6

Breeding Horticultural Plants

Purpose and Expected Outcomes

This chapter is designed to review basic genetic principles and concepts and how they are applied in the breeding of new horticultural plants.

After studying this chapter, the student should be able to

1. Discuss the importance of genetics in horticultural plant improvement.
2. Explain the genetic basis of biological variation.
3. Describe and discuss the steps in a simple plant breeding program.
4. Discuss the use of molecular biotechnological tools in plant improvement.
5. Discuss specific practical applications of classical genetics and molecular biology in plant improvement.

Overview

Plant breeding or improvement is a science and an art. Genetics is the underlying science of plant breeding. In fact, breeders are sometimes referred to as applied geneticists. They try to nudge nature to the advantage of humans by manipulating plants to perform according to their schedule and needs. They manipulate the nature (heredity) of plants and thereby create new types that are adapted to new environments (nurture) and produce higher-quality products that are disease resistant. Every year, new and improved flower and vegetable garden cultivars are released by plant breeders for use by growers. These are products of calculated and deliberate manipulation of plants by scientists who understand the genetics and environment of those plants.

As agents of heritable change, plant breeders are sometimes also described as applied evolutionists. This chapter is devoted to describing how these scientists operate, highlighting the role genetics plays in their endeavors. This is not to say that plant breeding is an exact science; it is also an art, as already stated. Experience (breeder's eye) is a valuable asset in breeding. Conventional or classical methods of breeding depend more on this artistic component. However, thanks to advances in science and technology, new and more effective methods of plant improvement are now available to breeders. Instead of

manipulating plants at the whole-plant level, breeders are able to manipulate plants directly at the DNA level (*molecular biotechnology*), thereby expanding the degree to which they are able to affect the course of nature. It should be emphasized that conventional and modern tools are used side by side for best results. As such, discussions in this chapter include both types of techniques.

6.1 What Is Plant Breeding?

Plant breeding (plant improvement) is the science and art of manipulating plant heredity to develop new and improved plant types for use by society. It is a deliberate effort by humans to nudge nature, with respect to the heredity of plants, to an advantage. Plant breeders manipulate plants to address specific needs of society.

1. *Address world food, feed, and nutritional needs.* Breeders develop new fruits, vegetables, and food crops that have higher nutritional value. Cereals are low in amino acids lysine and threonine, while rice lacks pro-vitamin A. Nutritional deficiency disorders such as blindness and rickets abound in regions where foods are staples. Breeders work to enhance the nutritional value of such foods, an example being the high-lysine corn.
2. *Address the food needs of a growing population.* As the world population continues to explode, the world food supplies must be expanded to meet the needs of society. Plant breeders develop new varieties that are higher yielding to increase agricultural productivity. For example, the yield of corn rose from about 2,000 kg/ha in the 1940s to 6,000 kg/ha in the 1990s.
3. *The need to adapt plants to environmental stresses.* Environmental stresses may be biotic or abiotic. Plant breeders develop new varieties that are adapted to abiotic environmental stresses like drought, cold, acid soils and salty soils, and biotic ones like diseases and insect pests. This action expands the production areas of plants.
4. *The need to adapt crops to specific production systems.* Different varieties are needed for different production systems. For example, a different variety of tomato is needed for hand harvesting, and another for mechanized production.
5. *Satisfying industrial and other end-use requirements.* The quality requirements for fresh produce meant for the table are different from the requirements for the food-processing industry. Potato for chipping should have low sugar content to avoid the browning from the caramelization of sugar upon heating that is undesirable. Different varieties are bred for baking, cooking, and fries.
6. *Developing new horticultural plants.* Apart from fruit and vegetables, horticulture is identified with ornamentals for beautifying the indoors and outdoors. Plant breeders develop new varieties of flowers each year.

6.2 The Art and Science of Plant Breeding

Plant breeding is firmly rooted in science, depending on principles of genetics especially, as well as biology, botany, statistics, biochemistry, agronomy, to name other major disciplines. Plant breeding is like evolution, the theory proposed by Charles Darwin to explain biological variation. The three principles of evolution are variation, heredity, and selection. Without variation, there can be no evolution! Natural variation arises through mutations. Some variations are more useful for survival than others. Natural selection will discriminate among variability to advance the ones that most fit the prevailing environment. Through heredity, the offspring of the selected individuals will resemble the parents more than other unrelated individuals.

Similarly, there can be no plant breeding without variation. If the breeder intends to develop a new variety of tomato that is short, there must be a source of the genes for shortness to access. Otherwise, such a desire cannot be fulfilled. Whereas evolution depends on random mutations to create variability, plant breeders can assemble and create variability through the use of specific techniques. Then, they impose artificial selection to pick out individuals that exhibit the traits of interest to advance and eventually develop a new variety for use by producers.

The significant differences between evolution and plant breeding include the duration of the processes. Evolution takes millions of years to bring about change in a direction; plant breeders take about ten years (or more or less), depending on the species, to develop a new variety. Evolution is driven by nature, following the path of chance. Plant breeders have a definite plan and objective in their breeding program, not leaving anything to chance. Selection is artificial (by humans–breeders) not by nature. The product of evolution is determined primarily by survivability as dictated by the environment. In plant breeding, the goal is first the value of the product or outcome to society. The variety may not survive on its own in nature, but modern advances in crop production allow us to provide supplemental inputs to overcome natural environmental factors, or to design plants to adapt to specific natural environments to produce desired products.

Plant breeding is not an exact science. Whereas genetic principles are depended upon in modern plant breeding, skill, intuition, good judgment, and keen observation are desirable qualities for successful plant breeding. Together, these qualities are called the "breeder's eye." Visual selection is the primary method of discriminating among variation in plant breeding.

There are two basic approaches to breeding—conventional and nonconventional. Conventional plant breeding entails using the sexual process to transfer and assemble genes of interest in a new plant individual. Traditionally, this occurs via crossing selected parents. Nonconventional plant breeding can circumvent the sexual process as a means of gene transfer. Genes of interest can be obtained from their sources and physically incorporated into the genetic system of another plant. Such genetic feats are accomplished in the often-controversial plant genetic manipulation called **genetic engineering.**

Genetic Engineering
The manipulation of genes, composed of DNA, to create heritable changes in biological organisms and products that are useful to people, living things, or the environment.

6.3 The Concept of Genetic Manipulation

The underlying principle of plant genetic manipulation by breeders can be summarized by the following simplified genetic relationship:

$$Phenotype = Genotype + Environment \; (P = G + E)$$

This means that what you see (phenotype or trait) is the product of the interaction of the genes that condition or control the trait (genotype), and the environment in which the genes are being expressed.

If you do not like what you see, you can change the genotype, the environment, or both. Changing the genotype amounts to developing a new variety by manipulating plant genetics to assemble genes in a new genetic matrix. Such a change is permanent and heritable. One can also change the environment. This is largely what agronomists do. Changing the environment includes providing production inputs like fertilizers, irrigation, and pesticides. Such changes are temporary, for if you desire to retain the level of expression of the trait (e.g., if you want to maintain the yield level), you must resupply the environmental factors. Their effects are not heritable and cannot be passed on from one generation to the next.

Plant breeders are interested in heritable changes, but, a new and improved variety is only as good as its environment. If you invest money to purchase a new and high-yielding hybrid seed, you will get the potential high yield inherent in the variety by virtue of the genes assembled by breeding methods, only if you provide an environment that supports high performance.

Application of Tissue Culture in Modern Genetic Improvement of Daylily (*Hemerocallis* sp).

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Importance of Daylily and Classical Breeding Challenges

Daylily (*Hemerocallis* sp) is a popular perennial monocot valued for the beauty of the colors and shapes of its flowers. It is also used as a vegetable in China. As in most crops, the improvement of daylily is usually achieved through classical breeding. This method has been used to produce many new cultivars with a wide range of colors (Stout, 1986). However, the time required to produce and introduce a new cultivar is very long, because only few new plants are produced annually under natural conditions (Apps and Heuser, 1975; Meyer, 1976). Even with the use of improved horticultural techniques whereby daylily is propagated per crown cottage, only 20 to 30 or a little more new plants could be obtained from hybrids per year in the greenhouse environment (Traub, 1936). There are also other limitations that have not been successfully and speedily resolved through classical breeding alone. For instance, after blooming, daylily flowers last only one day (Panavas *et al*., 1999) despite the fact that its beauty, thus its value, is based upon this organ. The approach of classical breeding to address this problem was to lengthen the flowering season. It has also been established that an increase in ubiquitin protein content speeds up the wilting of daylily flowers (Courtney *et al*., 1994). Thus the lack of longer flower life remains an unsolved challenge that might be tackled through modern techniques.

