

## **PBG 704: (Principles of Plant Breeding) 3(2-1)**

### **Session # 1: Tutorial**

#### **Role of Plant Breeding in crop improvement**

##### **The Art and Science of Plant Breeding:**

**Plant breeding** is the art and the science of improving the heredity of plants for the benefit of humankind. The changes made in plants are permanent and heritable. The professionals who conduct this task are called **plant breeders**.

This effort at adjusting the status quo is instigated by a desire of humans to improve certain aspects of plants to perform new roles or enhance existing ones. Consequently, the term “plant breeding” is often<sup>1</sup> used synonymously with “plant improvement” in<sup>2</sup> modern society. It needs to be emphasized that the<sup>3</sup> goals of plant breeding are focused and purposeful. Even though the phrase “to breed plants” often connotes the involvement of the sexual process in effecting a desired change, modern plant breeding also includes the manipulation of asexually reproducing. The art of plant breeding lies in the breeder's skill in observing plants with unique economic, environmental, nutritional, or aesthetic characteristics. Before plant breeders possessed the scientific knowledge that is available to them today, they relied solely on skill and judgement

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in selecting novel plants which could be propagated through seeds or vegetative parts. Thus, selection became the earliest form of plant breeding. The successful plant breeders were keen observers, quick to recognize variant plants of the same species which would improve performance in the field or garden. For them, plant breeding was purely an art. Many of the early breeders were amateurs—a cultivator who found an "offtype" plant in the field or a gardener who found a "sport" in the bed. Some, like Luther Burbank, were professionals who searched far and wide for unusual plant types that could be propagated and exploited for commercial gain.

**Plant breeding developed into a science** as knowledge progressed in classical genetics and related plant sciences. The foundation of plant breeding was based on recognition of the gene as the unit of heredity, on procedures for gene manipulation, and on rules of genetic behavior that permitted accurate prediction of the results from gene manipulations. The genes were identified by their effects on the visible expression of plant traits, such as whether a plant was tall or dwarf, or the flower color was white or pink. Through systematic crosspollination, particular combinations of genes for different meritorious traits could be combined into a single plant cultivar. Hybridization then became the principal plant breeding procedure. It was no longer necessary for the breeder to rely so completely on skill in finding chance variants with which to establish new cultivars. It now became possible to plan and synthesize new plant types more or less at will. Plant breeding became more of a science and less of an art.

## **The goals of plant breeding**

The plant breeder uses various technologies and methodologies to achieve targeted and directional changes in the nature of plants. As science and technology advance, new tools are developed while old ones are refined for use by breeders. Before initiating a breeding project, clear breeding objectives are defined based on factors such as producer needs, consumer preferences and needs, and environmental impact. Breeders aim to make the crop producer's job easier and more effective in various ways. They may modify plant structure, so it will resist lodging and thereby facilitate mechanical harvesting. They may develop plants that resist pests, so that the farmer does not have to apply pesticides, or applies smaller amounts of these chemicals. Not applying pesticides in crop production means less environmental pollution from agricultural sources. Breeders may also develop high yielding varieties (or cultivars), so the farmer can produce more for the market to meet consumer demands while improving his or her income.

## **Achievements of modern plant breeders**

The achievements of plant breeders are numerous, but may be grouped into several major areas of impact – yield increase, enhancement of compositional traits, crop adaptation, and the impact on crop production systems.

## **Yield increase**

Yield increase in crops has been accomplished in a variety of ways, including targeting yield per se or its components, or making plants resistant to economic diseases and insect pests, and breeding for plants that are responsive to the production environment. Yields of major crops (e.g., corn, rice, sorghum, wheat, and soybean) have significantly increased in the USA over the years). For example, the yield of corn rose from about 2000 kg/ha in the 1940s to about 7000 kg/ha in the 1990s. In England, it took only 40 years for wheat yields to rise from 2 metric tons/ ha to 6 metric tons/ha. Food and Agriculture Organization (FAO) data comparing crop yield increases between 1961 and 2000 show dramatic changes for different crops in different regions of the world. For example, wheat yield increased by 681% in China, 301% in India, 299% in Europe, 235% in Africa, 209% in South America, and 175% in the USA. These yield increases are not totally due to the genetic potential of the new crop cultivars (about 50% is attributed to plant breeding) but are also due to the improved agronomic practices (e.g., application of fertilizer, irrigation). Crops have been armed with disease resistance to reduce yield loss. Lodging resistance also reduces yield loss resulting from harvest losses.

## **Enhancement of compositional traits**

Breeding for plant compositional traits to enhance nutritional quality or meet an industrial need are major plant breeding goals. High protein crop varieties (e.g., high lysine or quality protein maize) have been produced for use in various parts of the world. Different kinds of wheat are needed for different kinds of products (e.g., bread, pasta, cookies, semolina). Breeders have

identified the quality traits associated with these uses and have produced cultivars with enhanced expression of these traits. Genetic engineering technology has been used to produce high oleic sunflower for industrial use; it is also being used to enhance the nutritional value of crops (e.g., pro-vitamin A golden rice). The shelf life of fruits (e.g., tomato) has been extended through the use of genetic engineering techniques to reduce the expression of compounds associated with fruit deterioration.

## **Crop adaptation**

Crop plants are being produced in regions to which they are not native, because breeders have developed cultivars with modified physiology to cope with variations in the duration of day length (photoperiod). Photoperiod insensitive cultivars will flower and produce seed under any day length conditions. The duration of the growing period varies from one region of the world to another. Early maturing cultivars of crop plants enable growers to produce a crop during a short window of opportunity, or even to produce two crops in one season. Furthermore, early maturing cultivars can be used to produce a full season crop in areas where adverse conditions are prevalent towards the end of the normal growing season. Soils formed under arid conditions tend to accumulate large amounts of salts; to use these lands for crop production, salt tolerant (saline and aluminum tolerance) crop cultivars have been developed for certain species. In crops such as barley and tomato there are commercial cultivars in use with drought, cold, and frost tolerance.

## Impact on crop production systems

Crop productivity is a function of the genotype (genetic potential of the cultivar) and the cultural environment. The Green Revolution is an example. The yield of major world food crops is steadily rising, as indicated by the increasing levels of crops produced in the US agricultural system. A significant portion of this rise is attributable to the use of improved crop cultivars by crop producers. (Source: Drawn with data from the USDA.) 12 CHAPTER 1 of an outstanding outcome of the combination of plant breeding efforts and production technology to increase food productivity. A chemically intensive production system (use of agrochemicals-like fertilizers) calls for crop cultivars that are responsive to such high input growing conditions. Plant breeders have developed cultivars with the architecture for such environments. Through the use of genetic engineering technology, breeders have reduced the need for pesticides in the production of major crops (e.g., corn, tobacco, soybean) with the development of GM pest resistant cultivars, thereby reducing environmental damage from agriculture. Cultivars have been developed for mechanized production systems.

