three methods are used to create the base population:

1. **Controlled biparental crosses** – In this case, male flowers are collected from the male parent and are used to pollinate female flowers of the chosen female parents. Seed yield from such crosses is limited because of the amount of labor involved. Many such crosses may be needed to produce a large base population. Pollination in cassava, however is viewed as one of easiest among all the crops because of its large flowers, large and sticky

pollen and no need of emasculation, therefore, this method can still be productive.

2. **Use of crossing blocks** – In this case, a set of cultivars are grown in an isolated crossing block. Male flowers are removed from all cultivars that are chosen to be female parents and seed harvested from the female parents are hybrid seeds that can be used to start the selection process. The physical separation of male and female flowers in a cassava plant makes it easy to remove male flowers. This method is commonly used in recurrent selection.
3. **Polycross** – In this case, each elite parent is replicated several times and they are randomly grown in a polycross nursery. Random pollination among parents ensure a fair representation of each parent in the progeny. This method is more efficient in producing sufficient quantity of hybrid seed than the biparental method. However, it does not prevent self-pollination.

Seedling evaluation trial (Year 1)

Once a base population is created using one of the methods described earlier, selection can immediately start. Up to 100, 000 hybrid seeds are directly sown in the field and seedlings will be screened for resistance to major disease such as cassava mosaic disease, cassava brown streak disease and for ideal growth habit, i.e., branching at a medium height (100cm for example) and low hydrogen cyanide (HCN) content in leaves. At harvest, additional screening is performed for plants producing compact roots with short necks.

In this step, selection is concentrated on eliminating poor genotypes rather than selecting for good genotypes. A total of up to 3,000 individuals may be selected for further testing.

Clonal evaluation trial (Year 2)

Entries selected from the seedling evaluation trial are multiplied by stem cuttings and each entry is grown in single-row plot without replication. Resistance to major diseases is again screened. In addition, root yield, root dry matter content as well as the HCN content in the root of each entry is evaluated. A locally-adapted leading cultivar is grown as a check to aid selection. A total of up to 100 individuals are selected.

Preliminary yield trial (Year 3)

Stem cuttings from each of the selected entries are grown into larger plots that are replicated at

least twice, along with a locally-adapted check cultivar to evaluate yield potential. This trial is conducted in multiple locations. The clonal entries within each location is randomized. Besides root yield, root quality and resistance to major disease and other pests will be continuously monitored. Evaluation of consumer acceptance is conducted at this stage. A total of 25 genotypes may be selected at the end of the trial.

Advanced yield trial (Year 4)

Stem cuttings from the selected genotypes are grown into larger plot size with more replications (for example 4) and more locations, along with a locally-adapted check cultivar. Selection is primarily focused on root yield and root quality traits. At the end of the trials, up to 5 genotypes may be selected.

Regional yield trial (Year 5)

At this stage, the best clones are grown on large-scale farms in multiple locations within the target region. At least four replications are used within each location.

Final selection will be made based on data for yield, root quality, and consumer acceptance. Planting materials will be rapidly multiplied and distributed to end-users.

Note that “upgraded” base populations can be continuously created by crossing elite entries selected at the end of each step and additional germplasm from other sources. A new cycle of selection can then be performed on the “upgraded” base populations.

Farmer Participatory Trial

Traditionally, farmers are not involved in the breeding process until a new cultivar is close to being released commercially, therefore farmers have limited influence on how new cultivars are developed.

Participatory Plant Breeding (PPB) is a process in which farmers play an active role early in the breeding process and it has become increasingly important in breeding grain crops as well as vegetatively propagated crops such as cassava. For example, in developing early bulking cassava cultivars in Kenya, farmers are invited to participate in the selection process after the 1st clonal evaluation trial. Farmers evaluated root quality traits including appearance, size, taste/texture,

and their inputs were used along with those of the breeders in making final selection decisions. A similar effort involving farmers early in the breeding process was also reported in developing cassava mosaic disease-resistant cultivars in Ghana.

Subsistence farmers in Africa often grow multiple crops/cultivars to counter the uncertain and adverse climatic conditions. Under such circumstances, PPB has been proven very useful and it also increased the adoption rate of new cultivars released by programs in which farmers have actively participated in selecting.