Ideal Daylily Tissue Culture Method for the Crop Improvement

Tissue culture is not required for genetic transfer in crops (Bent, 2000). However, efficient and large scale transformations of crops have been achieved via tissue culture (Vain, 2007). Unlike other tissue culture-free methods, transformation methods via tissue culture are generally reliable, reproducible, and can involve large scale plant materials for transformation with subsequent regeneration, thus, are very efficient. An efficient and reproducible tissue culture method in daylily should produce a high frequency of new shoots in a short period of time, preferably involving a few steps. It should induce repetitive or multiple shoots or embryos with a minimum amount of variationS. The method should also offer a wide choice of explant tissues such that the availability of tissues should not be restricted to the reproductive cycle of the crop.

Application, Challenges, and Prospects of Daylily Tissue Culture for Genetic Improvement

The cloning and subsequent transfer into plants of ubiquitin inhibitor gene(s) and perhaps other genes including those influencing flower color and forms, might prolong the life and diversity of the color and shape of the flowers. In addition, the use of modern techniques might also avoid the incorporation of unwanted genes into the crop, which could occur during conventional plant breeding.

Meristem and Inflorescence Explants and Related Challenges

In daylily, methods for *in vitro* organogenesis have been established (Aziz *et al*., 2003; Krikorian and Kann, 1981; Meyer, 1976). The vegetative meristem can be used as explant of choice for *in vitro* plant regeneration (Smith and Krekorian, 1991). However, since it is located underground, meristematic tissues are difficult to obtain in a sterile condition and excision destroys the mother plant (Meyer, 1976). Of all the floral tissues investigated, the use of the whole inflorescence has become common in part because it is available en masse and less difficult to obtain in sterile condition. Also, it is relatively easier to manipulate the whole inflorescence than petals or sepals. However, limitations with the use of floral tissues are obvious. For instance, those tissues are not available all year-round and, further, they are reproductive cycle dependent. Thus, this limits the freedom of scientists working with daylily to plan and effectively carry out experiments when needed.

Leaf Explant Potential

Leaf tissue may be the key to overcome such problems because: (1) with some exceptions, it is the only daylily organ above-ground that is available year-round; (2) it might be easier to obtain such tissues in sterile condition; (3) the use of the leaf as an explant might not affect the survival of the mother plant; and (4) the fact that leaf is flat and flexible might make it a more suitable target for particle bombardment and gene transfer, than the round and tough inflorescence. Further, it might be much easier to produce leaf discs providing an efficient wound area for *Agrobacterium* infection than the inflorescence for gene transformation. Despite all these advantages, the use of leaf tissue in daylily tissue culture is limited. This tissue is flexible and has successfully been used to produce protoplasts for subsequent production of callus and shoots (Ling and Sauve, 1995). However, the induction of shoots (Matand, 1999) or callus directly from leaf tissue has not been widely investigated (Matand, 1999).

Morphogenic Potency of Selected Growth Regulators

Morphogenesis in daylily has been manipulated using mostly common types of growth regulators such as NAA (naphthaleneacetic acid), 2, 4-D (2, 4-dichlorophenylacetic acid), BA (6-benzyl aminopurine), and KIN (kinetin), (Aziz *et al*., 2003; (Griesbach, 1989; Meyer, 1976). Thidiazuron (TDZ) has been proven to be a very powerful cytokinin. It is up to 10,000 times more active than DPU (N, N'-diphenylurea), and 10 times more active than

zeatin (Pierik, 1987). In general, thidiazuron is 1000 times more active than other standard growth regulators (Huettman and Preece, 1993). The use of TDZ in daylily is also limited (Matand, 1999, Aziz *et al*., 2003) but has several advantages. TDZ is characterized by a unique rapid induction of repetitive multiple shoots across explants and plant species, which needs to be fully explored also in daylily. This chemical has consistently induced shoots faster in higher frequencies than other cytokinins commonly used. TDZ is successfully used across species to induce direct as well as indirect adventitious shoots (Matand and Prakash, 2007). It is used for shoot regeneration even at lower concentrations. The lowest TDZ recommended concentrations (established after investigation) in plant propagation is 10^{-8}M (Huetteman and Preece, 1993). TDZ concentrations lower than the preceding proposed is also applicable. Reports also suggest that when TDZ is being used for the first time in a new species, the initial experiment should be designed using 10^{-7} M as a middle concentration and evaluating two orders of magnitude above and below that concentration. The normally recommended concentration range of TDZ activity is 10^{-9} to 10^{-5} M (Huetteman and Preece, 1993). However, depending on the species, this range might proliferate more calli than shoots. Thus to reduce callus proliferation in favor of shoot growth, it is recommended to use concentrations up to 10^{-4}M (Huetteman and Preece, 1993).

Physical State of the Medium

In some plant species, the success or failure of tissue culture might depend upon whether a liquid or an agar medium is employed (Murashige, 1974). For, instance, most bromeliads studied in laboratories could be started in cultures only in a liquid nutrient (Murashige, 1974). This has been true also with the cattleman orchid (Murashige, 1974). In contrast, shoot tip cultures of *Asparagus* (the closest relative of daylily) and *Gerbera* required initiation on agar gel medium (Chen and Galston, 1967). It has also been reported that the same species may require a different physical form of medium during each of the three growth stages *in vitro*. This change of the physical state of the nutrient medium in progressive stages of culture has been illustrated with *Asparagus officinalis* and *Daucus carota* (Murashige, 1974). In daylily, Krihorian and Kann (1981) demonstrated that besides semi-solid, liquid medium could be used to produce plants. Their studies suggested that the cultural conditions might be cultivar specific. They reported also that it is possible to use both solid and liquid medium to induce new shoots in daylily. Liquid medium was preferred over solid medium because of its more efficient use of space and the enhancement in shoot production (Krihorian and Kann, 1981). Also liquid medium has the capacity of diluting plant cell-excreted chemical toxic waste compared to solid medium. In liquid, explant cells are fully exposed to nutrients than on solid medium. However, their report failed to determine a shoot production ratio of liquid to solid medium in daylily. A rationale in the use of either physical medium needs further investigation in crop.

The Influence of Transformation Method in Regeneration Explant Selection

As indicated earlier, the selection of tissue culture method one should utilize for modern gene transfer into daylily should be dictated by the transformation technique. For instance, callus cells are more suitable for particle bombardment because a large number of cells that might be susceptible to subsequent plant regeneration can be efficiently arranged on the target plate for efficient gene blasts. This is also true for leaf tissue, except that in most cases plant regeneration is more likely to occur at leaf wound sites than non-wound areas. However, practically leaf wound cells are less likely to be

hit with target DNA. Therefore, it is recommended that leaf discs be lightly wounded across lamina to enhance chances of regenerating transgenic plants. The latter might not be necessary when the regeneration protocol involves complete de-differentiation of explant tissue. When callus is used for *Agrobacterium,* although some success might be possible, it should be remembered that (1) such bacteria are less infectious of monocots, (2) callus cells especially those that have lasted longer in *in vitro* culture produce very limited phenolic compounds, as those from wound response. The possibilities become even more restricted when cell suspension cultures are proposed for *Agrobacterium* infection; because limited phenolic compounds produced by cells are instantly diluted in the liquid medium. Phenolic compounds from fresh plant tissue wounds have been reported to be essential in carrying out special molecular signals that are thought to activate *Vir* genes in *Agrobacterium* for subsequent T-DNA excision and transfer into plant cells (Anand *et al*., 2007). Huge amounts of phenolic compounds are usually associated with fresh tissues. Thus, fresh tissues such as leaf, stem, or inflorescence are generally recommended as explants for *Agrobacterium* infection.

Daylily Experimental Approaches

This report also focuses on the investigation of eight-month-old callus potential for shoot regeneration, using kinetin or TDZ with or without NAA (see treatment layout Table 1). **Plant material:** Initial callus was developed from inflorescence tissues of daylily cultivar 'Incredible Charm' using Meyer (1976)'s protocol. This cultivar was selected from the Alabama A & M University's daylily germplasm (Normal, Alabama), which comprised a dozen of cultivars. Most of them were obtained as gifts from local growers in North Alabama. This crop had been maintained *in vitro* for eight months on Murashige and Skoog (MS) medium (1962). During the first seven months and half callus and very lmited shoots were induced and maintained on MS medium containing 2.68 µM NAA+0.46 µM KIN (Meyer, 1976), and then callus was subcultured onto basal MS medium for maintenance for the two weeks preceding this study. It was such callus that was used to initiate cultures for the present report. **Culture media:** Murashige and Skoog (1962) medium with the addition of myo-inositol (100 mg/l), sugar (30 mg/l), and agar (4 g/l) was used. The pH of the medium was adjusted to 5.8 prior to autoclaving. Three growth regulators including NAA, kinetin, and TDZ at various combinations, as shown in Table 1, were included in the media. Morphogenesis in daylily is commonly achieved using two types of media with respect to the kinds and concentrations of growth regulators that include (1) MS medium including 2.68 µM NAA+0.46 µM KIN, generally used to induce cell organization and differentiation (Meyer, 1976); (2) MS medium including 50 µM NAA+0.46 µM KIN, generally used for cell growth and division (Meyer, 1976). Those media are usually used to induce plants through callus phase. **Cultural conditions:** All cultures were conditioned similarly. 400 mg of callus was placed into a baby jar and used as a single experimental unit. Twenty mls of agar medium were assigned to each jar prior to culturing callus. Twenty experimental units were randomly assigned to each growth regulator treatment. Cultures were incubated in growth chamber at 25±30˚C under 16h/8h photoperiod and subcultured on similar medium every three weeks. Data were collected at two months of culture for each experimental unit.