## Norman Ernest Borlaug: The man and his passion:

*"For more than half a century, I have worked with the production of more and better wheat for feeding the hungry world, but wheat is merely a catalyst, a part of the picture. I am interested in the total development of human beings. Only by attacking the whole problem can we raise the standard of living for all people, in all communities, so that they will be able to live decent lives. This is something we want for all people on this planet".*

*Norman E. Borlaug.*

Dr Norman E. Borlaug has been described in the literature in many ways, including as “the father of the Green Revolution”, “the forgotten benefactor of humanity”, “one of the greatest benefactors of human race in modern times”, and “a distinguished scientist-philosopher”. He has been presented before world leaders and received numerous prestigious academic honors from all over the world. He belongs to an exclusive league, with the likes of Henry Kissinger, Elie Wiesel, and President Jimmy Carter – all Nobel Peace laureates. Yet, Dr Borlaug is hardly a household name in the United States. But, this is not a case of a prophet being without honor in his country. It might be more because this outstanding human being chooses to direct the spot light on his passion, rather than his person. As previously stated in his own words, Dr Borlaug has a passion for helping to achieve of a decent living status for the people of the world, starting with the alleviation of hunger. To this end, his theatre of operation is the developing countries, which are characterized by poverty, political instability, chronic food shortages, malnutrition, and prevalence of preventable diseases. These places are hardly priority sources for news for the first world media, unless an epidemic or catastrophe occurs.

Dr Borlaug was born on March 25, 1914, to Henry and Clara Borlaug, Norwegian immigrants in the city of Saude, near Cresco, Iowa. He holds a BS degree in Forestry, which he earned in 1937. He pursued an MS in Forest Pathology, and later earned a PhD in Pathology and Genetics in 1942 from the University of Minnesota. After a brief stint with the E.I. du Pont de Nemours in Delaware, Dr Borlaug joined the Rockefeller Foundation team in Mexico in 1944, a move that would set him on course to accomplish one of the most notable accomplishments in history.

He became the director of the Cooperative Wheat Research and Production Program in 1944, a program initiated to develop highyielding cultivars of wheat for producers in the area. In 1965, the Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT) was established in Mexico, as the second of the currently 16 International Agricultural Research Centers (IARC) by the Consultative Group on International Agricultural Research (CGIAR). The purpose of the center was to undertake wheat and maize research to meet the production needs of developing countries. Dr Borlaug served as the director of the Wheat Program at CIMMYT until 1979 when he retired from active research, but not until he had accomplished his landmark achievement, dubbed the Green Revolution. The key technological strategies employed by Dr Borlaug and his team were to develop high yielding varieties of wheat, and an appropriate agronomic package (fertilizer, irrigation, tillage, pest control) for optimizing the yield potential of the varieties. Adopting an interdisciplinary approach, the team assembled germplasm of wheat from all over the world. Key contributors to the efforts included Dr Burton Bayles and Dr Orville Vogel, both of the USDA, who provided the critical genotypes used in the breeding program. These genotypes were crossed with Mexican genotypes to develop lodging-resistant, semi-dwarf wheat varieties that were adapted to the Mexican production region. Using the improved varieties and appropriate agronomic package, wheat production in Mexico increased dramatically from its low 750 kg/ha to about 3200 kg/ha. The successful cultivars were introduced INTRODUCTION 13 into other part of the world, including Pakistan, India, and Turkey in 1966, with equally dramatic results. So successful was the effort in wheat that the

model was duplicated in rice in the Philippines in 1960. In 1970, Dr Norman Borlaug was honored with the Nobel Peace Prize for contributing to curbing hunger in Asia and other parts of the world where his improved wheat varieties were introduced (Figure B1.2). Whereas the Green Revolution was a life-saver for countries in Asia and some Latin-American countries, another part of the world that is plagued by periodic food shortages, the sub-Saharan Africa, did not benefit from this event. After retiring from CIMMYT in 1979, Dr Borlaug focused his energies on alleviating hunger and promoting the general well-being of the people on the continent of Africa. Unfortunately, this time around, he had to go without the support of these traditional allies, the Ford Foundation, the Rockefeller Foundation, and the World Bank. It appeared the activism of powerful environmental groups in the developed world had managed to persuade these donors from supporting what, in their view, was an environmentally intrusive practice advocated by people such as Dr Borlaug. These environmentalists promoted the notion that high yield agriculture for Africa, whereby the agronomic package included inorganic fertilizers, would be ecologically disastrous.

## **Session # 2 & 3: Laboratory**

### **Hybridization techniques used in self pollinated crops:**

Crop improvement typically involves the transfer of genes from one source or genetic background to another, or combining genes from different sources that complement each other, with the hope that the new cultivar will combine the best of both parents, while being distinct from both. When a plant breeder has decided on the combination of traits that are to be incorporated in the new cultivar to be developed, the next crucial step is to find one or more sources of the appropriate gene(s) for such characters. In flowering species, the conventional method of gene transfer or gene combination is by crossing or sexual hybridization. This procedure causes genes from the two parents to be assembled into a new genetic matrix. It follows that if parents are not genetically compatible, gene transfer by sexual means cannot occur at all or, at best, may be fraught with complications. The product of hybridization is called a hybrid. Sexual hybridization can occur naturally through agents of pollination. Even though self-pollinating species may be casually viewed as “self-hybridizing”, the term hybridization is reserved for crossing between unidentical parents (the degree of divergence is variable). Artificial sexual hybridization is the most common conventional method of generating a segregating population for selection in breeding flowering species. In some breeding programs, the hybrid ( $F_1$ ) is the final product of plant breeding (see hybrid breeding in Chapter 18). However, in most situations the  $F_1$  is selfed (to give an  $F_2$ ) to generate recombinants (as a result of recombination of the parental genomes) or a segregating population, in which selection is practiced. In clonally propagated crops, the  $F_1$  usually segregates sufficiently and its clonally produced descendants will be submitted to selection without further crossing or selfing. The tools of modern biotechnology now enable the breeder to transfer genes by circumventing the sexual process (i.e., without crossing). More significantly, gene transfer can transcend natural reproductive or genetic barriers. Transfers can occur between unrelated plants and even between plants and animals.

## **Applications of crossing in plant breeding**

Sometimes, crossing is done for specific purposes, within the general framework of generating variability. Hybridization precedes certain methods of selection in plant breeding to generate general variability. \_ Gene transfer. Sometimes, only a specific gene (or a few) needs to be incorporated into an adapted cultivar. Crossing is used for the gene transfer process, followed by additional strategic crossing to retrieve the desirable genes of the adapted cultivar.