Germplasm Conservation Centers and Practice

Genetic diversity is essential to plant breeding. Modern agricultural practices and the destruction of native cassava habitats in the centers of diversity cause significant erosion of cassava genetic resource. Therefore, collection and conservation of cassava germplasm is of paramount importance to sustained successes in cassava breeding. Great efforts have been put forth to collect and conserve cassava germplasm. Table 1 lists the number of accessions maintained at each location. The international institutes, the Centro Internacional de Agricultura Tropical (CIAT), Colombia, and the International Institute for Tropical Agriculture (IITA), Nigeria, both of which hold the UN mandate for cassava maintain a large number of accessions. These accessions are available freely to the public.

Cassava germplasm is typically conserved in one of the three methods:

Field genebanks

Cassava plants representing each accession are grown and maintained in the field. As is practiced at IITA, eleven plants of each accession are grown on a 2.5m row plot with a 25cm space between plants and 50cm space between rows. Field genebanks require a large amount of field space and germplasm may be lost due to various biotic and abiotic stresses.

Seed genebank

Seed of each accession are maintained in controlled environment with low temperatures and humidity. It has been reported that cassava seeds remain viable after 14 years of hermetic

storage at -20°C with 6% moisture content in the seed. As is practiced at IITA, seeds representing each accession are harvested in bulk from all plants of an accession and are therefore heterogeneous.

In vitro genebanks

Table 1 The second report on the state of the world's plant genetic resources for food and agriculture. Adapted from FAOSTAT 2010, Rome.

Location	Number of accessions	Type of accession (%)				
		Wild species	Landraces/ old cultivars	Research materials/ breeding lines	Advanced cultivars	Others/ mixture
CIAT	5436	1	87	11	0	1
Brazil	2889	0	0	0	0	100
IITA	2756	0	28	47	0	25
India	1327	0	0	0	0	100
Nigeria	1174	0	0	0	0	100
Uganda	1136	0	4	89	7	0
Malawi	978	0	22	72	6	0
Indonesia	954	0	0	0	100	0
Thailand	609	0	0	100	0	0
Benin	600	0	100	0	0	0
Togo	435	0	100	0	0	0
Other	14148	6	26	3	14	51

In vitro cultures established from apical buds or nodes containing axillary buds are maintained on culture media or culture conditions that slow down the growth of the culture. Such cultures can be maintained for 8-12 months without the need for subculture. Following disease and virus indexing, these cultures are suitable for exchange among collaborators across countries.

Cassava Genetics

Marker-assisted breeding in cassava

DNA markers are variations in DNA sequences, and such variations among individuals can be easily detected using polymerase chain reaction (PCR) or high throughput sequencing technology. DNA markers, unlike phenotypes are not affected by environment or developmental stages; therefore they can be assessed at any time and from any plant tissue. They are used for cultivar fingerprinting, assessing genetic diversity or marker-assisted selection for traits for which markers are available.

In cassava, many simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers have been developed. Recently, a high-resolution composite map covering 2412cM and organizing 22,403 genetic markers on 18 chromosomes has been described for cassava (International Cassava Genetic Map Consortium). This map will greatly facilitate marker-assisted selection in cassava, particularly for selecting plants resistant to the cassava mosaic disease for which linked DNA markers have been developed.

Sequencing of the cassava genome

The genome size of cassava is estimated to be ~770Mbp. It has recently been sequenced from a partially inbred line AM560-2 developed at CIAT. The complete sequence is made available by the Joint Genome Institute (JGI). A resequencing project in 2012 report a genome size of 760 Mbp. The sequenced genome will undoubtedly enable a whole array of research aimed at improving the crop.

Application of Biotechnology in Cassava

Traditional plant breeding is a very long and imprecise process and it could take more than a decade to release an improved new cultivar. Genetic transformation, however can introduce a trait very efficiently and rapidly. Furthermore it can break the reproductive barrier and transfer traits from unrelated species to cassava. Because cassava is a vegetatively propagated species, traits introduced into an existing elite cultivar or a farmer-favored landrace can be “fixed”

immediately without the need of inbreeding or backcross as is the case in sexually propagated species.

Successful genetic transformation has been accomplished in cassava using either Agrobacterium or particle bombardment. The key to the success relies on an efficient plant regeneration protocol. The current standard practice is to use the friable embryogenic callus (FEC) derived from immature leaf explants cultured in vitro for gene transfer.

The potential of using a transgenic approach to produce novel cassava germplasm has been demonstrated in transgenic lines with insect and disease resistance, herbicide tolerance, altered starch content and increased protein level as well as reduced cyanogenic content in cassava storage roots. Another noted example is the biofortified cassava generated by the BioCassava Plus (BC+) program supported by the Bill and Melinda Gates Foundation. These biofortified cassava varieties demonstrated the potential of developing cassava as a more nutritionally complete crop with increased zinc, iron, proteins and vitamin A.

