Chapter 2

Breeding and Crop Improvement

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Abstract

Plant breeding has played a pivotal role in horticultural crop improvement by introduction, selection, domestication of better phenotypes and creation of novel genetic diversity. Enhanced understanding in genetics, advances in molecular biology and development of molecular breeding tools, have accelerated the breeding process in economically important fruit and vegetable crops. However, comprehensive crop improvement programs in the indigenous material, native as well as major crops are still lacking. This chapter elaborates the basic concepts in breeding including, diversity in reproductive systems, incompatibility, male sterility, breeding methods, and significance of plant genetic resources in breeding programs. Web links of important global genetic resource centers having collections for specific crops as well as databases have been provided. The applications of modern breeding tools like mutation breeding, polyploidization and molecular breeding, for horticultural crop improvement, have been discussed. Lists of recent reports of some mutants and polyploids have also been provided. An integrated approach using conventional and modern breeding tools and utilizing indigenous wild genetic resources could help in crop improvement and development of biotic and abiotic stress resistance in elite cultivars.

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2.1. Introduction

Before Mendelian laws, breeding was merely involving phenotypic selection and domestication processes. The milestone fundamental discoveries of Darwin and Mendel provided genetic basis for better prediction in hybridization and efficient utilization of breeding tools for crop improvement. Methods in plant breeding involve creation of heterozygosity, phenotypic selection, genetic characterization and fixation of elite genotypes for the development of better producing cultivars for the growers and other stakeholders. Primary goals of breeding in horticultural crops involve development of germplasm resources, short duration/early maturing varieties, dwarf varieties for adaptation to mechanical harvesting, abiotic and biotic stress resistant varieties, fertilizer responsive varieties for higher yields and better produce quality. Breeder's role in crop improvement has been outstanding over time. This chapter highlights the basic concepts in hybridization and different conventional and modern breeding tools involved in horticultural crop improvement.

2.2. Reproductive Systems in Horticultural Plants

Plant breeders require a complete understanding of the floral biology and reproductive systems of plants because genetic architecture of a plant (open pollinated variety or a hybrid) depends on its mode of reproduction. The mode of reproduction also has impact on methods of maintenance and multiplication of cultivars. Basically there are two modes of reproduction in plants *viz.,* asexual and sexual.

2.2.1. Asexual Reproduction

Plants develop asexually from different vegetative parts or from unfertilized gametes, ovular tissues or modified cells (as in Apomixis). Varieties of asexually propagated species are highly heterozygous and show high segregation when reproduce sexually (Allard 1960).

2.2.1.1 Reproduction through vegetative organs

Some horticultural crops are propagated and regenerated from different vegetative parts such as underground tuberous roots (sweet potatoes, dahlia), tubers (potato), bulbs (onion, garlic, narcissus, lilium, amarayllus, tuberose), corm (yams, arum, crocus, gladiolus), rhizomes (ginger, turmeric, canna), fleshy roots (ranunculus), stem cuttings (tapioca, watercress, tapioca, rose, *Ficus* spp.), runners (strawberry, spider plant), and root suckers (globe artichoke).

2.2.1.2 Apomixis

The formation of embryo and finally a seed, from maternal tissue of the ovule, without entering the processes of meiosis and fertilization is termed as apomixis. It increases the proportion of maternal tissues through inhibition or modification of

meiosis and the fusion of gametes. This phenomenon has been reported in more than 400 flowering plant taxa, including representatives of more than 40 families (Carman 1997) of both monocotyledonous and eudicotyledonous plants. But, it seems to be absent among the gymnosperms (Bicknell and Koltunow 2004). Among the gametophytic plants that exhibit apomixis, 75% of the plants belong to three families *viz*., the Asteraceae, Rosaceae, and Poaceae (Czapik 1994), which constitute only 10% of the flowering plant species (Bicknell and Koltunow 2004). Apomixis is frequently observed in species which possess certain mechanisms that limit self-fertilization (autogamy), *i.e.,* in the form of self-incompatibility, dioecy, or heterostyly (Asker and Jerling 1992). Arctic environment is suited to apomixis as it helps to generate a few but very fit genotypes. Apomictic species are mostly perennials, and reproduce asexually through vegetative organs such as stolon or rhizome. There are two basic types of apomixis *viz.,* sporophytic and gametophytic (Fig. 2.1).