### **Recombination.**

Genetically diverse parents may be crossed in order to recombine their desirable traits. The goal of recombination, which is a key basis of plant breeding, is to forge desirable linkage blocks. \_ Break undesirable linkages. Whereas forging desirable linkage blocks is a primary goal of plant breeding, sometimes crossing is applied to provide opportunities for undesirable linkages to be broken. \_ For heterosis. Hybrid vigor (heterosis) is the basis of hybrid seed development. Specially developed parents are crossed in a predetermined fashion to capitalize on the phenomenon of heterosis for cultivar development. \_ For maintenance of parental lines. In hybrid seed development programs, crossing is needed to maintain special parents used in the breeding program (e.g., CMS lines, maintainer lines). \_ For maintenance of diversity in a gene pool. Plant breeders may use a strategy of introgression (crossing and backcrossing selected entries with desired traits into adapted stocks) and incorporation to develop dynamic gene pools from which they can draw materials for crop improvement. \_ For evaluation of parental lines. Inbred lines for hybrid seed development are evaluated by conducting planned

crosses to estimate combining abilities in order to select appropriate parents for used in hybrid seed development. \_ For genetic analysis. Geneticists make planned crosses to study the inheritance and genetic behavior of traits of interest.

## **Artificial hybridization**

Artificial hybridization is the deliberate crossing of selected parents (controlled pollination).

There are specific methods for crossing that depend on the species in which the cross is being made, which differ according to factors including floral morphology, floral biology, possible genetic barriers, and environmental factors. Methods for selected species are described later in this book. However, there are certain basic factors to consider in preparation for hybridization:

\_ Parents should belong to the same or closely related plant species. In the case that they belong to different (related) plant species, all kinds of techniques may be required to obtain hybrid progeny. \_ The parents, obviously, together should supply the critical genes needed to accomplish the breeding objective. \_ One parent is usually designated as female. Whereas some breeding methods may not require this designation, breeders usually select one parent to be a female and the other a male (pollen source). This is especially so when hybridizing self-pollinated species. Whenever genetic markers are available (e.g., white flowers, white seeds), the female exhibits the recessive morphological trait. In some cases, selected parents of cross-pollinated species may be isolated and allowed to randomly cross-pollinate each other. \_ The female parent usually needs some special preparation. In complete flowers (those having both male and female organs), the flowers of the parent selected to be female are prepared for

hybridization by removing the anthers, a tedious procedure called emasculation (discussed next). Emasculation is eliminated in some crossing programs by taking advantage of male sterility (renders pollen sterile) when it occurs in the species. \_ Pollen is often physically or manually transferred. Artificial hybridization often includes artificial pollination, whereby the breeder physically deposits pollen from the male parent onto the female stigma. However, when hybridization is conducted on large scale (e.g., commercial hybrid seed development), hand pollination is rarely a feasible option.

#### 7.4 Artificial pollination control techniques

As previously indicated, crossing is a major procedure employed in the transfer of genes from one parent to another in the breeding of sexual species. A critical aspect of crossing is pollination control to ensure that only the desired pollen is involved in the cross. In hybrid seed production, success depends on the presence of an efficient, reliable, practical, and economic pollination control system for large-scale pollination.

### **Pollination control may be accomplished in three general ways:**

#### **Mechanical control.**

This approach entails manually removing anthers from bisexual flowers to prevent pollination, a technique called emasculation, or removing one sexual part (e.g., detasselling in corn), or excluding unwanted pollen by covering the female part. These methods are time consuming, expensive, and tedious, limiting the number of plants that can be crossed. It should be mentioned that in crops such as corn, mechanical detasselling is widely used in the industry to produce hybrid seed.

## **(ii) Chemical control.**

A variety of chemicals called chemical hybridizing agents, or by other names (e.g., male gametocides, male sterilants, pollenocides, androcides), are used to temporally induce male sterility in some species. Examples of such chemicals include Dalapon<sub>1</sub>, Estrone<sub>1</sub>, Ethephon<sub>1</sub>, Hybrex<sub>1</sub>, and Generis<sub>1</sub>. The application of these agents induces male sterility in plants, thereby enforcing cross pollination. The effectiveness is variable among products.

## **(iii) Genetical control.**

Certain genes are known to impose constraints on sexual biology by incapacitating the sexual organ (as in male sterility) or inhibiting the union of normal gametes (as in self-incompatibility). The flower has a central role in hybridization. The success of a crossing program depends on the condition of the flower regarding its overall health, readiness or receptiveness to pollination, maturity, and other factors. The actual technique of crossing depends on floral biology (time of pollen shedding, complete or incomplete flower, self- or crosspollinated, size and shape of individual flowers and of the inflorescence).

## **Flower health and induction:**

It is important that plants in a crossing block (or to be crossed) be in excellent health and be properly developed. This is especially so when flowers are to be manually emasculated. Once successfully crossed, an adequate amount of seed should be obtained for planting the first generation. The parents to be mated should receive proper lighting, moisture supply, temperature, nutrition, and protection from pests. Parents should be fertilized with the proper amounts of nitrogen, phosphorus, and potassium for vigorous plant growth to develop an adequate number of healthy flowers. Plants growing in the greenhouse should be provided with

the proper intensity and duration of light. If the species is photoperiod sensitive, the lighting should be adjusted accordingly. Proper temperature is required for proper plant growth and development. In some species, a special temperature treatment (vernalization, usually some period of low temperature) is required for flower induction. Furthermore, temperature affects pollen shed in flowers. Consequently, extreme temperatures may cause inadequate amounts of pollen to be shed for successful artificial pollination. Pollen quantity and quality are influenced by the relative humidity of the growing environment. Extreme moisture conditions should be avoided.

## **Synchronization of flowering**

In artificial pollination, the breeder should be familiar with the species to know its flowering habits regarding time from planting to flowering, duration of flowering, mechanisms and timing of natural anther dehiscence and fertilization, and time of peak pollen production, in order to take advantage of the window of opportunity of anthesis (pollen shed) for best crossing outcomes. To ensure that parents in a crossing program will have flowers at the same time, the practice of staggered planting – to plant sets of parents at different times – is recommended. This way, a late-planted early flowering genotype may be pollinated by an early-planted late flowering genotype. When depending on natural pollination, interspersed planting on different dates will favor even pollen distribution. Photoperiod may be manipulated in photoperiod sensitive species to delay or advance flowering as appropriate, in order to synchronize flowering of the parents in a cross. Other techniques that have been used in specific cases

include manipulation of temperature and planting density, removal of older flowers to induce new flushes of flowers, and pinching (e.g., removal of plant apex to induce tillering or branching for additional flowers). In corn, the silk of an early flowering inbred parent may be cut back to delay the time to readiness for pollination.