Major accomplishments in cassava breeding

Notable is the consistent increase in production and harvested area despite the low yields in Africa compared to Asia and Latin America/Caribbean (Figs 19, 20, 21). Thus there is potential for even greater increases in Africa with the increased availability of improve cultivars.

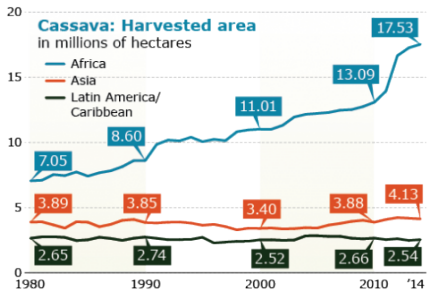

Fig. 19 Harvested area of cassava. Data source: FAOSTAT statistical database.

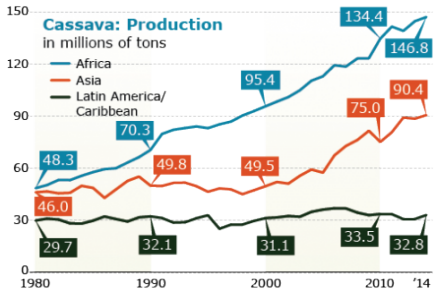

Fig. 20 Cassava production. Data source: FAOSTAT statistical database.

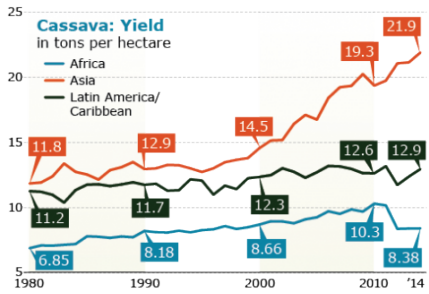

Fig. 21 Cassava yields. Data source: FAOSTAT statistical database.

References

Allem, A.C. 2002. The origins and taxonomy of cassava. In: R. J. Hillocks, J. M. Thresh and A. C. Bellotti, editors, Cassava: Biology, Production and Utilization. CABI Publishing. p. 1-16.

Ceballos, H., E. Okogbenin, J.C. Pérez, L.A.B. López-Valle, and D. Debouck. Cassava 2010. In J. E. Bradshaw editor, Root and Tuber Crops, Handbook of Plant Breeding. Springer New York Dordrecht Heidelberg London. p. 53-96.

FAOSTAT. https://www.fao.org/faostat/en/#data/QCL/visualize

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Chapter 16: Seed Systems and Certification

Teshale Mamo; Asheesh Singh; and Anthony A. Mahama

Seed is a basic and fundamental input for agriculture. Accessibility of high-quality seed is one of the basic requirements to increase crop productivity, production and use (Pelmer, 2005). The dissemination and use of high-quality seeds have great benefits to increase and continue crop production, improve household incomes, minimize risks of insect pests and plant diseases, and enhance the crop production patterns, which would increase agriculture sustainability. Therefore, viable seed supply system strategies are important to ensure the availability of good quality varieties of seed to farmers in a timely and affordable fashion (FAO, 1999).

Learning Objectives

- Differentiate between formal (commercial), informal, and semi-formal (integrated) seed systems and their development
- Develop knowledge of seed regulation systems
- Know the International Union For The Protection Of New Varieties of Plants (UPOV)
- Demonstration knowledge of Breeders' Right
- Know the different classes of seed

Current Seed System in Sub-Saharan Africa

Formal Seed System

The seed system represents involvement and interconnection among different organizations, institutions, and individuals associated with the development of new varieties and producing, testing, processing, storage, certifying and marketing seed to the farmers. Public and private sectors are highly involved in the production of different classes of seed for domestic/local use and export market. In sub-Saharan Africa, the majority of smallholder farmers are involved in various kinds of seed systems, which benefit them to produce and obtain the seed they need. There are three broad categories of seed systems in sub-Saharan Africa: Formal (commercial)

seed system, informal seed system (local seed supply system), and integrated seed system (community-based) (Table 1). The detailed description of each seed system is outlined below.