TABLE 1 Treatments layout

NAA(M)	Kinetin(M)		Thidiazuron (M)	
	0	46×10^{-8}	10^{-7}	10^{-8}
0	*	*	*	*
2.68×10^{-8}	*	*	*	*

However, regular observations of the experiment were made as needed. During the visual inspection, the physical growth and development of each explant were assessed. Assessment criteria included callus cell division, and/or organ formation, color development from callus, contamination of some tissue or media, development of vitrification, and the intensity of phenolic compounds waste accumulation.

The Influence of TDZ on Daylily Callus Organ Formation

Route 3 on the flow chart for daylily plant regeneration shown in Figure 1,was the approach applied in this investigation. A unique aspect of this investigation was to assess the potential of older callus (eight-month old) for shoot induction. This has a special meaning as older callus cells are known for losing their morphogenic potential and inducing more variations compared to fresh younger callus. The study showed that large callus multiplication was observed primarily from treatments that included at least one growth hormone as shown in Table 2. However, average or limited callus growth was observed from control samples.

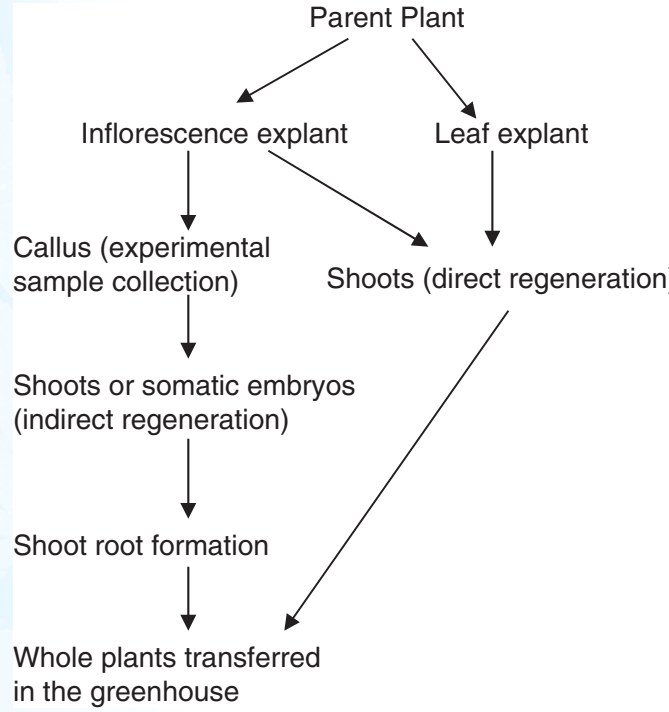

FIGURE 1 Daylily morphogenic flow-chart

TABLE 2 Eight-month callus morphogenic response

NAA	NAA/KIN	KIN	NAA/TDZ[7]	NAA/TDZ[8]	TDZ	TDZ[8]	Control
Callus multiplication							
++	++	++	++	++	++	++	++
Shoot formation							
4.9±3	6.3±5	3.7± 4	9.3±1	15±2	7.6±5	9.3±4	1.8±5
Root formation							
0	3.4±3	5.5± 3	5.6±3	0	0	0	0

Legends: −: no callus multiplication; +: limited average callus multiplication; ++: large callus multiplication; NAA: 2.68×10^{-8}M; KIN; 46×10^{-8}M; TDZ[8]: 10^{-8}M; TDZ[7]: 10^{-7}M; Control: MS basal medium; Shoot and root formation averages with standard errors were observed per callus unit.

Generally, within ten days of experimental culture organ primordia were observed. Shoot primordia particularly were identified by the development of localized greenish cells from callus, as shown in Figure 2. The development of greenish cells was the precursor for callus shoot meristem. All shoots reported in this investigation were observed from a single treatment and are only those that were initially formed after the experimental initiation. Repetitive shoots and those that formed after two months were not included in this study. Shoots were observed on all the treatments including the control, and the shoot averages ranged from 1.8 to 15 per callus unit (Table 2). The greatest shoot average of 15 per callus unit was observed from calli treated with 2.68 μM NAA and 10^{-8} μM TDZ, followed by 9.3 from those cultured on MS medium containing 10^{-8} μM TDZ or 2.68 μM NAA and 10^{-8} μM TDZ. All treatment media containing TDZ formed greater shoot averages than those containing NAA, NAA and KIN, KIN, or the control. The greatest shoot average influenced by KIN was 6.3, and was observed when KIN was combined with NAA. KIN alone induced relatively fewer shoots than NAA used alone. Almost two shoots per callus were observed from the control. Although there are not other obvious reasons, we believe that shoot formation on control media was influenced by residual effect of 2.68 μM NAA+0.46 μM KIN that were previously included into the initial medium, prior to transferring experimental sample callus onto the MS basal medium for two weeks maintenance. It was callus from the latter maintenance medium that was used for this study. During the study, some calli formed first roots then shoots (Figure 3) while some others formed only roots (Figure 4). Except Kinetin, most of the rooting calli were observed from samples cultured on treatments that included both auxin and cytokinin (Table 2). No roots were observed on control. No variations were observed from either callus or adventitious shoots. Shoot rooting was achieved on similar shoot inducing media or by transferring shoots onto MS basal medium (Figure 5). All plants that were transferred into the greenhouse recovered without much difficulty.

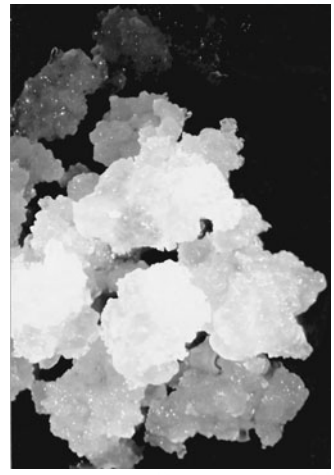

FIGURE 2 Callus tissues showing localized meristemoids, prerequisite for shoot formation *(Source: George Acquaah)*

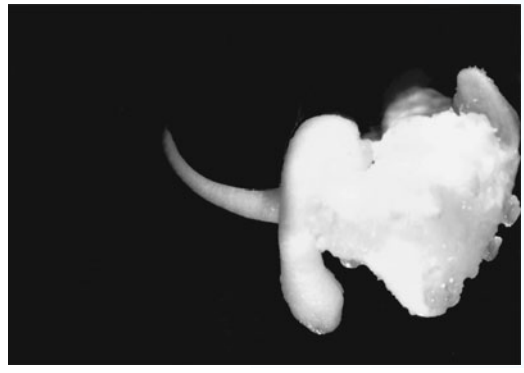

FIGURE 3 Picture showing root formation prior to shoot development *(Source: George Acquaah)*

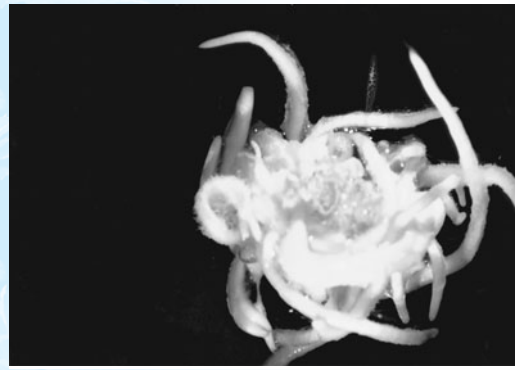

FIGURE 4 Picture showing only root development from callus *(Source: George Acquaah)*

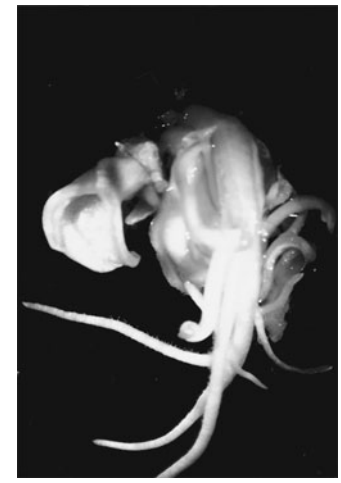

FIGURE 5 Picture showing roots forming on shootlet kept on shoot-including medium *(Source: George Acquaah)*

By experience plant regeneration in daylily can be achieved directly or indirectly (Matand, 1999) as outlined in daylily plant regeneration flow chart (Figure 1). Indirect regeneration is the most common approach in daylily (Smith and Krikorian, 1991). However, direct shoot regeneration has also been demonstrated and proven to occur much faster than the indirect regeneration (Matand, 1999), In that report, shoots were directly observed from both florescence and leaf explants. Those shoots formed within two weeks when TDZ treatments, as described in this report, were applied.