### **Selecting female parents and suitable flowers:**

After selecting lines to be parents in a cross, it is necessary in artificial crosses to designate one parent as female (as previously stated), as well as identify which type of flowers on the parent would be most desirable to cross. In crossing programs in which the CMS system is being used, it is critical to know which plants to use as females (these would be the male sterile genotypes, or A and B lines; Chapter 17). Because the pollen or male gamete is practically without cytoplasm, and because certain genes occur in the extranuclear genome (such as CMS), it is critical that parents selected as female plants be selected judiciously. Markers are important to plant breeding as was discussed previously. Some markers may be used to distinguish between selfed and hybrid seed on the female plant. For example, in sorghum, waxy endosperm is conditioned by a recessive allele while normal endosperm is under the control of the dominant allele. If a waxy female is crossed with a normal male, all  $F_1$  seed with waxy endosperm would be products of selfing (undesirable) while normal seed would indicate a successful hybrid. Other markers, molecular and morphological, may be strategically included in a crossing program to allow the authentication of hybridity. In terms of flower characteristics, bigger flowers are easier to handle than tiny ones. Whenever possible, the

parent with bigger flowers should be used as female. Another critical aspect of flower physiology is the age of the flower when it is most receptive to pollination. The breeder usually determines the optimal stage of flower maturity by examining its physical appearance. Tell-tale signs depend on species. Usually, fully opened flowers would have already been pollinated by undesirable pollen. In most plant species flowers are emasculated in the bud stage just as the petals begin to show through the bud. Rice is ready in the boot stage, whereas wheat is best emasculated when florets are light green with well-developed but still green anthers and feathery stigmas that extend about a quarter of the length of the florets. Furthermore, flowers in the same inflorescence usually have different maturity levels. In species such as the broad bean (*Vicia faba*), the first inflorescence is more suitable for crossing than later ones. Also, flowers at the base and middle of inflorescences give better results than those at the top. Flowers in the inflorescence that are not used for crossing may be removed, while the ones that are used in crossing may be marked with a label or small clip or peg.

## **Emasculation**

The process of making a bisexual flower female by removing the male parts or incapacitating them is called emasculation. It should be pointed out right away that emasculation is not a universal requirement for artificial crossing of plants. Species with fertility-regulating mechanisms (e.g., male sterility, self-incompatibility, protogyny, monoecy, dioecy) may be crossed without the often tedious and time consuming process of emasculation.

## **Factors to consider for success:**

Apart from picking the right flowers, it is critical to know the duration of stigma receptivity and pollen viability. The maximum time between emasculation and pollination that can be tolerated varies among species. Since the anthers were removed before they were mature, the female parts often are not yet receptive at the moment of emasculation. This makes it necessary to pollinate at a later time, either during the same day or even later. The caution to observe is that prolonged delay between the two operations increases the chance of contamination from undesirable pollen. To reduce this risk, emasculated flowers may be covered with bags (e.g., glassine, paper or cloth bag). Pollen quality and quantity vary with the weather and time of day. For example, in chickpea, some breeders prefer to emasculate in the evening and pollinate in the morning. Because emasculation is done before anthers are mature in species such as wheat and barley, pollination is done 2–3 days later when the stigma is receptive. In extreme cases, such as in sugar beet, pollination may immediately follow emasculation or be delayed for up to 12 days.

## **Methods of emasculation**

There are several techniques of emasculation used by plant breeders that include the use of instruments or chemicals. A pair of forceps or tweezers is one of the most widely used instruments in the emasculation of flowers. Different shapes and sizes are used according to the size and structure of the flower.

**The methods of emasculation may be classified as direct or indirect.**

### **Direct anther emasculation:**

The technique of removing anthers from selected flowers is the most common procedure for emasculation of flowers (usually using a pair of forceps). When handling plants with inflorescence, it is important to firstly thin out the bunch by removing immature flowers as well as old ones. This improves the survival of the emasculated flowers. Breeders of various crops have developed convenient ways of removing the anthers. Sometimes, the sepals are first removed, followed by the petals before access is gained to the anthers. In soybean and sesame, a skilled person may be able to remove the petals and anthers in one attempt. In flowers such as soybean, the pedicel is easily broken as a result of physical handling of the delicate flower during emasculation. In wheat and barley, the florets are clipped with scissors.

### **Indirect anther emasculation:**

In these methods, the anthers are incapacitated without being removed from the flower. Incapacitation is achieved in several ways: \_ Thermal inactivation. The inflorescence is first thinned out to leave only flowers at the proper stage for emasculation. It is then immersed in hot water (e.g., held in a thermos bottle) to kill the pollen without injuring the pistil. The temperature and time of immersion are variable (e.g., 43 °C for 5 minutes in rice; 47–48 °C for 10 minutes in sorghum). The inflorescence is allowed to dry before pollinating about 30–60 minutes later. \_ Alcohol emasculation. In species such as alfalfa the raceme is immersed in 57% ethanol for 10 seconds and then rinsed in water for a few seconds. \_ Commercial gametocides. These are chemicals designed to kill the anthers (e.g., sodium methyl arsenate). If pollination is not to follow emasculation immediately, the flowers

should be covered to exclude contaminating pollen from elsewhere. Once properly pollinated, the flower should be tagged for identification.

## **Pollination :**

Successful pollination depends on pollen maturity, quality (freshness), and timing of pollination, among other factors.

## **Collection and storage:**

In some species (e.g., soybean) pollination immediately follows emasculation. In this case, there is no need for storage. Fresh pollen gives the best success of crossing. Good pollen flowers may be picked and placed in a Petri dish or some suitable container for use. In some species, mechanical vibrations may be used to collect pollen. Pollen is most copious at peak anthesis. Generally, pollen loses viability quickly. However, in some species, pollen may be stored at a cool temperature and an appropriate humidity for the species for an extended period.

## **Application of pollen:**

Commonly, pollen is applied directly to the stigma by using a fine brush or dusting off the pollen onto the stigma directly from the flower of the pollen source (e.g., the staminal column may be used as brush). Sometimes, an object such as a cotton bulb or a tooth pick is used to deposit pollen on the stigma. In some flowers, pollen deposition is made without direct contact with the stigma. Instead, pollen may be injected or dusted into a sack covering the emasculated

inflorescence and agitated to distribute the pollen over the inflorescence. A key precaution against contamination during pollination is for the operators to disinfect their hands and tools between pollinations when different varieties are involved. It is critical to tag the pollinated flower for identification at the time of harvesting.

### **Tagging after pollination:**

After depositing the desired pollen, it is critical to identify the flowers that were pollinated with an appropriate tag or label. The information on the label should include the date of emasculation, date of pollination, name of seed parent, and the name of pollen parent. The tag should be attached to the pedicel of the emasculated flower not the branch.

7.8 Number of  $F_1$  crosses to make

There are practical factors to consider in deciding on the number of crosses to make for a breeding project. These include the ease of making the crosses from the standpoint of floral biology and the constraints of resources (labor, equipment, facilities, and funds). It will be easier to make more crosses in species in which emasculation is not needed (e.g., monoecious and dioecious species) than in bisexual species. Some breeders make a small number of carefully planned crosses, while others make thousands of cross combinations. Generally, a few hundred cross combinations per crop per year would be adequate for most purposes for species in which the  $F_1$  is not the commercial product. More crosses may be needed for species in which hybrids are commonly produced, for the purpose of discovering heterotic combinations. As is discussed next, breeding programs that go beyond the  $F_1$  usually require very large  $F_2$  populations. Regarding the number of flowers per cross combination, there is

variation according to fecundity. Species such as tomato may need only one or two crosses, since each fruit contains over 100 seeds. Plants that tiller also produce large numbers of seed. Each crop species has its own reproduction rate, which may be huge (e.g., tobacco: 1000s of seeds produced per plant, 100s per bowl) or relatively small (e.g., pea: about 100 per plant, about 2–5 per pod).