The Formal (commercial) Seed System

The formal seed supply system is highly regulated and covers seed production and supply mechanisms. This system involves a chain of activities leading to clearly defined products, i.e., certified seed of verified cultivars (Louwaars, 1994). It involves formal plant breeding, multiplication by seed companies, following established procedures including processing, bagging, labeling, and marketing. The formal systems also follow the standard of distinctness, uniformity and stability (DUS) of varieties. The system also assures that the cultivar identity and purity are kept throughout various levels of seed multiplication (Breeder/Prebasic to Foundation/Basic to Registered and/or certified seed). The main participants in a formal seed supply system are private and public sectors, and mainly focus on major economically viable crop species with good recurrent seed demands, such as hybrid maize. This kind of seed system is dominant in developed countries. It is a more complex system compared to the informal seed system. The formal seed system produces about 10-20% of the seed demands in Africa (Wekundah, 2012).

Informal Seed System

Informal seed supply system is also sometimes called as 'farmer seed system' or 'traditional seed system'. It is a chain of seed multiplication and marketing steps that involve farmers who produce, disseminate or access seed through farmer-to-farmer seed exchange based on barter system and through local grain/seed markets mainly based on indigenous knowledge and local diffusion mechanism. In addition, small private companies and farmers cooperatives are involved in seed production in many countries for example, Tanzania, Uganda and Malawi. The informal seed supply system is mostly characterized by its flexibility and operates under non-law regulated conditions. Cultivars may be landraces/local varieties or mixture of different varieties of the same races or may be heterogeneous. Besides, the seed may be of variable quality in terms of purity, physical and physiological quality (Almekinders and Louwaars, 1999). Though the informal seed supply is not formally framed, it covers the majority of seed related activities in most of sub-Saharan Africa and contributes about 80-100% of seed supply to the farmers (Maredia et al. 1999; Wekundah, 2012). It can also enhance wide diffusion of seed over relatively wide areas and promote the small scale seed businesses in the region (Sperling and Cooper,

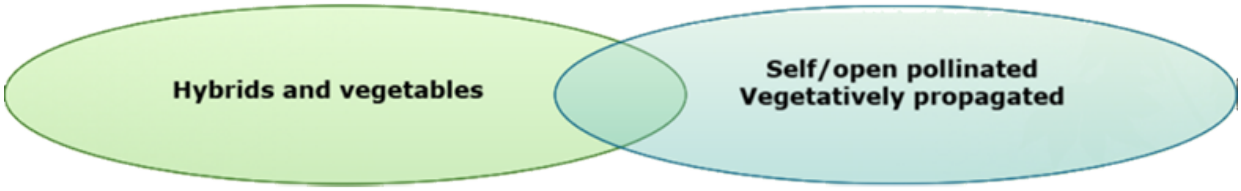
2004). However, little is known about the system, production and marketing chain due to lack of regulation.

The informal seed system is believed to help the farmers due to the following factors:

- Retain seed on farm from previous harvest/farm saved seeds
- Farmer-to-farmer exchange networks
- The seed do not go under certification process so it is less expensive

Seed Sector Development

Table 1 Range of seed sector development. Data from FAO, 2010.

Formal seed system		**Informal seed system**
		
Medium to large companies	Small scale enterprise	On-farm management

Plant breeding capacity	Limited to public sector	Farmer selection
Variety registration and release process	Not applicable	Inexistent
Early generation seed production capacity	Not applicable	Inexistent
Seed policy and regulatory framework adapted	Inexistent or not always adapted	Inexistent
Seed quality assurance and capacity	Inadequate capacity	Not applicable
Seed production capacity	Limited	Farmer saved seed
Seed conditioning, storage capacity	Limited	Farmer's practice
Entrepreneurial capacity	Limited	Not applicable
Access to credit	Limited	Not applicable
Market access	Limited	Seed exchange or local market
		

Integrated Seed Supply Systems (Semi-formal Seed Supply System)

This is a mix of informal and formal seed supply systems. Small farmers and community-based organizations such as small famers' cooperatives multiply and sell a small amount of good affirmed seed of improved cultivars to other farmers within a restricted production area with the least possible quality control (Alemkinders and Louwaars, 1999).

Variety Release Regulations

Seed Regulation Systems

Most sub-Saharan African countries differ highly in seed regulation systems. This seed regulation system is comprised of seed quality control/certification and cultivar regulation. Cultivar regulation system follows steps to control the release of cultivars both by private seed companies and government-owned research institution breeding programs. The cultivar registration requires new cultivars to show distinctiveness, uniformity and stability (DUS), and

value for cultivation and use (VCU) before being officially registered (Setimela et al., 2009). Each cultivar registration is performed by national and private breeding programs. Meanwhile, the national cultivar releasing committees have different criteria to register a new variety. Depending on the country's variety release regulations, the DUS and the VCU tests may take one to three years (three seasons) before enough data are available for cultivar registration. The seed law in terms of evaluation and release of varieties are different and inconsistent among sub-Saharan African countries. These different regulations and inconsistent seed laws (and implementation) among countries make it costly and discouraging for private seed companies to release and market their new cultivars.