Overall, this report has presented select key challenges and considerations that have to be taken into account when one considers culturing daylily *in vitro* for either standard commercial or research micropropagation, or the crop genetic improvement using modern techniques. The report also succinctly addressed some considerations for the selection of plant regeneration method based upon its compatibility with the corresponding gene transfer method, while ensuring the availability of plant materials year-round. One of the highlights of this report is that daylily callus as old as eight months still has the potential to respond exogenous inducing hormones for organ formation. Although there is no clear explanation, it should be noted also that, under the conditions of the present study, daylily callus, which had been pre-cultured on hormone-inducing medium and then maintained on MS basal medium for two weeks retained the potential to form shoots without additional shoot-inducing hormones. TDZ as in many other species has proved to possess higher potency for shoot formation in daylily than KIN. It even induces shoots directly adn faster in both leaf and inflorescence tissues.

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6.4 Review of General Genetic Principles

The dominant scientific discipline in plant breeding is genetics, since breeding is all about manipulating the genetics of the organism in a predetermined way. The *cell* is the unit of organization of living things. Some organisms, like bacteria, are entirely one cell (unicellular) while others comprise numerous cells (multicellular). In one group of organisms, prokaryotes, the cellular components coexist without barriers, while in eukaryotes, the components are compartmentalized into discrete units called *organelles*, with membranous walls. The organelles include the nucleus, mitochondria, and chloroplasts, which are the three parts of a plant cell that contain DNA, the hereditary material.

Each nucleus contains a set of chromosomes (or genes) that is characteristic of the species, called the *genome*. A sexually reproducing plant has two basic types of cells, the gametes (pollen, ovules) of the sex cell have half the number (haploid, *n*) of the chromosomes of the somatic (body) cells (diploid, 2*n*). The nuclear chromosomes are subject to the laws of genetics as described by Mendel and are transmitted through the hereditary process in a predictable fashion (Mendelian inheritance). The genes in the mitochondria and chloroplast (extranuclear) are not subject to Mendel's laws (cytoplasmic or extranuclear inheritance).

The totality of all the genes an individual possesses constitutes its genotype. However, this total number is hard to determine. The term is more commonly used to represent the specific combinations of alleles present at a locus or loci of interest. That is, if the alleles of height are *H* and *h*, the genotype of an individual with respect to height, could be *HH, Hh,* or *hh*. What is observed is called the **phenotype**.

Phenotype
A biological characteristic or a trait possessed by an organism that results from the expression of a specific gene.

The cells in a sexually reproducing organism may undergo one of two basic kinds of cell division. Mitosis is the process in which the nucleus divides to create two identical daughter nuclei that contain the same number of chromosomes as the mother cell. It occurs only in the somatic tissue. Meiosis, the other division process, occurs only in the gametes and results in four cells with the haploid number of chromosomes as well as being non-identical. This is because of the critical genetic phenomenon called *crossing over* in which certain chromosomes physically exchange parts. There is a shuffling of chromosomes in a new genetic matrix (recombination) to create new cells that are unlike the mother cell. Recombination is the primary source of variation in sexually reproducing organisms. It is the reason why no two individuals are genetically alike (except identical twins).

6.5 Brief Review of Mendelian Genetics

The fundamental unit of heredity is called a *gene*, a segment of DNA that codes for a specific trait. There are alternate forms of a gene, called *alleles*. A diploid organism has two of these forms at a specific site on the chromosome (locus). Alleles control the same trait (e.g., height) but with different effects (e.g., short, tall). A diploid has only two alleles at one time at each locus, and may have identical or different effects (Figure 6–1). If different, one of the alleles (dominant) may mask the expression of the other (recessive). The locus with identical alleles is said to be homozygous, while one with different alleles is said to be heterozygous.

An individual that is heterozygous for the locus of interest will produce two distinct types of alleles because the alleles segregate into different gametes during meiosis (Mendel's law of segregation). Consequently, when two heterozygous individuals are crossed, random combinations will allow the two alleles to form new combinations, including one in which two recessive alleles will occupy the same locus. By being homozygous, the recessive allele whose effect was suppressed previously by the dominant allele in the heterozygous state, is now able to fully express its effect. When more than one locus of interest is simultaneously considered, the same principle operates. The genes for the different traits are inherited independently of each other. This is called *Mendel's law of independent assortment*. Plant breeders, knowing these laws, can select parents for use in crosses to produce predictable outcomes.

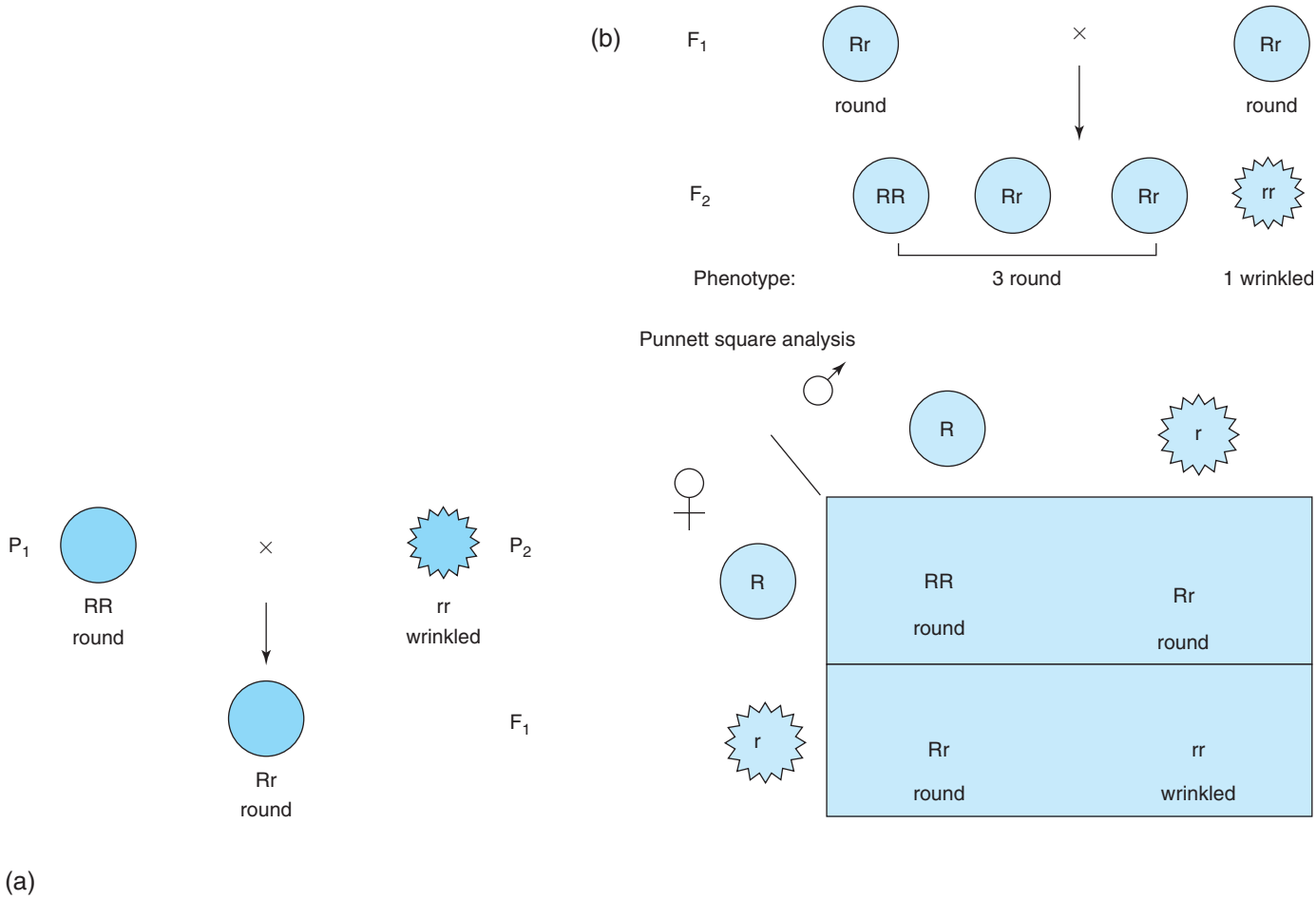

FIGURE 6–1 (a) Homozygous loci (RR,rr) interact to produce heterozygous locus (Rr); (b) heterozygous loci interact to reveal homozygous locus (rr).