### **Genetic issues in hybridization:**

Because hybridization involves combining two sets of genes in a new genetic matrix through the meiotic process, it is accompanied by a variety of genetic based effects.

### **Immediate effect:**

The immediate effect of hybridization is the assembly of two different genomes into a newly created individual. Several genetic consequences may result from such a union of diverse genomes, some of which may be desirable, some of which may not be desirable. The key ones are: \_ Expression of recessive lethal gene. Crossing may bring together recessive lethal genes (that were in the heterozygous state) into the expressible homozygous state. The resulting hybrid may die or loose vigor. By the same token, hybridization can also mask the expression of a recessive allele by creating a heterozygous locus. Individuals carry a certain genetic load (or genetic burden), representing the average number of recessive lethal genes carried in the heterozygous condition by an individual in a population. Selfing or inbreeding predisposes an

individual to having deleterious recessive alleles that were protected in the heterozygous state to becoming expressed in the homozygous recessive form \_

### **Hybrid necrosis.**

The crossing of parents that are somewhat distantly related (but still the same crop species) may result, especially, in the phenomenon of hybrid necrosis. Interactions between pairs of genes in both parents may work out unfavorably to the physiology of the plant. This phenomenon has been reported not only in wheat and rye but also in Arabidopsis. \_ Heterosis.

Genes in the newly constituted hybrid may complement each other to enhance the vigor of the hybrid. The phenomenon of hybrid vigor (heterosis) is exploited in hybrid seed development \_

Transgressive segregation. Hybrids have features that may represent an average of the parental features, or a bias toward the features of one parent, or even new features that are unlike either parent (transgressive segregates). When the parents “nick” in a cross, transgressive segregates with performance superseding either parent is likely to occur in the segregating population. \_

### **Genome-plastome incompatibility.**

Plastomes (the genetic material found in plastids such as in chloroplasts) and genomes in most genera function to form normal plants, regardless of the taxonomical distances between the plastid and nuclear genomes. However, in some genera, plastomes and genomes, having co-evolved to a significant degree, are only compatible within a specific combination.

## **Subsequent effect:**

The subsequent effect of hybridization, which is often the reason for hybridizing parents by breeders, occurs in the  $F_2$  and later generations. By selfing the  $F_1$  hybrid, the parental genes are reorganized into new genetic matrices in the offspring. This occurs through the process of meiosis, a nuclear division process that occurs in flowering plants. Contrasting alleles segregate and subsequently recombine in the next generation to generate new variability. Furthermore, the phenomenon of crossing over that leads to the physical exchange of parts of chromatids from homologous chromosomes provides an opportunity for recombination of linked genes, also leading to the generation of new variation.

### 7.9.3 Gene recombination in the $F_2$

The goal of crossing for generating variability for selection is to produce a large number of gene recombinations from the parents used in the cross. In hybrid seed programs, the  $F_1$  is the end product for commercial use. However, in other crosses, the  $F_2$  and subsequent generations are evaluated to select genotypes that represent the most desirable recombination of parental genes. The  $F_2$  generation has the largest number of different gene combinations of any generation following a cross. The critical question in plant breeding is what size of  $F_2$  population to generate in order to have the chance of including that ideal recombinant this is homozygous for all the desirable genes in the parent. Three factors determine the number of gene recombinations that would be observed in an  $F_2$  population: (i) The number of gene loci for which the parents in a cross differ. (ii) The number of alleles at each locus. (iii) The linkage of the gene loci. Plant breeders are often said to play the numbers game. Table 7.1 summarizes the challenges of breeding in

terms of the size of the  $F_2$  population to grow. If the parents differ by only one pair of allelic genes, the breeder needs to grow at least 16 plants in the  $F_2$  to have the chance to observe all the possible gene combinations (according to Mendel's laws). On the other hand, if the parents differ in 10 allelic pairs, the  $F_2$  population size needed is 59 049 (obtained by the formula  $3^n$ , where  $n$  is the number of loci). The frequencies illustrate how daunting a task it is to select for quantitative traits. The total possible genotypes in the  $F_2$  based on the number of alleles per locus is given by the relationship

## **Session # 4 & 5: Tutorial**

### **Variability in natural populations and its exploitation**

#### **Genetic variability:**

Variability that can be attributed to genes that encode specific traits and can be transmitted from one generation to the next is described as genetic or heritable variation. Because genes are expressed in an environment, the degree of expression of a heritable trait is impacted by its environment, some more so than others (Figure 4.1). Heritable variability is indispensable to plant breeding. As previously noted, breeders seek to change the phenotype (trait) permanently and heritably by changing the genotype (genes) that encode it. Heritable variability is consistently expressed generation after generation. For example, a purple-flowered genotype will always produce purple flowers. However, a mutation can permanently alter an original expression. For example, a purple-flowered plant may be altered by mutation to become a white-flowered plant. Genetic variation can be detected at the molecular as well as the gross morphological level. The availability of biotechnological tools (e.g., DNA markers) allows plant breeders to assess genetic diversity of their materials at the molecular level. Some genetic variation is manifested as visible variation in morphological traits (e.g., height, color, size), while compositional or chemical traits (e.g., protein content, sugar content of a plant part) require various tests or devices for evaluating them.

#### **Origins of genetic variability:**

There are three ways in which genetic or heritable variability originates in nature – gene recombination, modifications in chromosome number, and mutations. The significant fact to note is that, rather than wait for them to occur naturally, plant breeders use a variety of techniques and methods to manipulate and make these three phenomena more and more targeted, as they generate genetic variation for their breeding programs. With advances in science and technology (e.g., gene transfer, somaclonal variation), new sources of genetic variability have become available to the plant breeder. Variability generated from these sources is, however, so far limited.

### **Genetic recombination:**

Genetic recombination applies only to sexually reproducing species and represents the primary source of variability for plant breeders in those species. As previously described, genetic recombination occurs via the cellular process of meiosis. This phenomenon is responsible for the creation of non-parental types in the progeny of a cross, through the physical exchange of parts of homologous chromosomes (by breakagefusion). The cytological evidence of this event is the characteristic crossing (X-configuration or chiasma) of the adjacent homologous chromosome strands, as described in Chapter 3, allowing genes that were transmitted together (non-independent assortment) in the previous generation to become independent. Consequently, sexual reproduction brings about gene reshuffling and generation of new genetic combinations (recombinants). Unlike mutations that cause changes in genes themselves in order to generation variability, recombination generation variability by assembling new

combinations of genes from different parents. In doing this, some gene associations are broken.

