Fundamentals of seed production I: Genetics, breeding and seed production

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(2) I- INTRODUCTION

(3) One of the fundamental goals of agriculture is the production of food, feed and fiber for an increasing number of people in the world. As seen in this graph, world population increased exponentially during the past century, reaching over 6 billion people in the year 2000. There is no indication that this increase will stop in the near future and it is estimated that by 2050, the world population will be 9 billion people. In addition to this increase in population, in the last 50 years, the demand for meat has quadrupled and it is estimated that 40% of the grain produced in the world will be used for livestock feed. (4) An obvious question from these trends is how will we address this increasing demand for food, feed and fiber? In order to supply this increasing demand, different areas of production management such as breeding, establishment techniques, irrigation, nutrient supply, pest control and postharvest must be improved. (5) Among these areas, breeding of cultivated plants has been most important, with estimates that it represents about 50% of the total increase in yield of most cultivated species during the last 50 to 60 years (Duvick, 1996).

Breeding can be defined as the art and science of the genetic improvement of plants (Fehr, 1987). Its origin is probably as old as the origin of agriculture when humans first selected one type of plant or seed in preference for another. Over time, the science of breeding has become increasingly important because of the advancement of our knowledge in plant biology and genetics. (6) During the last 50 years, the main objectives of breeding have been focused on yield increase. This figure shows the dramatic increase in maize yields between 1870 and 1997. The development of double cross hybrids from the 1930s to 1960s in maize resulted in substantial yield improvement compared to old open-pollinated cultivars. Later, the adoption of single cross hybrids increased maize yields even more.
During the 1960s and 1970s, dramatic yield improvements in wheat and rice also played an important role in augmenting world food production, a phenomenon now known as the Green Revolution. These yield increases were associated with the development of short-statured cultivars that performed extraordinarily well in highly productive environments.

(7) Other common objectives of breeding during the past 50 years have been (Fehr, 1987):
Disease and insect resistance
Seed Composition
Forage quality
Tolerance to environmental stresses
Adaptability to mechanization
Lodging resistance
Photoperiod response

(8) Today, there is a tendency to focus breeding objectives more on quality traits, such as increasing feed efficiency, improvement of flavor or healthier composition of products. In

addition, more desirable traits have been accumulated or "stacked" into individual cultivars. Such trait stacking is evident not only in multiple disease-resistance traits, but also in transgenic input traits available in commercial cultivars for insect and herbicide resistance (Baenziger et al. 2006).

Breeding objectives have also shifted from more traditional uses for feed and food crops to new areas such as ornamental, recreational, medicinal and industrial uses. Examples of these new exciting applications include the production of polymers and biofuels. In switchgrass (*Panicum virgatum* L.), for example, new efforts in breeding are for biomass production and composition traits that improve the use of this plant as a source for ethanol production (Baenziger et al. 2006).

(9) During the last 50 years, an applicaton that has had a major impact in plant breeding is biotechnology (Baenziger et al. 2006). Since its introduction in 1995, production of genetically modified crops has increased dramatically. In the United States, for example, commercial production of transgenic soybean, cotton and maize represented 87, 79, and 52% of the total national acreage for 2005, respectively.

Today, companies are using biotechnology to enhance traits important for the producer, processor and consumer, including enhanced yield, pest control, disease resistance, resistance to abiotic stresses such as heat and drought, and improved compositional quality for processing and health benefits (Baenziger et al. 2006).

Development and release of new cultivars is a process that requires a significant investment of time and money and seed companies must recover this investment. (10) In this context, seeds have become more than a fundamental input of agriculture because they represent the carriers of these genetic enhancements. Therefore, seeds must be of high quality, genetically pure and capable of performing under diverse environments.

A fundamental objective of seed production is to increase the number of seeds of a particular genotype. During this process, particular traits or attributes that characterize a cultivar must be conserved, which is acheived by maintaining high genetic purity. If the seed produced does not represent the genetic purity of the cultivar, it will loose value, even if it has high germination and vigor. Because of this, seed producers understand the value of the genotype or cultivar they increase as well as its economic value. As a result, this presentation introduces basic concepts of genetics and plant breeding which are important for understanding the fundaments of seed production.