6.6 Complex Inheritance

Mendel's work focused on traits that were controlled by one or a few genes. Such traits are called *simply inherited* or *Mendelian traits*. The genetics are straightforward and easy to breed. Unfortunately, many traits are controlled by many genes and hence have complex inheritance that is not readily amenable to Mendelian treatment. At the simpler end of the complex inheritance spectrum is incomplete dominance (or partial dominance), whereby the effect of the dominant allele over the recessive allele is not decisive, resulting in an average or blending of the two effects (Figure 6–2). In another situation, the two contrasting alleles are equally expressed (codominance). There are situations in nature whereby one gene has more than two alternative forms (multiple alleles), one being the ABO blood groups in humans.

On the other end of the inheritance spectrum are traits that are governed by numerous genes whose individual effects are difficult to distinguish (polygenes, quantitative genes). The trait they control is called **quantitative traits.** Each gene contributes little effect to the overall phenotype. Such traits are best measured (metrical traits) rather than counted as in Mendelian traits because they are difficult to breed.

Quantitative Traits
Traits that are controlled by many genes, each contributing a small effect to the overall phenotypic expressions of the trait.

Mendelian traits can be neatly categorized into distinct classes (ratios) by counting. Sometimes the expected ratios do not manifest because of various interferences. The phenomenon of epistasis, whereby alleles at different loci (nonallelic genes) interact, can modify genetic outcomes and consequently the expected ratios. For example, instead of the

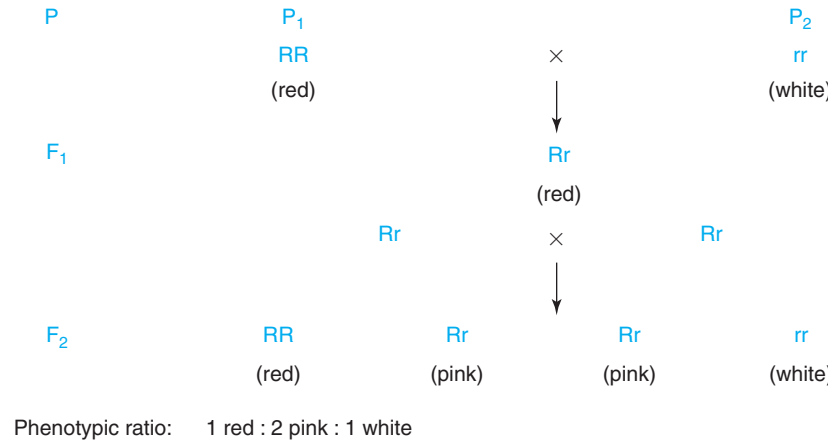

FIGURE 6–2 Incomplete dominance gene action produces pink flowers instead of red in the case of complete dominance.

9:3:3:1 ratio expected when two loci of interest are considered (i.e., a dihybrid cross), alleles at another locus can contribute to the expression of the phenotype, resulting in modifications of the ratio. Examples of modifications are 9:7 (complementary genes), 13:1 (suppressor genes), and 15:1 (duplicate genes). Such modifications can complicate breeding efforts.

Genes that are located on the same chromosome are said to be linked (genetic linkage) and are expected to be inherited from one generation to the next as a block. However, the event of crossing over can interfere with such smooth, intact transmission of gene blocks. Some genes are more tightly linked than others. The former are difficult to interrupt or break. It is a blessing to plant breeding when good gene blocks occur. Unfortunately, there are occasions in which good and desirable genes are linked with undesirable genes! Plant breeders used techniques like repeated crossing to provide opportunities for genetic recombination to occur to break undesirable genetic associations.

6.7 The Importance of Reproductive Systems

Plant breeders must be familiar with the reproductive systems of the plants they seek to manipulate. The genetic structure of plants depends on their mode of reproduction. Plants may be sexually reproducing or asexually reproducing, the latter not involving the sexual process or crossing. Asexually reproducing species remain "pure" or genetically identical from one generation to the next (clones) because the sexual process involves meiosis, the process that creates natural genetic variability.

To breed sexually reproducing species, the breeder must understand the floral biology and other factors associated with their flowering. To cross plants, you must know when the flowers open, how long the pollen stays viable, when the stigma is receptive, and other factors. Some sexually reproducing species are called self-pollinating because they receive pollen grains from their own flowers or from like plants. This habit makes them have a narrow genetic base. They are homozygous at most loci and produce relatively uniform products. Other species are cross-pollinating, receiving pollen from an available source. Cross-pollinated species are heterozygous at most loci and have a broad genetic base. These two basic reproductive behaviors dictate how the species are genetically manipulated. If you treat a cross-pollinated species as self-pollinated, once you remove the artificial restriction, it would revert back to its old ways!

In addition to the state genetic differences, there is a critical factor that plant breeders exploit in breeding plants. It is known that the farther apart genetically parents in a cross are, the more vigorous the product of their mating. Because cross-pollinated species are heterozygous, they can maintain a high genetic load without consequences. Lethal recessive alleles can enjoy a heterozygous advantage, remain innocuous while being suppressed by a dominant allele. However, when selfed (crossed with itself,

e.g., *Aa* × *Aa*), the lethal allele can become homozygous (*aa*) and be expressed! The consequence of selfing cross-pollinated species is a reduction in vigor because of the expression of deleterious gene. This event is called *inbreeding depression*. There is no depression of vigor when self-pollinated species are selfed, because that is their natural way of life, and further, selfing over the years has eliminated the deleterious genes from the population.

There are several mechanisms in nature that promote one mode of reproduction over the other. *Monoecy* is the anatomical condition in which the male and female parts of a flower are located on the same plant but on different parts. For example, in corn, the male flower occurs on top of the plant, while the female flower occurs on the side of the stalk, producing the cob. Such a condition promotes cross-pollination. Some species have separate male and female plants (dioecy; dioecious plants). Similarly, some species have a condition called self-incompatibility, whereby a plant is unable to fertilize itself with its own pollen. Self-incompatibility occurs in some nut trees and other species like broccoli. Yet, some species have a genetic problem, male sterility that renders pollen grains infertile. All these natural conditions are exploited by plant breeders in their breeding work, as will be discussed shortly. Further, it is always advisable in fruit and nut production to be certain you have provided adequate pollen sources for optimal yield.

6.8 Role of Variation in Plant Breeding

As previously indicated, there can be no plant breeding without variability! Genetic recombination produces variability in sexually reproducing species. But, the ultimate source of biological variation is mutation. Plant breeders start by assembling variability for their breeding program according to their state objectives. The recommendation is to always start looking for suitable parents from commercial cultivars, having gone through the rigorous testing and adaptation processes that are part of plant breeding. Germplasm banks are repositories of variability in plant species. Some banks hold thousands of different genotypes of a particular species. A breeder, if need be, can consult these centers for materials to use in a project.

6.9 The Plant Breeder as a Decision Maker

Modern plant breeding is a carefully planned and executed activity. Breeding is tedious and time consuming, often taking over ten years to produce a variety for use by producers. It is imperative that sound decisions be made to avoid waste and prolonging the process. Some of the specific decisions that are made are in the following areas:

1. *Organization design*. The breeder plans the entire operation, regarding personnel, equipment, field and nurseries needed, and so on.
2. *Planning and control.* The breeder must set clear objectives and define the strategies for achieving them.
3. *Behavioral process*. The team engaged in the breeding program should work and relate well to each other.

Breeding objectives drive the project. Not all plant problems are amenable to plant breeding. Some are better resolved by changing the plant cultural environment. Objectives may be decided upon following consultation with consumers and producers. Do not spend ten years to develop a new variety and then look for customers. Specific objectives could be higher yield, disease resistance, augmenting nutritional quality, early maturity, and adaptation to abiotic stress (cold tolerance, drought resistance, etc.).

6.10 General Steps in a Breeding Program

Regardless of the breeding approach, plant breeders follow certain general steps.

1. *Objectives.* Set clearly defined and attainable objectives.
2. *Germplasm.* Assemble appropriate germplasm to use to initiate the project. That is, if you desire to breed for resistance to a disease, you must have a source of resistance to this disease.
3. *Selection.* When you assemble variability and you make crosses to create more of it, you have to discriminate among the variability to find which individual plant has accumulated all the desirable gene combinations, according to the breeding objectives.
4. *Evaluation.* Breeders evaluate germplasm in the field to determine which ones to release for use by producers.
5. *Certification and cultivar release.* Once satisfied with the "winner," the next step is to pursue certification to demonstrate compliance with existing **seed laws,** as well as to obtain some protection from unauthorized use of your invention, if so desired.