Consider a cross between two parents of contrasting genotypes AAbb and aaBB. A cross between them will produce an F<sub>1</sub> of genotype AaBb. In the F<sub>2</sub> segregating population, and according to Mendel's law, the gametes (AB, Ab, aB, and ab) will combine to generate variability, some of which will be old (like the parents – parental), while others will be new (unlike the parents – recombinants)

### **Ploidy modifications:**

New variability may arise naturally through modifications in chromosome number as a result of hybridization (between unidentical genotypes), or abnormalities in the nuclear division processes (spindle malfunction). Failure of the spindle mechanism, during karyokinesis or even prior to that, can lead to errors in chromosome numbers transmitted to cells, such as polyploidy (individuals with multiples of the basic set of chromosomes for the species in their cells) (Figure 9.3). Sometimes, instead of variations involving complete sets of chromosomes, plants may be produced with multiples of only certain chromosomes or deficiencies of others (called aneuploidy). Sometimes, plants are produced with half the number of chromosomes in the somatic cells (called haploids). Like genetic recombination, plant breeders are able to induce various kinds of chromosome modification

### **Mutation:**

Mutation is the ultimate source of biological variation. Mutations are important in biological evolution as sources of heritable variation. They arise spontaneously in nature as a result of errors in cellular processes such as DNA replication (or duplication) and by chromosomal aberrations (deletion, duplication, inversion, translocation). The molecular basis of mutation may be described by mechanisms such as modification of the structure of DNA or a component base of DNA, substitution of one base for a different base, deletion or addition of one base in one DNA strand, deletion or addition in one or more base pairs in both DNA strands, and inversion of a sequence of nucleotide base pairs within the DNA molecule.

### **Transposable elements:**

The phenomenon of transposable elements (genes with the capacity to relocate within the genome), creates new variability. Transposable genetic elements (transposable elements, transposons, or “jumping genes”) are known to be nearly universal in occurrence. These mobile genetic units relocate within the genome by the process called transposition. The presence of transposable elements indicates that genetic information is not fixed within the genome of an organism. Barbara McClintock, working with corn in the 1940s, was the first to detect transposable elements, which she initially identified as controlling elements. This discovery was about 20 years ahead of the discovery of transposable elements in prokaryotes. Controlling elements may be grouped into families. The members of each family may be divided into two classes: autonomous elements or non-autonomous elements. Autonomous elements have the

ability to transpose whereas the non-autonomous elements are stable (but can transpose with the aid of an autonomous element through trans-activation).

## **Biotechnology for creating genetic variability:**

### **Gene transfer:**

The rDNA technology is the state-of-the-art in gene transfer to generate genetic variability for plant breeding. With minor exceptions, the DNA is universal. Consequently, DNA from an animal may be transferred to a plant! The tools of biotechnology may be used to incorporate genes from distant sources into adapted cultivars. An increasing acreage of cotton, soybean, and maize are being sown to genetically modified (GM) cultivars, indicating the importance of this technology for creating variability for plant breeding. Economic gene transfers have been made from bacteria to plants to confer disease and herbicide resistance to plants. The most common GM products on the market are RoundupReady<sup>®</sup> cultivars (e.g., cotton, soybean) with herbicide tolerance, and Bt products (e.g., corn) with resistance to lepidopteran pests. The technique of site-directed mutagenesis allows scientists to introduce mutations into specified genes, primarily for the purpose of studying gene function, and not for generating variability for breeding per se. Other tissue culture based techniques include protoplast fusion, cybrid formation, and use of transposons.

### **Somaclonal variation:**

In vitro culture of plants is supposed to produce clones (genetically identical derivatives) from the parent material. However, the tissue culture environment has been known to cause heritable

variation called somaclonal variation. The causes cited for these changes include karyotypic changes, cryptic chromosomal rearrangements, somatic crossing over and sister chromatid exchange, transposable elements, and gene amplification. Some of these variations have been stable and fertile enough to be included in breeding programs.



## **Session # 6 & 7: Tutorial**

### **Creation of genetic variation using conventional techniques.**

Variability that can be attributed to genes that encode specific traits and can be transmitted from one generation to the next is described as genetic or heritable variation. Because genes are expressed in an environment, the degree of expression of a heritable trait is impacted by its environment, some more so than others (Figure 4.1). Heritable variability is indispensable to plant breeding. As previously noted, breeders seek to change the phenotype (trait) permanently and heritably by changing the genotype (genes) that encode it. Heritable variability is consistently expressed generation after generation. For example, a purple-flowered genotype will always produce purple flowers. However, a mutation can permanently alter an original expression. For example, a purple-flowered plant may be altered by mutation to become a white-flowered plant. Genetic variation can be detected at the molecular as well as the gross morphological level. The availability of biotechnological tools (e.g., DNA markers) allows plant breeders to assess genetic diversity of their materials at the molecular level. Some genetic variation is manifested as visible variation in morphological traits (e.g., height, color, size), while compositional or chemical traits (e.g., protein content, sugar content of a plant part) require various tests or devices for evaluating them.

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## **Session # 8 & 9: Tutorial**

### **Creation of genetic variation using non conventional techniques.**

Mutation is non- conventional method of creating genetic variation. Mutations are important in biological evolution as sources of heritable variation. They arise spontaneously in nature as a result of errors in cellular processes such as DNA replication (or duplication) and by chromosomal aberrations (deletion, duplication, inversion, translocation). The molecular basis of mutation may be described by mechanisms such as modification of the structure of DNA or a component base of DNA, substitution of one base for a different base, deletion or addition of one base in one DNA strand, deletion or addition in one or more base pairs in both DNA strands, and inversion of a sequence of nucleotide base pairs within the DNA molecule.

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In vitro culture of plants is supposed to produce clones (genetically identical derivatives) from the parent material. However, the tissue culture environment has been known to cause heritable variation called somaclonal variation. The causes cited for these changes include karyotypic changes, cryptic chromosomal rearrangements, somatic crossing over and sister chromatid exchange, transposable elements, and gene amplification. Some of these variations have been stable and fertile enough to be included in breeding programs.

## **Session # 9 & 10: Tutorial**

### **Breeding methods in self- pollinated crops**

#### **Mass selection**

In mass selection, seeds are collected from (usually a few dozen to a few hundred) desirable appearing individuals in a population, and the next generation is sown from the stock of mixed seed. This procedure, sometimes referred to as phenotypic selection, is based on how each individual looks. Mass selection has been used widely to improve old “land” varieties, varieties that have been passed down from one generation of farmers to the next over long periods.

An alternative approach that has no doubt been practiced for thousands of years is simply to eliminate undesirable types by destroying them in the field. The results are similar whether superior plants are saved or inferior plants are eliminated: seeds of the better plants become the planting stock for the next season.