(11) Activities involved in seed production

This figure shows the procedures involved in the production and handling of commercial seed lots. Breeding or genetic selection is the very first step in the process, and its objective is to develop superior cultivars. Breeding involves selection of parents, specific breeding procedures, and genotype stabilization or "fixing" (Kester et al, 2002). Once a new cultivar is obtained, the following activities increase the availability of seed of that superior genotype, ultimately making it available to the farmers. If, during the process, the genetic purity of the new cultivar is lost, the seed value is lost.

(12) Genetically pure seed has the following attributes (Kester et al, 2002):

Trueness to name: this means that the seeds in question have been labeled with the correct species name, cultivar name, or whatever is appropriate.

Trueness to type: this means that the seeds in question also produce progeny that conform to visual and genetic standards for that cultivar or species.

Freedom from contaminants: this includes pathogens, mixtures of other crop seeds, and weed seeds.

(13) II- GENETICS OF PLANT POPULATIONS AND BREEDING SYSTEMS

(14) In general, the objective of any propagation technique (sexual or asexual) is to multiply a specific genotype in order to produce the kind of plant or phenotype of interest.

(15) Genotype, may be defined as the genetic constitution of a cell or individual, whereas (16) *Phenotype* is the total appearance of an organism, i.e. what we see (color, size, shape, etc). (17) The phenotype is determined by the interaction between genotype and environment. (18) Different phenotypes may result from identical genotypes in different environments, but it is unlikely that two individuals would share all their phenotypic characters without having an identical genotype.

(19) This figure demonstrates how the cross between two individuals of similar phenotype results in progeny with characteristics similar to the parents. In this case, the genotypes of the parents and the progeny likely are similar, and we could say that we have achieved our objective during propagation.

However, (20) it may also be that the progeny of these apparently similar individuals is different from the parents. (21) What may be the explanation for this observation? One explanation is associated with something that we cannot see, i.e. the genotype of these plants. Assuming that the environment is the same, the genotypes of the parents and progeny in this example are not the same.

(22) An individual genotype is determined by the specific combination of genes present in the cells of that individual. Inside the cell, genes are arranged along *chromosomes*. In each vegetative cell are pairs of each individual chromosome. These pairs are called *homologous chromosomes.*

(23) Homologous chromosomes have the same gene or genes affecting the same traits at corresponding positions or *locus*. Genes are termed *alleles* to each other if they occupy the same locus on homologous chromosomes and affect the same trait. Allelic genes can be dominant or recessive to each other.

(24) For example, in this figure, “a1” and “a2” are allelic genes; note that both are in the same position or locus of homologous chromosomes. The phenotypic trait determined by this locus will depend on the combination of allelic genes; for example, if the trait were flower color, and supposing there is no dominance, it could be red when a1 is in both homologous chromosomes, white when a2 is in both homologous chromosomes, or pink if a1 and a2 are both in one homologous chromosome.
(25) On the other hand, if a1 is dominant and a2 is recessive, the homozygous for a1 and the heterozygous genotypes will have the same phenotype, which is red flowers in this example. The homozygous genotypes for the a2 allele will have white flowers.

(26) When two alleles at a particular locus are identical, that locus is said to be *homozygous*. When they are different, that locus is said to be *heterozygous*.

(27) When most of the loci of individual chromosomes are filled with identical allelic genes, that individual is said to have a *homozygous genotype*. (28) When most of the allelic genes are different, that individual is said to have a *heterozygous genotype*.

(29) Homozygosis and self-pollination

(30) When the gametes of homozygous genotypes are formed, all of them have a similar genetic constitution, which is explained by the lack of genetic variability in the somatic cells of the parent. Basically, for each phenotypic trait, both allelic genes are identical. (31) In this way, when fertilization or the union of two gametes from the same genotype occurs, the new individuals will all be similar, because for each trait of those genotypes there is only one possible combination of genes. This is what happens when homozygous individuals are propagated by self-pollination, i.e. the pollen germinates and fertilizes the same flower where it was produced or another flower of the same plant. Depending on flower morphology and developmental characteristics which will be discussed later, self-pollination may vary among species. There are species that are primarily propagated by self-pollination, with less than 4% cross-pollination, such as many cereal grains, grasses, and legumes (Kester et al. 2002).