Most crop breeding programs in sub-Saharan Africa differ in their capacity. Some national and regional crop breeding programs focus on testing lines introduced from other countries, while others have established their own crossing/hybridization programs to develop breeding lines targeting specific and wide crop growing environments. The consultative Group for International Agricultural Research (CGIAR) such as International Maize and Wheat Improvement Center (CIMMYT), International Institute for Tropical Agriculture (IITA), International Rice Research Institute (IRRI), International Crops Research Institute for Semi-Arid Tropics (ICRISAT), International Center for Tropical Agriculture (CIAT) and International Center for Agricultural Research in the Dry Areas (ICARDA) have helped and contributed for crop varieties and other crop technology development (David and Sperlings, 1999).

Common Features of Regulations

In most African countries the following common features of regulations for cultivar release have been established:

- Developed guidelines and standard procedures for testing cultivars proposed for release.
- Independent national varietal releasing committee (NVRC) formed with a mandate to recommend for release or reject based on test results.
- Officially released cultivars that have been recommended by NVRC should be registered and made available to the public. Sufficient information on morphological description, year of release, variety name, and releasing institutions should be clearly indicated.

The International Union For The Protection Of New Varieties of Plants (UPOV)

The International Union for the Protection of New Varieties of Plants (UPOV) is an intergovernmental organization with a goal of providing and promoting plant variety protection (UPVO, 2015). The main objective of UPVO is to strengthen the development of new cultivars that benefit the farmers (UPVO, 2015). The UPOV helps to recognize the rights of plant breeders for the varieties they develop. The UPVO convention provides intellectual property rights to the breeder that enables her/him to have full authority on seed multiplication of her/his cultivar. The breeder's right is implemented if the variety is new, distinct, uniform and stable (UPVO, 2015). Among sub-Saharan African countries, South Africa, Kenya, Morocco, and Tunisia are the only members of UPOV (UPOV 2017). However, Africa has its own regional organization called African regional intellectual property organization (ARIPO) with the main objective of pooling resources of its member countries to solve intellectual property (IP) and related issues through harmonizing IP laws and facilitating IP activities within member countries and distributing of information associated with IP. There are 19 member countries in ARIPO: Botswana, The Gambia, Ghana, Kenya, Lesotho, Malawi, Mozambique, Namibia, Sierra Leone, Liberia, Rwanda, São Tomé and Príncipe, Somalia, Sudan, Swaziland, Tanzania, Uganda, Zambia and Zimbabwe.

Plant Breeder's Right

Plant breeder's right: is an intellectual property right granted to a crop breeder in respect to new plant varieties developed by him/her against exploitation without his/her permission. The breeder has exclusive control over his/her new plant materials such as seed, cuttings, tissue culture and harvested materials including fruit and foliage for a number of years. This provides to a plant breeder a recognition and economic reward for his effort and also energizes the plant breeder to continue developing new and better high yield good quality varieties. According to South Africa's plant breeders' Rights Act (Act 15 of 1976), once the plant materials or cultivars are approved then the plant breeder is given a certificate of plant breeder's right. This plant breeder's right is valid for 25 years in the case of vines and trees, and for 20 years for annual crops, which is started from the date on which a certificate of registration is given.

- Eligibility for protection; the cultivar
  - must be new,

    - distinct
    - uniform
    - Stable and have acceptable variety name
- **Distinct**: it is distinguishable from any other existing cultivars of common knowledge at the time of application.
- The new variety should be **uniform**: It should be adequately uniform in its unique characteristics.
- The new variety should be s**table** (DUS): The essential characteristics of the new varieties should remain unchanged after repeated propagation or multiplications.

Who Can Apply

Who can apply for a plant breeder's right: a breeder who bred the cultivars and the employer of the breeder who bred the varieties.