A modern refinement of mass selection is to harvest the best plants separately and to grow and compare their progenies. The poorer progenies are destroyed and the seeds of the remainder are harvested. It should be noted that selection is now based not solely on the appearance of the parent plants but also on the appearance and performance of their progeny. Progeny selection is usually more effective than phenotypic selection when dealing with quantitative characters of low heritability. It should be noted, however, that progeny testing requires an extra generation; hence gain per cycle of selection must be double that of simple phenotypic selection to achieve the same rate of gain per unit time.

Mass selection, with or without progeny test, is perhaps the simplest and least expensive of plant-breeding procedures. It finds wide use in the breeding of certain forage species, which are not important enough economically to justify more detailed attention.

## **Pure-line selection**

Pure-line selection generally involves three more or less distinct steps: (1) numerous superior appearing plants are selected from a genetically variable population; (2) progenies of the individual plant selections are grown and evaluated by simple observation, frequently over a period of several years; and (3) when selection can no longer be made on the basis of observation alone, extensive trials are undertaken, involving careful measurements to determine whether the remaining selections are superior in yielding ability and other aspects of performance.

Any progeny superior to an existing variety is then released as a new “pure-line” variety. Much of the success of this method during the early 1900s depended on the existence of genetically variable land varieties that were waiting to be exploited. They provided a rich source of superior pure-line varieties, some of which are still represented among commercial varieties. In recent years the pure-line method as outlined above has decreased in importance in the breeding of major cultivated species; however, the method is still widely used with the less important species that have not yet been heavily selected.

A variation of the pure-line selection method that dates back centuries is the selection of single-chance variants, mutations or “sports” in the original variety. A very large number of varieties

that differ from the original strain in characteristics such as colour, lack of thorns or barbs, dwarfness, and disease resistance have originated in this fashion.

## **Hybridization**

During the 20th century planned hybridization between carefully selected parents has become dominant in the breeding of self-pollinated species. The object of hybridization is to combine desirable genes found in two or more different varieties and to produce pure-breeding progeny superior in many respects to the parental types.

Genes, however, are always in the company of other genes in a collection called a genotype.

The plant breeder's problem is largely one of efficiently managing the enormous numbers of genotypes that occur in the generations following hybridization. As an example of the power of hybridization in creating variability, a cross between hypothetical wheat varieties differing by only 21 genes is capable of producing more than 10,000,000,000 different genotypes in the second generation. At spacing normally used by farmers, more than 50,000,000 acres would be required to grow a population large enough to permit every genotype to occur in its expected frequency. While the great majority of these second generation genotypes are hybrid (heterozygous) for one or more traits, it is statistically possible that 2,097,152 different pure-breeding (homozygous) genotypes can occur, each potentially a new pure-line variety. These numbers illustrate the importance of efficient techniques in managing hybrid populations, for which purpose the pedigree procedure is most widely used.

## **Pedigree breeding:**

starts with the crossing of two genotypes, each of which have one or more desirable characters lacked by the other. If the two original parents do not provide all of the desired characters, a third parent can be included by crossing it to one of the hybrid progeny of the first generation (F<sub>1</sub>). In the pedigree method superior types are selected in successive generations, and a record is maintained of parent–progeny relationships.

The F<sub>2</sub> generation (progeny of the crossing of two F<sub>1</sub> individuals) affords the first opportunity for selection in pedigree programs. In this generation the emphasis is on the elimination of individuals carrying undesirable major genes. In the succeeding generations the hybrid condition gives way to pure breeding as a result of natural self-pollination, and families derived from different F<sub>2</sub> plants begin to display their unique character. Usually one or two superior plants are selected within each superior family in these generations. By the F<sub>5</sub> generation the pure-breeding condition (homozygosity) is extensive, and emphasis shifts almost entirely to selection between families. The pedigree record is useful in making these eliminations. At this stage each selected family is usually harvested in mass to obtain the larger amounts of seed needed to evaluate families for quantitative characters. This evaluation is usually carried out in plots grown under conditions that simulate commercial planting practice as closely as possible. When the number of families has been reduced to manageable proportions by visual selection, usually by the F<sub>7</sub> or F<sub>8</sub> generation, precise evaluation for performance and quality begins. The final evaluation of promising strains involves (1) observation, usually in a number of years and

locations, to detect weaknesses that may not have appeared previously; (2) precise yield testing; and (3) quality testing. Many plant breeders test for five years at five representative locations before releasing a new variety for commercial production.

### **The bulk-population method:**

It differs from the pedigree method primarily in the handling of generations following hybridization. The F<sub>2</sub> generation is sown at normal commercial planting rates in a large plot. At maturity the crop is harvested in mass, and the seeds are used to establish the next generation in a similar plot. No record of ancestry is kept. During the period of bulk propagation natural selection tends to eliminate plants having poor survival value. Two types of artificial selection also are often applied: (1) destruction of plants that carry undesirable major genes and (2) mass techniques such as harvesting when only part of the seeds are mature to select for early maturing plants or the use of screens to select for increased seed size. Single plant selections are then made and evaluated in the same way as in the pedigree method of breeding. The chief advantage of the bulk population method is that it allows the breeder to handle very large numbers of individuals inexpensively.

Often an outstanding variety can be improved by transferring to it some specific desirable character that it lacks. This can be accomplished by first crossing a plant of the superior variety to a plant of the donor variety, which carries the trait in question, and then mating the progeny back to a plant having the genotype of the superior parent. This process is called **backcrossing**. After five or six backcrosses the progeny will be hybrid for the character

being transferred but like the superior parent for all other genes. Selfing the last backcross generation, coupled with selection, will give some progeny pure breeding for the genes being transferred. The advantages of the backcross method are its rapidity, the small number of plants required, and the predictability of the outcome. A serious disadvantage is that the procedure diminishes the occurrence of chance combinations of genes, which sometimes leads to striking improvements in performance.

## **Hybrid varieties**

The development of hybrid varieties differs from hybridization. The F1 hybrid of crosses between different genotypes is often much more vigorous than its parents. This hybrid vigour, or **heterosis**, can be manifested in many ways, including increased rate of growth, greater uniformity, earlier flowering, and increased yield, the last being of greatest importance in agriculture.

## **Session # 11 & 12: Laboratory**

### **Breeding methods in cross pollinated crops.**

This article throws light upon the top three breeding methods used for cross-pollinated crops. The methods are:

#### **1. Mass Pedigree Method**

#### **2. Inbreeding**

#### **3. Recurrent Selection.**

#### **1. Mass Pedigree Method:**

In this method of breeding, the best individuals with desired characters are selected on the basis of phenotypic performance in a source population. Open-pollinated seeds of the selected individual plants are divided into two halves. Second year replicated progeny row trial is conducted using one set of half seeds from each plant.

On the basis of the progeny performance, the best parental individuals are identified. The remnant half seeds from the superior parental plants are mixed and grown in isolation for random mating during the third year. This method of breeding is equivalent to ear-to-row selection in context of maize originally proposed by C.G. Hopkins at the Illinois Agricultural Experiment Station in 1896 to improve protein and oil content of maize. This method has been named as mass-pedigree method by S.S. Rajan in India. This very method is called line breeding when selection is based on progeny tests and a group of progeny lines is composited.