(32) As may be seen in this table, homozygosis in a self pollinated cultivar is "fixed" by consecutive generations of self-fertilization. Fixing may be defined as the process of stabilizing a genotype of a seedling population to make it homozygous so that it will "breed true" (Kester et al 2002). (33) In this example, after an initial cross of the homozygous pea genotypes Tall (DD) and Dwarf (dd), there is a first generation, F1, which is 100% heterozygous. (34) The next generation, F2, will have homozygous (25% DD, and 25% dd) and heterozygous (50% Dd) individuals. (35) The proportion of homozygous individuals will increase in consecutive generations, while the proportion with heterozygous genotypes will decrease by a factor of one-half with each successive generation. In this way, to produce a "true-breeding" homozygous cultivar, plant breeders will start with a single plant and then eliminate the off-type plants each generation for a period of six to ten generations in a process known as roguing (Kester et al 2002).

(36) Once the homozygosis in a self-pollinated cultivar is fixed, propagation of this cultivar to maintain its genetic purity is relatively simple. In this case, the seeds produced by the cultivar contain the same genetic information as the progenitor, so they will produce a progeny that possesses the cultivar genotype. (37) However, because there is a small percentage of contamination present, some precautions must be taken. Depending on the degree of self-pollination that the species exhibits, (38) isolation from other plants of the same species, but different genotypes, should be practiced. (39) In addition, because of risk of mutations or the presence of unwanted genes, (40) roguing or elimination of off-type plants must be regularly performed during the production of the seeds. Finally, during harvest, cleaning and all stages of seed handling, any contamination with seeds from another genotype must be avoided.

(41) Heterozygosis and cross-pollination

In the case of heterozygous genotypes, most of the allelic genes are different. (42) As a consequence, heterozygous individuals produce a diversity of different gametes that, when fertilized, produce progeny that in most cases differs from the parents and from each other. In nature, heterozygous genotypes propagate primarily by cross-pollination, i.e., plants are pollinated by pollen from a different individual. In evolution, heterozygosis increases the chances of individuals and populations adapting to environmental change. Additionally, heterozygous genotypes tend to be more vigorous (Kester et al, 2002).

For the breeding of naturally cross-pollinated species, breeders select those individuals with desirable phenotype and try to fix those traits of interest by eliminating off-type plants during successive generations. In the end, the number of different allelic genes for those traits of interest become reduced and plants of the same cultivar share a restricted pool of genes, which makes individual plants generally similar to one another. The individuals of the same cultivar may not necessarily have an identical genotype, but must share some set of traits that characterize the cultivar, such as color, earliness, shape, pest or disease resistance, etc.

The propagation of these types of cultivars is more complicated than for homozygous self-pollinated cultivars and may be performed in different ways:

(44) Asexual propagation

In the case of cross pollinated cultivars with a high level of heterozygosis, sometimes attempting to maintain the characteristics of the cultivar in the progeny by sexual reproduction is not possible or impractical. For example, in some vegetables such as artichoke or potato, and many ornamental and fruit species, specific traits that make a cultivar valuable, such as color, shape or flavor, are lost in the progeny where individuals show high heterozygosis and diversity of phenotypes. In these cases, an alternative is to use some form of asexual propagation, such as bulbs, corms, tubers, cuttings, etc. (45) When asexual reproduction is used, the progeny is genotypically identical to the parent. There are cases where asexual propagation is preferred because seed production is not possible, e.g., garlic, or the use of seeds is impractical. For instance, in species like potatoes or tulips, establishment by seed is more difficult and requires a longer time for production than with the use of tubers or bulbs.

Another example of asexual propagation is apomixis, which will be discussed later.

(46) Open Pollinated seed production

There are cases in which asexual propagation of cross-pollinated cultivars is difficult or impractical. During the breeding of these species, the level of heterozygosis of the individuals is reduced, especially for those traits of interest. Plants of the same cultivar will share a restricted pool of genes, which makes them relatively uniform and possessing those desirable traits that define the cultivar. (47) Seed production of these cultivars is performed by self- and cross-pollination among selected genotypes. Avoiding contamination with external genes, different from those that define the variety, is fundamental. This would result in traits different from those that define the cultivar, thereby affecting the genetic purity of the seed and reducing its value. This type of seed is known as *open pollinated* or OP seed. Isolation during production of OP seed is very important and how much isolation is required depends, in part, on the type of cross-pollination that the species exhibits.