Seed law

Seed law is a legislation that regulates the labeling, possessing for sale, sale and offering or exposing for sale or otherwise providing for planting purposes of agricultural seeds, vegetable seeds and screenings; to prevent misrepresentation thereof; and for other purposes

6.11 Selection in Breeding

As previously indicated, plant breeding is like evolution. In evolution, the arbiter of what survives (makes the cut, so to speak) is natural selection. In plant breeding, plant breeders are the ultimate decision makers, deciding which genotype becomes a commercial variety of cultivar. A *cultivar* is the term for the product of plant breeding. Variety (botanical variety) occurs in nature. However, the term *variety* is used in society to refer to the products of plant breeding.

Selection is a discriminating force. In plant breeding, breeders have a state objective and hence seek to bias the choices they make at every stage of the process toward achieving their objective. This focused approach to discriminating among variation is called *directional selection*. When two plants are crossed, their genes are mixed up and reorganized into new genetic matrices. As previously stated, some traits are controlled by one or a few genes, while others are controlled by numerous genes. The chance of finding the individual that combines all the desirable genes increases as the number of genes that control the traits increases. For example, if the trait is controlled by one gene, only four combinations are possible in the F_2. To find that ideal plant, the breeder needs to plant only four plants. If three genes are involved, sixty-four plants are needed. If n genes are involved, the formula for calculating the F_2 population size is $(1/4)^n$. For quantitative traits, breeders routinely produce, grow, and evaluate hundreds of thousands and even millions of plants! To find the proverbial needle in a haystack requires an excellent breeder's eye. Modern breeders use various scientific techniques to reduce the guesswork to make the selection process more efficient (e.g., genetic markers for selection).

From the thousands of plants generated in the F_2, only a fraction is selected for more careful evaluation in the F_3. The numbers decrease progressively with each generation. The goal is that progress (genetic gain) is made with every cycle of selection; that is, the breeding objective is continuously advanced, each step moving the breeder closer to attaining it. The progress from one generation to the next is called the *response to selection (R)* or the *genetic gain*. A mathematical relationship will make this clearer:

$$R = ih^2\sigma$$

where R = the advance in one generation of selection, h^2 = heritability, i = intensity of selection, and σ = phenotypic standard deviation. Heritability measures the proportion of the observed variation in a progeny that is inherited. This is sometimes called the

breeder's equation. Simply stated, the higher the heritability estimate, the higher the success one can have by using plant breeding approaches to solving the breeding problem. If heritability is low, the impact of the environmental effect would be so strong that what you observe would not always be an accurate reflection of the genes the plant has. Selection intensity reflects the proportion of the plants that you select to advance to the next generation. Obviously, the smaller the better because of cost and the labor involved. But, ultimately, you want to be sure the plant with all the desired combinations is always included in the sample. If you advance only a few plants, you have a higher chance of leaving it behind. Plant breeders are always looking for ways of enhancing the selection process to make it more efficient and effective.

6.12 Hybridization in Plant Breeding

Hybridization (crossing) is the principal tool or technique used to achieve gene transfer in conventional breeding; it can occur naturally. Artificial crossing entails exercising control over the parents used and how they are crossed (controlled pollination). Parents to be crossed should be reproductively compatible. Once the parents are chosen, the breeder decides how to cross them. A critical decision is what parent to use as male or female. As previously pointed out, DNA (genes) occurs in both the nucleus and the cytoplasm (in mitochondria and chloroplasts). During gamete formation, the male gametes (pollen) are produced from only the nucleus without the cytoplasm. The egg or ovule has both parts. If there is a gene of interest on the mitochondrion, pollen grains will not have it. Using a parent with such a gene means you cannot pass the gene on to the offspring. In this instance, the parent should be the female. Some male sterility genes reside on the mitochondria.

Pollen transfer is often by physical or mechanical means (hand pollination). However, in a large-scale program, hand pollination may not always be practical. Once a female parent has been identified, the next thing is to prevent self-pollination. This is achieved in species whose flowers are hermaphrodites (have both sexes) by the tedious technique called *emasculation*. This entails removing the anthers from the flower to leave only the exposed pistil. Emasculating a large field of plants is feasible in places like India where labor is cheap. Alternatively, breeders resort to techniques like male sterility and self-incompatibility to render one sex infertile. If a parent is made sterile by incorporating a male sterility gene, this effectively genetically emasculates the plant, leaving no need for the tedious mechanical emasculation that usually has a low success rate.

6.13 Methods of Breeding

The methods of breeding differ according to the reproductive biology of the species. Breeding methods are essentially methods of selection after creating an initial population. The initial population sets the tone for the selection program. It can be as simple as a landrace (farmer-developed variety). Often, it is generated by planned crosses following the judicious selection of parents.

Plant breeders develop six basic types of cultivars: pure line, open pollinated, hybrid, clonal, apomictic, and multilines.

1. *Pure-line cultivars.* These are developed for self-pollinated species. They are homogeneous and homozygous in genetic structure and have a narrow genetic base. They are preferred for markets and products where uniformity has a premium.
2. *Open-pollinated cultivars.* As the name implies, these cultivars are developed for cross-pollinated species. They are heterogeneous and heterozygous and have a broad genetic base.

3. *Hybrid cultivars.* Hybrids are known for their high yield. Most of the highly successful ones are developed for cross-pollinated species like corn that are easy to pollinate.
4. *Clonal cultivars.* These are cultivars that are propagated by asexual (vegetative) methods. Some species have the capacity to bear viable seed, so they are improved through crossing. However, they are propagated asexually so that the vigor created through hybridization will be kept intact. Remember, genes are reshuffled anytime we cross parents.
5. *Apomictic cultivars.* Seed is normally produced after pollination and fertilization. However, the phenomenon of apomixis is the production of seed without fertilization. This behavior is common in perennial forage grasses. In effect, apomictic seeds are clones of the parent plant, and are equivalent to vegetative propagation through seed.
6. *Multilines.* This cultivar is developed for self-pollinated species. A multiline cultivar consists of a mixture of specially developed genotypes called *isolines* (or *near isogenic lines*) because they differ only in a single gene or a defined set of genes. They are developed primarily for disease control. Instead of incorporating one gene for protection, breeders insert multiple genes for protection against the same disease. Should one succumb while in use in production, there are several "backup" genes that can provide some protection.

6.14 Breeding Hybrids

Heterosis (hybrid vigor)
The increase in size, vigor, fertility, and overall productivity of a hybrid plant over the average performance of the two parents.

Inbreeding depression
Opposite and complementary to heterosis, this is the reduction in fitness of an individual as a result of inbreeding.

Hybrids are very intriguing cultivars. They are developed based on the phenomenon called **heterosis** or **hybrid vigor.** This is the phenomenon whereby the hybrid (the F_1; product of the cross of two parents) exceeds both parents in the expression of the trait of interest. Commercially, the parents for a hybrid in a cross-pollinated species are developed by first selfing them repeatedly to make them pure or homozygous (called *inbred lines*). Such repeated selfing exposes the deleterious alleles by making them homozygous. These parents experience **inbreeding depression** and look very weak and unproductive. However, when these inbred lines are crossed, the hybrid becomes invigorated (hybrid vigor) and excels in productivity. A suggested scientific explanation is that crossing the two inbred lines results in a highly heterozygous hybrid. Heterozygosity per se, is thought to cause this dramatic vigor.

Hybrid vigor is most pronounced in the F_1 generation. Seed companies sell the F_1 seed to farmers to grow to experience the benefit of heterosis. However, if seed is saved from the F_1 or hybrid for planting the next season, this beneficial heterotic effect is reduced by 50 percent! This compels farmers to go back and purchase fresh hybrid seed each season. That is why companies make money, to recoup their investment in developing inbred lines and other activities associated with hybrid development.

6.15 Cultivar Release and Certification

Once genotypes with potential have been identified, the breeder proceeds to conduct performance evaluation trials, often at various locations including where the new cultivar will be released for use by farmers. This evaluation is conducted over seasons and years. After this activity, one of the potential genotypes is released as a cultivar. It is often given a name that can be readily identified by customers. Sometimes the name reflects the breeding process and includes some codes for tracking by the breeder or company.

Before the seed or product becomes available for sale, it goes through a certification process. Certification is conducted by certifying agencies according to prescribed guidelines for the crop or species. For seed products, the breeder retains the most authentic version of the cultivar called the *breeder seed*. Some of the seed is increased by a contracted producer to obtain the *foundation seed*. This seed is further increased by farmers under contract to yield the *registered seed*. Registered is further increased to obtain *certified seed* for sale to farmers. These are called *seed classes*.