## **2. Inbreeding:**

The mating of individuals more closely related than individuals mating at random is known as inbreeding. The lines produced by continued inbreeding are known as inbred lines. Self-fertilization is the most intense form of inbreeding.

In plant breeding nearly homozygous lines are produced by continued self-fertilization accompanied by selection for five to six generations. This can be used as the method of breeding only in those crops, which do not show any loss of vigour due to inbreeding, like cucurbits.

### **The three important uses of inbreeding in cross-pollinated crops are as follows:**

- (i) To attain uniformity in plant characters.
- (ii) To improve yield etc. by individual plant selection as in cucurbits in which there is no inbreeding depression.
- (iii) To develop suitable inbred lines in production of hybrids and synthetics.

## **Synthetic Variety:**

The term 'synthetic variety' has come to be used to designate a variety that is maintained from open pollinated seed following its synthesis by hybridization in all combinations among a number of selected genotypes, which have been tested for combining ability.

The components of a synthetic variety could be inbred (usually), clones, mass selected populations or various other materials. The component units are maintained so that the synthetic may be reconstituted at regular intervals.

The inbreeds to be used as component lines are chosen on the basis of combining ability tests.

The component inbred are crossed in all possible combinations. This inter-crossed seed is called as Syn 0.

Equal quantity of seed from all crosses is composited and the mixture is allowed open-pollination in isolation and seed is harvested. This becomes Syn 1 generation. In absence of reconstitution of a synthetic at regular intervals, the population becomes an open-pollinated variety.

The testing for combining ability is the decisive criterion for a synthetic variety by which it can be distinguished from a conventional variety of a cross-pollinating species, which originates in a continuous selection of individuals and subsequent progeny tests. The greater variability caused by crossing several components with high general combining ability makes the synthetic varieties more adaptable compared to conventional varieties.

Similar to hybrids, the yield of a synthetic variety generally also decreases after the Syn 2, until an equilibrium is reached which, in partially self-fertile species, depends on selfing rate and inbreeding (minimum depression), but also on the number of components used in the Syn 0.

## **Session # 13 & 14: Tutorial**

### **Hybridization techniques used in cross pollinated crops; Heterosis and its exploitation in crop improvement.**

#### **EMASCULATION AND POLLINATION TECHNIQUES IN CEREALS**

##### **1. RICE (*Oryza sativa*) (2n = 24) (Family – Poaceae)**

In rice anthesis commences shortly after emergence of panicle. Spikelets at the tip bloom first and proceed downwards. Anthesis time 8-10 am. Each spikelet remain open 30 minutes and then closes. The anther dehiscence takes place immediately after the opening of the spikelets. Receptivity remains for one day.

#### **Emasculation and Crossing techniques**

Emasculation is necessarily followed by controlled pollination. Emasculation is done during early morning between 6 and 8 AM in spikelets, due to open on the same day. Emasculation should be over well ahead of the time of anthesis. Crossing techniques in rice differ based on the method of emasculation. Since maximum number of spikelets open on the 3rd or 4th day of anthesis, panicles of that stage are selected for emasculation. The following methods are widely used for hybridization in rice.

##### **1) Clipping method**

In the previous day evening, top 1/3rd and bottom 1/3rd portions in the panicle of the desired female parent are clipped off by using scissors leaving the middle spikelets. With the help of scissors again, top 1/3 portion in each spikelet is clipped-off in a slanting position. The six

anthers present in each spikelet are removed with the help of the needle (Emasculation). Care must be taken during emasculation for not to damage the gynoecium. Then to prevent contamination from the foreign pollen, the emasculated spikelets are covered with a butter paper bag. In the next day morning (usually at 9.00AM), the bloomed panicle from the desired male parent is taken. The top portion of the butter paper bag which was originally inserted in the emasculated female parent is now cut to expose the panicle. The male parent panicle is inserted in an inverted position into the butter paper bag and turned in both ways in order to disperse the pollen. After ensuring the abundant disbursement of pollen, the opened butter paper bag is closed using a pin. Coloured thread may be tied at the base of the panicle to identify the crossed ones. After ensuring pollination, the bag may be removed.

## **2) Hot water method**

A method of hot water emasculation is used to about the same extent as the clipping method. Panicles in 3rd (or) 4th day of blooming are chosen as female parents. An hour or so before blooming (i.e. normally at 7. A.M.), the panicle is selected and under developed and opened spikelets are removed. Now, the tiller is bent over (carefully to avoid breaking) and the selected panicle is immersed in hot water contained in a thermos bottle at 40-44°C for a period of 5 to 10 minutes. This treatment causes the florets to open in a normal manner and avoids injury. Then, emasculation is done by removing the six stamens by fine forceps or needles and then dusting should be done.

#### **4) Vacuum emasculation method**

This works on the principle of suction pressure. The spikelets are clipped off prior to operation. The minute pipette is to be shown at the point of clipping and pollen is sucked in. Six panicles can be emasculated at a time. By hand emascualtion, 100 flowers can be emasculated by a person. With the vaccum emasculator, six persons can operate and emasculate 3000 to 3600 florets/hour.

#### **6) Brown paper method**

The panicles are enclosed in a Brown paper cover before a couple of hours of blooming. Heat develops inside due to which the anthers extrude, but donot dehisce. This happens in 15-30 minute then the anthers are easily clipped off. Stigmatic surface is then dusted with pollen grains collected from the chosen male parent. The crossed panicle is then properly tagged and protected with paper cover which is retained in a position for 7 – 10 days.

### **WHEAT (*Triticum aestivum*) (2n = 42 Hexaploid) (Family – Poaceae)**

Much of the pollen grains shed within the floret and the crop is largely self pollinated. The glumes normally open during the flowering process, the anthers protrude from the glumes and part of the pollen grains is shed outside the flowers. Entry of foreign pollen at flower opening may result in a small extent of cross pollination which is normally less than one per cent.

## **Selfing**

The inflorescence is covered with a butter paper cover prior to anthesis, and kept undisturbed till the flower opening completed.

## **Emasculation**

On emergence of the ear upper 1/3rd of the spikelet is cut and lower spikelets are also removed. Of the remaining spikelets alternate ones on both sides of the axis are removed. The top spikele is held with forceps and pulled downwards and upwards to remove the upper florets of the spikelets. The glumes are separated and anthers left exposed are removed carefully and covered with butter paper cover.

## **Crossing**

On the next day earhead selected from the pollen parent are used for crossing. The upper half of the glumes of the few medium spikelets are cut of and the ripened bright yellow anthers are rubbed on the styles of the emasculated florets and then covered.

## **Session # 15:**

**Tutorial: Course/Discussion from session 1 to 14 (Mid Term Exam)**