For example, maize pollen is transported by wind. Because these pollen grains are relatively heavy, it is uncommon for them to move long distances, so isolations of 200 to 400 meters are usually recommended. Pollen of other species, such as sugar beet (*Beta vulgaris* L. supsp. *esculenta*) and spinach beet (*B. vulgaris* L. supsp. *cycla*), is relatively small and may move long distances transported by wind; in these cases, isolation distances may vary from 500 to 5000 meters. For other species pollinated by insects, such as onions and carrots, isolation distances from 400 to 1500 meters may be required.

(48) Hybrid seed production

Forced self-pollination of naturally cross-pollinated plants through consecutive generations may result in homozygous plants and populations of genotypically similar individuals. These are known as *inbred lines*. Vigor and productivity of inbred lines is usually reduced, a phenomenon known as *inbreeding depression* (Kester et al, 2002). (49) Because of their high homozygosis,

inbred lines produce gametes that are alike genetically. (50) If gametes of two different inbred lines join and fertilize in a process called *hybridization*, the progeny will be highly heterozygotic and very uniform. Additionally, the vigor and productivity of this progeny may be higher than the parents. This is known as *hybrid vigor* or *heterosis*. (51) For instance, this figure shows how the first generation of single crosses between inbred lines of maize outperform the inbred parents, more than doubling their yield.
From the development of the first hybrids of maize at the beginning of the 20th century, the transition from open pollinated to hybrid maize cultivars was very rapid. For example, in Iowa, the proportion of hybrid maize acres grew from less than 10% in 1935 to more than 90% 4 years later (Crow, 1998). The main reasons for this rapid adoption of hybrid cultivars were higher yields and greater uniformity, which is particularly important for machine harvesting.

(52) A hybrid cultivar may be defined as the first generation from a cross between progenitors with different genotypes through controlled pollination. The seed obtained from that cross is the only commercial seed that may be designated as hybrid.

Depending on the type of progenitors, hybrid cultivars may be classified as:

(53) Single-cross hybrid, from the cross between two inbred lines

(54) Three-way cross hybrid, from the cross between a single-cross hybrid and an inbred line,

(55) Double-cross hybrid, from the cross between two single-cross hybrids, and

(56) Top-cross hybrid, from the cross between an inbred line and an open pollinated cultivar.

(57) Apomixis (extracted from Kester et al, 2002).
Apomixis is an asexual form of reproduction that may be a common sexual reproduction mechanism, but occurs without fertilization and/or meiosis. During apomixis, seeds of normal appearance are formed, however the embryo does not develop from the fertilization of gametes but asexually from a somatic single cell of the mother plant. The progeny of these seeds are genetically identical to the mother plant. Apomixis is considered facultative when both sexual and apomictic seeds are produced, and obligate when all the seeds formed are apomictic.
For breeding of apomictic cultivars, a genetic source of apomictic reproduction is required. Apparently, a relatively low number of genes control apomixis, and the trait may be incorporated into selected cultivars. Once the apomictic trait is introduced, the progeny is immediately stabilized as a "true-breeding" line, without genotypic variation among individuals.

Apomixis has been reported to occur in 35 families and 300 species, and has been important in breeding of grasses, forage crops, and sorghum.

(58) III - MECHANISMS FAVOURING SELF- OR CROSS-POLLINATION

The mechanisms for pollination of plants are an important factor determining the type of cultivar commercially grown and the breeding method used to develop the cultivar (Fehr, 1987). (59) In sexual reproduction, seeds are classified according to the source of pollen responsible for fertilization (Fehr, 1987):
Self-pollinated seeds are formed when the pollen is produced on the same plant as the ovule which it fertilizes.

Cross-pollinated seeds result when the pollen of one plant fertilizes the ovule of another plant.