According to the South Africa breeder's right: the following steps must be authorized by the breeder:

- Seed production and reproduction
- Permission for sale
- Exporting and importing

Right of plant breeders: the breeder who developed new varieties has the following rights:

- The right to sell his/her new varieties including the right to delegate other persons to sell or multiply his/her new varieties.
- Full right to multiply his/her new cultivars including the right to authorize other persons to multiply or propagate his/her varieties for sale

Variety Performance Testing

This is a variety trial focusing on the selection of new cultivars with desirable traits that could meet the requirements of farmers or consumers. This test ensures that the new cultivar is similar to or better than the existing cultivars in terms of agronomic characteristics such as grain yield and diseases and insect pest resistance. In the majority of sub-Saharan African

countries such as in Uganda, Malawi, Ghana, Kenya, Tanzania, Rwanda, Burundi, Nigeria, Cameroon, Angola, and Zambia, multi-environment and multi-year variety trials are conducted across different agro-ecological zones to select better performing cultivars (FAO, 1994). The new cultivars have to show better performance in acceptable number of tests in comparison to existing/commercial cultivar(s). The variety performance testing usually includes testing for two to three years in regional or national varietal trials at least in 3 or 4 locations before being recommended for release (Bishaw and Gastel, 2009).

The cultivar which is proposed to be released should be uniform, stable and distinctly better than the existing commercial cultivars in the environments where it is intended to be grown and should have also good agronomic characters and fulfill farmers' requirements. The decision for variety release is made by National Variety Releasing Committee (NVRC). However, in some countries where not enough number of released varieties are available with the unique quality of a particular crop, the NVRC may consider releasing of varieties despite not being better than the existing commercial varieties (Setimela et al., 2009). All sub-Saharan African countries have their own variety release procedure in place even if it is done by an ad hoc committee or by officially assigned authority (Bishaw and Gastel, 2009).

Variety Release

After the variety is officially released and registered, the breeder or institution makes appropriate quantities of the breeder seed and basic seed available for commercial seed multiplication and marketing (Bishaw and Gastel, 2009). In a new development, Drought Tolerant Maize for Africa (DTMA) governed under CIMMYT has proposed a regional harmonization of seed laws in eastern, southern and western African countries, and they will get advantages from the free flow or exchange of maize germplasm across the regions if the regional maize variety release process is implemented. The seed laws allow a maize cultivar released in one country to be considered for automatic release in neighboring countries with similar environments. This helps to release varieties in mega-environments that cover large adaptation zones across country boundaries and also helps to link and create a big maize seed market and fast variety release across the regions (Setimela et al., 2009)

Condition for Release

- Appropriate documents and based on guide, clear morphological description,

distinguishing characters, vegetative description and quality test (palatability, taste etc.)
- Sufficient season data from multiple sites [check country registration system guidelines], wide adaptation (at national level), specific adaptation (regional level).
- The new cultivar has to show better performance compared to existing commercial cultivars in environments where it is intended to be grown.
- The cultivars should show distinctiveness, uniformity, Stability (DUS), and value for cultivation and use (VCU).

Seed Quality and Certification

Seed certification is a tool to produce genetically pure and good quality standard seed of improved varieties for farmers. Also, it is appraised for true to type physical purity, germination, seed health and moisture contents, true labeling, backed with appropriate laws and regulation and DUS. The newly released variety must have excellent seed quality attributes which is critical to crop production whereas if the seed is of poor quality, it lowers the potential yield of the variety.

Seed Quality Attributes

- Genetic purity: The seeds have to be genetically pure, this means true-to-type of the specific seed lot. For example breeder seeds must be 100%, foundation seed 99.5%, and certified seed varieties 98 % genetically pure (Brijesh Tiwari, 2014).
- Physical purity or physical qualities: This is characterized by minimum of damaged seed (broken, cracked or shriveled seed), minimal noxious weed seed or other crop seeds and inert matter, minimal diseased seed (discolored or stained seed) in a sample seed lot.
- Physiological attributes/physical qualities: This includes high germination and vigor of the seed.
- Seed health: This refers to free from diseases and insect pests. Example, seedborne diseases could have impact on the health and productivity of the crop which may cause contamination of the seed lot.

Seed Production

Current seed production systems in sub-Saharan African countries include both the formal,

which involves both the public institutions and private seed companies, or informal, which includes small scale informal village and community level seed production (Table 3). Hybrid maize seed production is mainly run by both public and private seed companies, whereas legume crops seeds are not extensively produced by public and private seed companies, and they are mostly produced by informal village and community level seed production. Legumes are not widely commercial crops in most Africa countries therefore market demand for good quality and uniform seeds is low (Muigai et al., 2010). In advanced or formal seed production system, five different classes of standard are known, though each country has its own specification based on the affiliate international protocols such as International Seed Testing Association (ISTA) or OECD seed schemes, or Union for the Protection of Varieties (UPOV). For example, the seed laboratory of Zimbabwe and Zambia is mandated for seed quality control and is accredited to the international Seed Testing Association (ISTA) (Muigai et al., 2010).