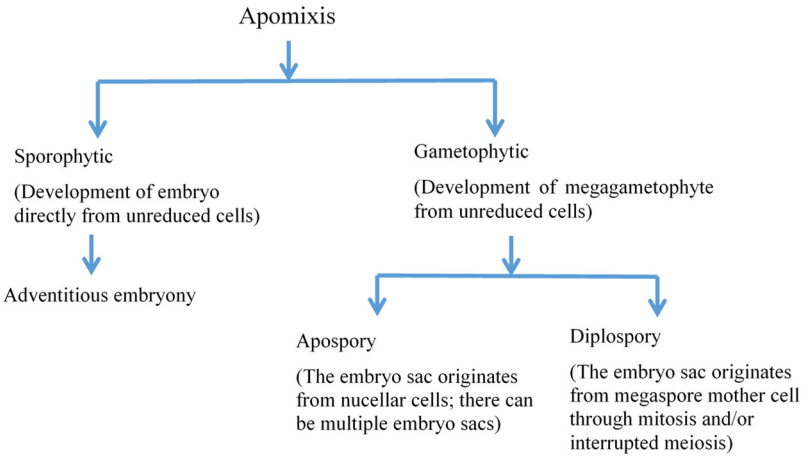

Fig. 2.1 Apomixis and its types.

In sporophytic apomixis, the asexual embryo is formed directly from unreduced (diploid) cells other than the cells of megagametophyte. The megaspore mother cell undergoes normal meiosis and forms a normal embryo sac. After fertilization, when first division starts in the embryo, cells in the nucellus or integument start abnormal divisions, which lead to formation of multiple embryos in the micropylar region of the ovule. Therefore, this type of apomixis is also termed as adventitious embryony (Kandemir and Saygili 2015). It has been reported in about 57 families (Carman 1997). Examples of adventitious embryony are citrus, *Commiphora wightii* ('Guggul'), mango, cacti and orchids.

In gametophytic apomixis, the nuclei in the embryo sac do not undergo meiosis but still occupy their position in the embryo sac. The unreduced (diploid) nuclei corresponding to the egg cell divides spontaneously or parthenogenically to form an embryo without fertilization with sperm nuclei. In this type of apomixis, endosperm

develops either through fertilization of unreduced polar nuclei with sperm (pseudogamy) or only forms polar nuclei without fertilization. Gametophytic apomixis is of two types namely, apospory and diplospory, distinguished on the basis of origin of the cells that give rise to apomictic embryo. In apospory, normal meiosis occurs in the megaspore mother cell but the resulting cells usually abort before fertilization. Besides the development of megaspore mother cell, one or several somatic cells of the ovule and their nuclei start to develop, which resemble megaspore mother cell, and are termed as aposporous initials. These cells enter mitosis to produce embryo sac. The aposporous embryo sac can initiate together with a sexual embryo sac or it can inhibit formation of sexual embryo sac. Apospory is the most common mechanism in higher plants (Kandemir and Saygili 2015) and has been reported in Rosaceae, Asteraceae, and Poaceae (Barcaccia and Albertini 2013).

In diplospory, embryo sac is unreduced (diploid) because megaspore mother cell divides without underging meiotic division or meiosis remains incomplete. Richards (2003) reported that meiosis proceeds to the dyads formation stage followed by semi-heterotypic division. Diplospory is common in the Asteraceae and in some grass species. Three major types of diplospory are *Taraxacum*, *Ixeris* and *Antennaria*, named after the plants in which they have been observed. Sometimes, diplosporous and aposporous embryo sacs coexist even in the same ovule, as in the grass *Paspalum minus* (Bonilla and Quarin 1997).

Gametophyte with diploid number of chromosomes in apospory or diplospory may give rise to parthenogenetic (do not need pollination) or pesudogamous (pollination is necessary but male nuclei does not take part) embryos. Parthenogenesis is the term used for development of embryo from an unfertilized egg. Parthenogenesis can also be haploid or diploid. Haploid parthenogenesis is due to high or low temperature, usage of foreign or irradiated pollen, and application of chemicals, which cause development of embryo from unfertilized egg. Diploid parthenogenesis, also known as matromorphy, is due to the formation of unreduced embryo sac and parthenogenetic development of haploid egg cells followed by their re-duplication. It is common in cole crops. It can be due to intergeneric or interspecific crosses, application of foreign pollen or growth regulators (especially GA_3) and temperature (Eenink 1974). Diploid androgenesis, also termed as patromorphy, refers to the situation in which male nucleus does not fertilize the egg cell but itself divides and form a haploid embryo. This phenomenon has been recorded in *Solanum*.