For certification, the agency inspects the variety in the field according to the guidelines for the crop. The breeder is also required to furnish specific product information including the history and origin of the cultivar and documentation of the processes of evaluation and plans for maintenance of the various seed classes.

The seed has to be submitted for testing (seed testing) to determine viability or germination percentage, purity, vigor, seed health, and noxious weed seed. Before being offered for sale, the bags are tagged, as described in Chapter 9.

6.16 Breeding Asexually Propagated Species

As previously indicated, variation is the lifeblood of plant breeding. Recombination is the primary source of variation for species that reproduce sexually. For species that do not reproduce sexually, variation can be generated through other means such as inducing artificial mutations. Mutations have been used, not only in asexually reproducing species but also in sexually producing species, to develop numerous commercial species of fruit trees, ornamentals, and other species. Tissue culture and other biotechnological techniques that can be used are discussed next.

6.17 Breeding Seedless Fruits

Seedlessness in fruits is a desirable trait in horticulture breeding. Seedless fruits are more convenient to eat because there are not seeds to spit out. Seedless cultivars are commercially available for fruits such as watermelon, grape, orange, and strawberry. The conventional method of breeding seedless fruits is the use of triploid hybrids. To obtain a triploid, a tetraploid ($4x$) parent is crossed with a diploid ($2x$) line. In watermelon, for example, the tetraploid is always the female parent ($4x = 44$; $2x = 22$). The reciprocal cross, with the female as male parent, does not produce seed (Figure 6–3). The triploid resulting from this cross ($3x = 33$) is female-sterile and hence the fruit is seedless.

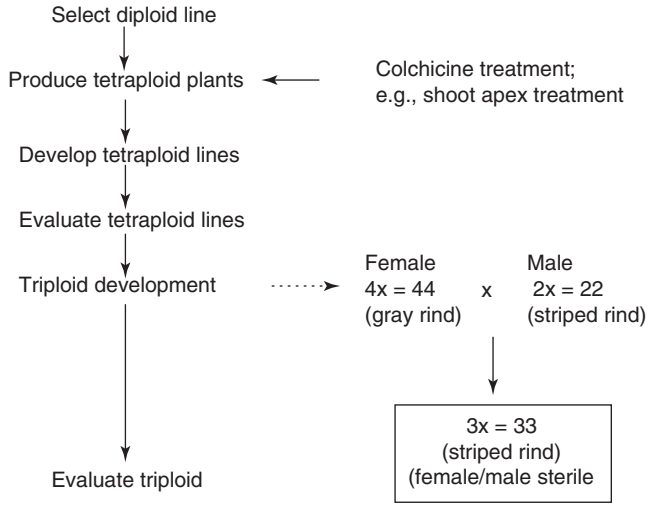

FIGURE 6–3 Breeding seedless watermelon.

Because the triploid is also male-sterile, growers of seedless watermelon must plant rows of diploid lines as pollinators for stimulation of fruit formation.

6.18 Biotechnology in Plant Breeding

So far, we have discussed the concepts and methods of conventional plant breeding. This section is devoted to discussing the principles and concepts of nonconventional breeding at an introductory level.

6.18.1 What is Biotechnology?

Biotechnology may be broadly defined as the use of techniques based on living systems to make products or improve other species. This definition would include the use of microbes to make products by the age-old process of fermentation. However, a narrower definition would restrict the term to the genetic manipulation of organisms for specific purposes. Again, this definition would include classic plant breeding by crossing. Another term is used to distinguish between the levels of genetic manipulation—indirect manipulation of plant genetics by conventional methods at the whole organism level using methods like crossing, and direct genetic manipulation at the molecular level (Figure 6–4). The term *genetic engineering* is used to describe the genetic manipulation of organisms at the molecular level, directly involving the DNA. Scientists, using the revolutionary technology of **recombinant DNA** (rDNA), are able to transfer genes from any organism to another.

Recombinant DNA
A hybrid DNA molecule produced in the laboratory by joining pieces of DNA from different sources.

What makes this radical gene transfer possible is the fact that DNA is "universal." Regardless of source, all DNAs obey the same rules and are chemically identical. So, it is possible to transfer DNA from an animal and insert it into a plant! This, theoretically, removes all biological barriers, reducing all life into just one humongous gene pool. The controversy with genetic engineering primarily stems from this radical approach to genetic manipulation.

The term *molecular breeding* is used to describe the application of a variety of tools for manipulating the DNA of plants, which may or may not involve rDNA, to improve them for specific purposes. Even though crossing two different parents produces new recombinants in the segregating population, the term *recombinant DNA* is reserved for the union of DNA segments of different biological origins.

A cultivar developed by genetic engineering protocols is called a *transgenic cultivar* or a *genetically modified (GM) cultivar*. Generally, an organism developed by rDNA procedures is called a *genetically modified organism (GMO)*.

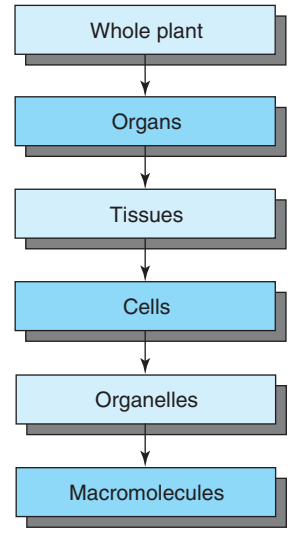

FIGURE 6–4 Levels of eukaryotic organization.

6.18.2 THE GENERAL STEPS OF RDNA TECHNOLOGY

General steps in rDNA manipulations are as follows:

1. The DNA segment or gene of interest is identified. This is called the *transgene*. It is extracted and snipped out of the source using restriction enzymes.
2. The transgene is inserted into a special DNA molecule called a *cloning vector* and joined to create a new rDNA molecule.
3. The rDNA molecule is transferred into and maintained in a host cell (bacterium) by the process of genetic transformation. The vector replicates to produce identical copies (clones) of the insert DNA.
4. The host cells with the cloned transgene are identified and isolated from untransformed cells.
5. The cloned transgene can be manipulated such that the protein product it encodes is expressed by a host cell.

There is modification to these general steps, according to the purpose of the research.

6.19 Breeding Genetically Modified Cultivars

Breeding genetically modified (GM) cultivars is a very lengthy process and involved process, compared to conventional breeding. Researchers need to establish the appropriate facilities and receive clearance from a local biosafety committee in order to conduct rDNA research. One may discover and isolate the gene of interest or procure it from another source. Either way, it can be very expensive. Recombinant DNA technology may sound like a simple event, but it involves numerous patented subprotocols. To be able to produce and market new products, one must be untangled from the web of patents, something that adds to the cost of product development.

Once an appropriate transgene has been obtained and incorporated into a plant, the breeder conducts a controlled environment (greenhouse) evaluation. After that, an application must be made to the appropriate federal agency (APHIS—Animal and Plant Health Inspection Service) to conduct a field test. After the agency is satisfied that the product is stable, nonpathogenic to animals or humans, and meets safety stipulations according to the nature of the GM product, approval is given for commercialization.

The convergence of conventional and molecular approaches to plant breeding should be pointed out. The critical difference between the two approaches is the method of gene transfer, one method within normal biological reproductive modes, and the other by outside biological boundaries. However, before the GM cultivar is released for use by producers, it undergoes the same field evaluation process as a conventional cultivar.

6.20 Use of Markers in Plant Breeding

Whereas rDNA research incorporates foreign DNA into plants, there are other applications of molecular genetics that do not incorporate transgenes. Molecular techniques are used to identify parents for hybridization. This method in plant breeding is called *marker assisted selection (MAS)*. As previously pointed out, plant breeders face the daunting task of finding that ideal plant with all the gene combinations from a sea of segregating plants. They employ various techniques to help them effectively and efficiently do their work. One such strategy is to use genetic markers.

Genetic markers are simply landmarks on chromosomes that serve as reference points to the location of other genes of interest. The rationale of markers is that an easy-to-observe trait (marker) is tightly linked or associated with a more difficult-to-observe trait

of interest to the breeder. By knowing the presence of such an association, breeders can select plants directly on the basis of the marker, and indirectly select them for the traits of interest. This greatly facilitates the selection process.

There are morphological markers that manifest outside the organism as adult phenotypes, and molecular markers that are identified at the subcellular level. Molecular markers can be assayed before the adult stage in the life cycle of the organism and hence are more advantageous, allowing breeders to make early decisions, saving time and money. Molecular makers may be proteins (isozymes) or DNA. DNA markers are more versatile, including RFLPs (restriction fragment length polymorphisms), AFLPs (amplified fragment length polymorphisms), SSRs (single sequence repeats), SCARs (sequence characterized amplified regions), STSs (sequence tagged sites), and SNPs (single nucleotide polymorphisms).