Different Classes of Seed

In sub-Saharan Africa, four major classes of seeds are currently being produced by public institutions and private companies. Even some of the countries such as South Africa, Kenya, and Zimbabwe have accredited seed certification by OECD (Organization for Economic Cooperation and Development (Europe) and AOSCA (Association of official seed Certifying Agency (Table 1).

Seed Classes

1. **Breeder seed:** This is the highest purity of the new cultivar and maintained and multiplied by breeder, and provided to the seed companies for multiplication by breeder's institutions. This class of seed is used to increase foundation seed.
2. **Foundation seed:** This is a class of seed produced from breeder seed. The breeder and research institutions are the ones who help to keep genetic purity and identity. Depending on the seed regulation of the country, foundation seeds could be produced by public or private seed companies.
3. **Registered seed:** This class of seed is produced from foundation seed and is produced by selected farmers and seed companies under the seed regulation agency to keep varietal identity and purity. In most countries, production of registered seed undergoes field and seed (lab) inspection by representative seed inspectors to ensure the fulfillment of the standards.

4. **Certified seed:** This class of seed is produced from foundation or registered seed, or sometimes from certified seed and is available to farmers for general production. It is grown by selected farmers who have experience and capacity to produce the certified seed. This helps to maintain varietal purity. Production of certified seed is subjected to field and seed (lab) inspection priority to approval by certifying agency.

Comparing Class Systems

Table 2 Seed class system of Organization for Economic Cooperation and Development (OECD) and comparable US seed classes. Data from www.oecd.library.org.

US Seed Class	Label color	Equivalent OECD Seed Classes	OECD Label color
Breeder	n/a	Prebasic	White with diagonal violet stripe
Foundation	White	Basic	White
Registered	Purple	Basic	White
Certified	Blue	1st Generation Certified Seed	Blue
Certified produced from certified	Blue	2nd Generation Certified Seed	Red

Table 3 Differences between the formal and informal sector. Data from Minot et al. , 2007

Component of seed system	Formal seed system	Informal seed system
Varietal development	Plant breeders employed by the public institution or private company select for specific traits and produced varietal pure "breeder seed"	Farmers select seed from plants with desirable traits, but the seed is not necessarily varietally pure
Seed production	State or private seed companies multiply seed under strict conditions to avoid mixture of varieties; sometimes contract farmers.	Farmers produce seed along with crops; in some cases the portion of the crop destined for seed is given special management
Processing	Seed is dried using mechanical dryers. Seed may be cleaned by hand, processing machinery used to remove dirt, dried in the sun, and sometimes rocks, and seeds of other plants. May be treated to extend shelf-life.	Seed may be cleaned by hand, dried in the sun, and sometimes treated to extend shelf-life.
Certification	Seed is usually subjected to some formal quality control procedure based on tests of purity and germination of random samples	Seed is generally not tested, certified, or labeled.
Distribution	Seed is bagged and labeled, and distributed by stockists, extension agents, NGOs, and cooperatives	Farmers use seed they save from previous harvest, acquired from other farmers through barter, gift, or sales, or acquired in local grain markets.

References

Almekinders, C., and N. Louwaars. 1999. Farmers' seed production: New approaches and practices. London: Intermediate Technology Publications, Ltd. Bishaw, Z. & van Gastel, A.J.G. 2009. Variety release and policy options. pp. 565587, in: S. Ceccarelli, E.P. Guimaraes and E. Weltzien (editors). Plant Breeding and Farmer Participation.

Bishaw, Z. & van Gastel, A.J.G. 2009. Variety release and policy options. pp. 565–587, *in:* S. Ceccarelli, E.P. Guimaraes and E. Weltzien (editors). *Plant Breeding and Farmer Participation.*

Brijesh Tiwari. 2014. Seed production and quality control. Published by Oxford Book Company, Technology and engineering_ 272.

David S., and L. Sperling. 1999. Improving the technology delivery mechanisms: lessons from

bean seed systems research in east and central Africa. Agriculture and Human Values 16: 381-388.

FAO seed review 1989-1990: Food and Agricultural Organization of the United Nations. 1994.

FAO. 1999. Seed policy and programmes for sub-Saharan Africa. Proceeding of the regional technical meeting on seed policy and programmes for sub-Saharan Africa. Abijan, Cote d'lvoire, 23-27 Nov. 1998.