Plants species are classified according to the relative frequency of self- or cross-pollination during seed production (Fehr, 1987). There is a continuum of variation among and within species, ranging from plants with almost 100% self-pollination to others with frequencies close to 100% cross-pollination. Even within the same genotype, there may be different frequencies of self- and cross-pollination depending on the environment where the plant develops. (60) Despite this variation, a species usually may be classified as:
i) self-pollinated or autogamous, when under normal conditions reproduction is predominantly by self-pollination, or
ii) cross-pollinated or allogamous, when reproduction is mainly by cross-pollination under normal conditions.

In nature, there are different mechanisms that favor autogamy or allogamy of species. Manipulation of these mechanisms may be important in breeding and seed production.

(61) One of the most simple and obvious characteristic affecting frequency of cross- or self-pollination is flower morphology. In the case of perfect or hermaphroditic flowers, both male (stamens) and female (pistil) structures are present, so self-pollination may occur. (62) On the other hand, imperfect or unisexual flowers have only stamens or pistils, making cross-pollination more probable. In dioecious species, i.e., plants that have male and female flowers on separate plants, self-pollination is not possible under natural conditions and seeds are always produced by cross-pollination.

(63) This table shows different modifications of perfect flowers that may favor cross- or self-pollination:

Among the mechanisms that favor self-pollination are *cleistogamy* and *homogamy*.
Cleistogamy is the production of flowers where the stigma is receptive and the pollen is shed in closed flowers. This trait is common in legumes, such as peanuts, peas, and beans.
Homogamy refers to the simultaneous maturation of male and female structures, i.e. the pollen is shed at the same moment that the stigma is receptive. This term is the opposite of dichogamy.

Some of the mechanisms that favor cross-pollination are:
Chasmogamy, which is the opposite of cleistogamy, i.e. during pollen shed, the stigma is receptive after the flower opens.
Dichogamy occurs when the stamens and stigma mature at different times. There are two possible forms of dichogamy:

> ***Protandry*** refers to the condition when pollen is shed before the stigma is receptive, and
>
> ***Protogeny*** refers to the conditon when the stigma matures and ceases to be receptive before pollen is shed.

In both cases, dichogamy may be partial or absolute. It is partial if the receptive stigma and the mature pollen coincide at least during a short period, making possible some level of self-pollination. It is absolute if they do not coincide at all.

Self-Incompatibility is the inability of a plant with functional female and male gametes to self-pollinate or cross-pollinate with genetically similar plants.

Sterility is the production of non-functional gametes or sexual structures.

Heterostyly is when individuals of the same species possess two or more different positional arrangements of their anthers and stigmas.

(64) Lettuce is an example of a species in which its flower structure ensures self-pollination. In these flowers, there is a staminal column through which the style and stigma emerge (Ryder, 1986). On its path through the anther sheath, the emerging receptive stigma is covered with mature pollen ensuring self-pollination.

(65) In this example of protogyny in *Magnolia grandiflora*, the stigmas are receptive in the first day of flowering while the anthers are not shedding pollen. In the second day of flowering, the anthers shed pollen, but the stigmas are no longer receptive.

(66) Primula veris L. is an example of a species that exhibits heterostyly as a mechanism favoring cross-pollination. This mechanism is present in several species of the Primula genus, and has been used to facilitate hybrid seed production of some Primula species. In the case of Primula veris, the plants have two possible arrangements of anthers and stigma (Silverside, 2002):
"***pin***" plants, in which the style is long and the anthers are short. (67) Here, you can see that the stigma is exposed at the mouth of the corolla tube.
In the case of "***thrum***" plants, (68) the anthers are long, positioned at the mouth of the corolla tube, while the style is short, just half the length of the corolla tube.

These flowers are pollinated by insects and, in this process, pollen from thrum plants is likely to be transferred to the stigma of pin plants, and vice versa, favoring cross-pollination. Additionally, in flowers of this species, the stigma is mature and receptive 2 to 3 days before its own anthers dehisce, i.e. they are protogynous. (69) Finally, as an extra mechanism to ensure cross-pollination, different pollen sizes restrict the chances of thrum *x* thrum or pin *x* pin crosses.