One application of markers is the authentication of hybridity. When a cross is made, breeders may run simple tests to ensure that the F_1 is a true hybrid, not a self. It is a tragedy and a waste of resources to advance a self-pollinated product as if it were a cross. When morphological markers are available, these are easiest to use. For example, it is known that a purple flower is dominant to a white flower color. If one is crossing two parents that happen to differ in flower color, the dominance test could be used. By using the white parent as female, you would expect the F_1 seed to be purple-flowered if it is truly a hybrid.

6.21 Success of Genetic Engineering of Plants

The first bioengineered food crop, *FlavrSavr* tomato, was introduced in 1985. It was designed to have reduced levels of the enzyme polygalacturonase that is associated with fruit ripening. By so doing, tomato could be harvested as vine-ripened and hence tastier than the green-harvested and forced-ripened tomato. The GM tomato also had an extended shelf life. This product literally ignited the GM food wars that continue today. Another feat that has been attempted is the development of the Golden Rice, a GM product that represents the first rice known to man to have the capacity to produce pro-vitamin A. No natural rice has this capacity, making blindness very common in societies that depend on rice as a staple food.

Pest resistance is an area where GM cultivars abound. Most notable are the Bt products, short for *Bacillus thuringiensis*, the bacterium from which the gene for resistance to the lepidopteran pest was derived. Bt products are resistant to the devastating attacks of the European corn borer. Other common products include the herbicide-tolerant transgenic such as Roundup Ready® products. These plants allow producers the additional flexibility in weed control by being able to apply the herbicides while the crop of interest is still growing in the field. There are several other achievements that have been commercialized, with many in the pipeline.

6.22 The Biotechnology Debate

There are many issues associated with the development and application of biotechnology in plant breeding. The issues of patents are more technical and can often be resolved. However, the issue of ethics continues to be debated on three main fronts:

1. *Scientific disagreements.* Society is concerned about the potential risk that the development and application of biotechnology poses to humans, animals, and the environment health. Even though resolvable empirically by scientific methods, some value judgment is in play, for example, regarding the level of risk deemed acceptable.

2. *Political disagreement.* There are political positions on the social and economic impacts of biotechnology on society, the pendulum swinging in favor of the dominant political ideology of the day.
3. *Religious, ethical, and philosophical disagreements.* These are faith-based issues about morality and whether scientists are playing God, or whether biotechnology is natural. In a pluralistic society, consensus is often difficult to achieve, leading some to pursue extremist acts of vigilantism (e.g., destroying biotech research labs). There are perceptions and fears about biotechnology in society. Some think the technology is alien, unnatural, and too radical. The normal direction of genetic information transfer (central dogma) is from DNA to RNA to proteins; genetic engineering can reverse this process, synthesizing DNA from protein. The transfer of DNA across natural biological boundaries is deemed playing God, as well. It should be pointed out that such a transfer occurs in nature whereby the bacterium *Agrobacterium tumifaciens* causes tumors in plants by transferring bacterial DNA into cells upon infection. In fact, this bacterium is widely used in genetic engineering in this natural role. There is the concern of the unknown, the possibility of creating monsters and superweeds. These and many other concerns prevail, keeping the debate alive.

Biotechnology is a very highly regulated industry. In the United States, the USDA (United States Department of Agriculture), EPA (Environmental Protection Agency), and the FDA (Food and Drug Administration) are all engaged. The USDA focuses on plant pests, plants, and veterinary biologics; the EPA on microbial/plant pesticides, novel microorganisms, and existing pesticide; and the FDA on food, feed, food additives, veterinary drugs, human drugs, and medical devices. GM products are evaluated and labeled as such before commercialization.

6.23 Tissue Culture in Plant Breeding

Tissue culture or micropropagation, as it is sometimes called, is a technology that is useful to plant breeders. The premise of this technology is that the cell is the fundamental unit of organization of the organism, and contains all the requisite genetic information to make the entire organism (totipotent). It is possible, and routinely done, to raise a whole plant from a single cell! This is critical to genetic engineering, for the transgene is inserted into a single cell that is nurtured and regenerated into a full blown plant in vitro (in the test tube).

Micropropagation is useful for multiplying a limited amount of plant material very rapidly. Pieces of the plant part (leaf, stem, roots, flower, etc.) can be obtained and nurtured into full plants. Plants so produced are clones (genetically identical). The plant part used to start tissue culture is called the *explant*. By manipulating the cultural environment using growth regulators, the researcher can induce a variety of morphological structures, including roots and shoots. The induction of organs from explants is called *organogenesis*. In genetic engineering applications, often the explant used is the callus, an amorphous mass of meristematic cells that are equivalent to human stem cells in that they can be manipulated to produce various organs. Pieces of DNA or genes can be inserted into single cells in the callus by direct bombardment in a special device called a *gene gun* (Figure 6–5). Another common approach is to use specially modified bacteria (*Agrobacterium*) to transfer the gene of interest into the cell.

Another application of tissue culture in breeding is embryo rescue. When plants that are genetically distant are crossed, there is often a number of problems, including lethality, failure of fertilization, or failure of the embryo to develop. Just like premature birth in humans, plant breeders may extract the immature embryo from the flower and culture on appropriate media to produce a full plant.

FIGURE 6–5 A gene gun. This tabletop model uses compressed helium gas to propel the DNA. *(Source: George Acquaah)*

SUMMARY

Plant improvement involves the manipulation of the genome of the plant with a specific goal in mind. A good understanding of genetics helps breeders to be effective and efficient in their endeavors. DNA, hereditary material, is located mostly in the nuclei of plants in linear structures called chromosomes. Genes, the factors that condition traits, are pieces of DNA arranged in a linear order in chromosomes. Gene—not traits—are inherited by the offspring from their parents. The transmission of genes in this fashion is governed by certain laws that were discovered by Mendel: gene pairs segregate during cell division so that only one from each pair ends up in each gamete, and genes assort independently during meiosis. Some genes (dominant) mask the expression of others (recessive) when they occur at the same locus.

Meiosis is the major source of biological variation. However, the ultimate source of variation is mutation. The expression of genes is subject to the environment in which they occur. Certain traits are governed by one or a few genes (simply inherited), whereas others are governed by many genes with small effects (quantitative traits).

The underlying principle in plant breeding is that the phenotype (what is observed) is a product of the interaction between the genotype (genetic makeup) of the individual and its environment. Phenotype can therefore be changed by manipulating either the genotype (plant breeding) or its environment (agronomy). Before starting a breeding program, breeders should first ascertain whether adequate heritable variation is available. Then they should have clear goals or objectives. The specific methods used depend on the mating system of the plant. Genetic changes may be made in flowering plants through crossing (hybridization).

Molecular biotechnological procedures are being utilized by plant breeders to accelerate their breeding programs through the use of molecular markers for effective selection. Further, breeders are able to import genes from outside of the genus with which they are working and thereby develop transgenic plants. Problems that once were impossible to solve with conventional breeding are being tackled with amazing success using molecular biotechnological tools.

It should be emphasized that conventional and molecular biotechnological methods of breeding are complementary tools. Geneticists and breeders work together to improve horticultural plants.

References and Suggested Reading

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Breeding watermelon
http://cuke.hort.ncsu.edu/cucurbit/wmelon/wmelonmain.html

Mendelian genetics
http://www.cliffsnotes.com/WileyCDA/CliffsReviewTopic/Mendelian-Genetics.topicArticleId-23791,articleId-23725.html

Rose breeding
http://aggie-horticulture.tamu.edu/rose/rosebreeding/index.html

Practical Experience

Laboratory

1. Select one (or more) vegetable and one ornamental flowering crop and assemble seeds of as many variant types as possible. For this activity, sources of seed include the market, farms, research stations, other universities, and germplasm banks. Place the collection in test tubes in a rack and label clearly.
2. Plant samples of selected specimens from activity 1 and describe the differences in morphology.

Field Work

Attempt to cross two different cultivars of tomato, pepper, or an ornamental flowering plant.

Outcomes Assessment

1. Why is plant breeding important to society?
2. In what way is plant breeding a science and an art?
3. Compare and contrast conventional and nonconventional approaches to plant breeding.
4. Discuss the importance of plant genetics in plant breeding.
5. Why is it more challenging to improve traits that are controlled by many genes?
6. Discuss the plant breeder as a decision maker.
7. Discuss the underlying genetic principles of hybrid breeding.
8. Why do growers have to purchase fresh hybrid seed each growing season, rather than save seed from the previous season for planting?
9. What is biotechnology?
10. Discuss the underlying genetic principle in genetic engineering.
11. Discuss specific achievements of genetic engineering of plants.
12. Discuss, giving specific examples, the biotechnology debate in society.