FAO. 2010. SoW-PGRFA, Chapter 2 Gaps & Needs; Country level assessments; Seed trends papers; Africa seed review papers; Countries' requests; Electronic consultations; FAO/OECD/UPOV/ISF/ISTA World Seed Conference, 8-10 September, 2009.

International Union for the Protection of New Varieties of Plants (UPVO). 2015. Publication No. 437(EN).

International Union for the Protection of New Varieties of Plants (UPVO). 2017. Publication No. 423(PDF).

Louwaars, N. 1994 Seed supply systems in the tropics: International course on seed production and seed technology. Wageningen, the Netherlands: International Agriculture Centre.

Maredia, M., J. Howard, D. Boughton, A. Naseen, M. Wanzala, and K. Kajisa. 1999. Increasing Seed System Efficiency in Africa: Concepts, strategies and issues. Michigan State University International Development Working Paper. Department of Agricultural Economics- MSU East Lansing Michigan, pp 12-13.

Minot, N., M. Smale, C. Eicher, T. Jayne, J. Kling, D. Horna, and R. Myers. 2007. Seed development programs in sub-Saharan Africa: A review of experiences.

Muigai, C., F. Ogana, B. Shiferaw, F. Simtowe, and S. Asfaw. 2010. Integrated innovations for improving Legume Productivity, Market Linkages and Risk Management in Eastern and research to downstream applications. PNAS 102 (44) 15739-15746.

Pelmer D.P. 2005. Agriculture in developing world: connecting innovation in plant breeding res.

Quality Declared Seed: Technical Guidelines for Standards and Procedures, 2006, FAO Plant Production and Protection Paper 185 Rome Italy.

Setimela P.S., B. Badu-Apraku, and W. Mwangi. 2009. Variety testing and release Approaches in DTMA project countries in sub-Saharan Africa. Harare, Zimbabwe, CIMMYT.

Southern Africa. Seed Production and Marketing: Handbook for Farmer Organizations and Agro-enterprise.

Sperling, L., and H.D. Cooper. 'Understanding seed systems and strengthening seed security: a Background paper.' In: Sperling, T. Osborn and H.D. Cooper eds. towards effective and sustainable seed relief activities. Rome, Italy: FAO plant production and protection paper, 181. 2004.

Wekundah, J. 2012. Why informal seed sector is important in food security. African Technology policy studies network biotechnology trust Africa. Special paper series. No. 43 pp 8.

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Suza is an Adjunct Associate Professor at Iowa State University. He teaches courses on Genetics and Crop Physiology in the Department of Agronomy. In addition to co-developing courses for the ISU Distance MS in Plant Breeding Program, Suza also served as the director of Plant Breeding e-Learning in Africa Program (PBEA) for 8 years. With PBEA, Suza helped provide access to open educational resources on topics related to the genetic improvement of crops. His research is on the metabolism and physiology of plant sterols. Suza holds a Ph.D. in the plant sciences area (with emphasis in molecular physiology) from the University of Nebraska-Lincoln.

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Lamkey is the Associate Dean for Facilities and Operations for the College of Agriculture and Life Sciences at Iowa State University. He works in collaboration with the dean, associate deans, department chairs, college-level centers, and other unit leaders to ensure that operations directly advance the mission of the college and that resources are deployed wisely and efficiently. Previously, he served as the chair for the Department of Agronomy at Iowa State University, where, in addition to advocating for research and the PBEA program, he oversaw the Agronomy Department's educational direction, its faculty, and Agronomy Extension and Outreach. Dr. Lamkey is a corn breeder and quantitative geneticist and conducts research on the quantitative genetics of selection response, inbreeding depression, and heterosis. He holds a Ph.D. in plant breeding from Iowa State University and a master's in plant breeding from the University of Illinois. Lamkey is a fellow of the American Society of Agronomy and the Crop Science Society of America and has served as an associate editor, technical editor, and editor for *Crop Science.*

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Applied Learning Activities

The following downloadable Applied Learning Activities (ALAs) are associated with the Crop Improvement course:

- ALA 1-(Breeding Objectives) [DOC]
- ALA 2-(MTA) [DOC]
- ALA 3-(Quantitative and Qualitative traits) [DOC]
- ALA 4-(Development of simulated QTL mapping populations) [DOC]
- ALA 5-(PPB1) [DOC]
- ALA 6-(PPB2) [DOC]
- ALA 10-(BMS platform – Make germplasm list from existing database) [DOC]
- ALA 11-(BMS platform – Import germplasm list made in excel to BMS) [DOC]
- ALA 12-(Use of marker for patent protection) [DOC]
- ALA 13-(Cultivar Development) [DOC]