This picture demonstrates differences in pollen size between types of flowers. Pin pollen is smaller than thrum pollen, which means that it has lower amounts of nutritional reserves, insufficient to support germ tube development through the long pin style. On the other hand, thrum pollen has enough reserves to successfully fertilize pin flowers with long styles. These reserves would be more than enough to fertilize the short styles of thrum flowers, however the larger thrum germ tube is mechanically unable to penetrate the surface of thrum styles, though it can penetrate pin styles (Silverside, 2002).

Self-incompatibility.
(70) Self-incompatibility or the inability of functional pollen to set seed after self-pollination is an effective mechanism that promotes cross-pollination in about 3000 species of flowering plants (Kalloo, 1988). Self-incompatibility may be found in crops such as *Solanum* spp., *Lycopersicon* spp., *Beta vulgaris*, *Brassica oleracea*, and

Raphanus sativus. In some of these, such as *Brassica oleracea*, this mechanism has been used for the commercial production of hybrid cultivars.

There are two types of self-incompatibility, gametophytic and sporophytic.

(71) Gametophytic self-incompatibility results from the interaction between the haploid genotype of the pollen grain and the diploid genotype of the pistil. In most of these cases, this form of self-incompatibility is controlled by a single locus with multiple alleles. (72) In this example, for the locus "S" there are three possible alleles: S1, S2, and S3. The incompatibility occurs when the allele of the pollen grain is the same as any of the alleles present in the female genotype. For instance, a pistil with the genotype S1S3 may only be fertilized by a pollen grain with the allele S2.

(73) This table shows the relationship of compatibility and incompatibility for a population with three alleles "S" for the locus of incompatibility.

(74) Sporophytic self-incompatibility results from the interaction between the diploid or maternal genotype of the pollen grain and the diploid genotype of the pistil. The alleles for the incompatibility trait may be independent among them, or some of them may be dominant to others. The incompatibility occurs when the active alleles of the pollen and the pistil are the same.
(75) In this example, there is no dominant allele, and only genotypes without common alleles are compatible.
(76) This type of incompatibility is common in brassica species and has been used for hybrid seed production in some of them. This table documents the different types of sporophytic self-incompatibility that may be present depending on whether the alleles are independent or dominant. The table shows what the compatibility would be assuming three possible alleles for the incompatibility trait.

(77) Male sterility.
Male sterility may be defined as the failure of a plant to produce functional pollen. This mechanism favors cross pollination in natural populations and has been identified in several species. It can be used to facilitate hybridization in breeding programs and commercial production of hybrid seed (Fehr, 1987). There are two types of male sterility, genetic and cytoplasmic.

(78) Genetic male sterility
The genetic male sterility trait is generally controlled by single recessive nuclear alleles. In this figure, this allele is represented by ms.
Because ms is recessive, only the homozygotic genotype "ms ms" is sterile, while the heterozygotic genotype "Ms ms" and the homozygotic "Ms Ms" are fertile. (79) When this type of male sterility is used for seed production of hybrid cultivars, the parental line used as the pollen donor must be homozygotic for the allele "Ms Ms", and the parental line that receives the pollen and produces the seed must be homozygotic for the recessive form of the allele. In this way, the line producing the seed is male sterile and can not

undergo self-pollination, which would cause genetic contamination of the hybrid seed. The seed produced is hybrid and heterozygotic for the allele ms, i.e. it is 100% fertile. This type of male sterility has been reported in several crops, such as tomato, eggplant, sweet pepper, soybean, muskmelon, cucumber, watermelon, summer squash, broccoli, cauliflower, and cabbage (Kalloo, 1988).

(80) Cytoplasmic male sterility
Cytoplasmic male sterility is controlled by the interaction of genes of the cellular nucleus and cytoplasm. The cytoplasm is transmitted only through the female parent, and it may be either normal fertile (N) or male sterile (S). In the nucleus, the alleles MS (dominant) and ms (recessive) interact with the cytoplasm to determine the plant phenotype. Only plants with the S cytoplasm and homozygous for recessive “ms” are sterile. (81) When cytoplasmic male sterility is used for hybrid seed production, the male line should be homozygous for the dominant Ms allele, while the female line should be male sterile, i.e. homozygous for the recessive ms allele and with the S cytoplasm.

Onions, carrots, and maize are examples of species where cytoplasmic male sterility has been reported and used in hybrid seed production.

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