Apomixis can be facultative or obligate. Facultative apomicts can develop normal embryo after fertilization and thus can also set seed, *e.g., Paspalum notatum* (Bahiagrass), *Mentha spp.*, *Mimulus spp.* and *Potentilla anserine*. Obligate apomixis is associated either with organs which displace the inflorescence or with seed production (agamospermy).

Sometimes, vegetative parts such as bulbils are produced in the inflorescence instead of flowers, and the condition is termed as vegetative apomixis. It also covers reproduction by vegetative organs such as, stolons, runners and rhizomes, in plants where sexual reproduction is markedly reduced or absent. Different types of apomixis, which are useful for plant breeders, include diplospory, apospory, haploid fusion or second division restitution (SDR), and semigamy. Second division

restitution may also lead to the development of aneuploids (organisms having other than exact multiple of the haploid number of chromosomes) discussed in section 2.9.2.

There are several merits of apomixis such as the rapid generation and multiplication of seed; the reduced cost and time of breeding; restricting the transfer of viruses in vegetatively propagated plants, *e.g.,* potatoes. It also avoids the complications associated with sexual reproduction including those related to pollinators and cross-compatibility. Moreover, apomixis mediates the formation of large genetically uniform populations and perpetuates hybrid vigour through successive seed generations. This can help the farmers to save their own seeds without losing hybrid vigour. Homozygous autodiploid (2n = 2x = 24) and tetrahaploid (2n = 4x = 48) have been produced from haploids through apomixis (Kalloo and Bergh 1993).

Following are the conditions that indicate the occurrence of apomixis in any crop plant:

1) When the progeny of a plant is more uniform than expectation, as can be facultative or obligate apomicts. If all off-springs are uniform, then it would be obligate apomixis. In facultative apomixis, off springs comprise of apomictic, zygotic and those developed from aberrant types originating from either fertilization or cytological variations in male and/or female gametes.
2) Apomixis is likely to occur if pollen parents have no influence on the progeny.
3) Apomictic plants, although highly heterozygous, yet do not show inbreeding depression upon self-pollination.
4) Multiple seedlings from a single seed also indicate apomictic behaviour.
5) Irregular number of chromosomes also indicates apomixis. In many species, a wide range of chromosome number can be found. For example, chromosome number (2n) varies from 28 to 154 in *Poa pratensis*. This uneven number of chromosome is also sometimes combined with undistributed seed setting in apomictic seedlings.

2.2.2. Sexual Reproduction

The life cycle of flowering plant consists of two basic growth phases *i.e.* vegetative and reproductive. The vegetative phase is the first one in which plant produces only vegetative organs such as stem, branches, and leaves, etc. While in reproductive phase, plants produce flowers. In almost all flowering plants, transition from vegetative to reproductive phase is gradual. This transition is triggered in some species by certain environmental factor(s), such as temperature and/or photoperiod, and can be manipulated by modification of the environment where plant is growing. Different species vary for the duration between phases. Sexual reproduction comprises of two processes; first one involves halving of the chromosome number (from 2*n* to 1*n*) through meiosis resulting in gametophytic generation. The second

process involves union of two gametes *i.e.* male and female, the fertilization, which restores the diploid chromosome number and thus starts the sporophytic generation.

Gametophytes: In higher plants (ferns, gymnosperms, angiosperms), the male gametophyte generation is comprised of three haploid nuclei (the microgametophyte) in a tiny pollen tube. While, the female gametophyte (the megagametophyte) is a single multinucleated cell, the embryo sac. The genotype of the gametophyte or sporophyte has significant impact on sexual reproduction of species which have self-incompatibility problems and has implications in breeding of several plant species. **Gametogenesis:** The process of gamete formation takes place in micro- or mega-sporangium and is termed as gametogenesis. The microspore mother cells in anthers and megaspore mother cells in the ovary are diploid ($2n$). The microspore mother cells after meiosis yield microspores which are haploid, and each of these divide by mitosis to produce an immature male gametophyte, the pollen grain. Usually, pollen is bi-nucleate, but sometimes one of the cells later divides again to produce two sperm cells showing trinucleate stage, like in grasses.

In the ovule (megasporangium), megaspore mother cell undergoes meiosis to produce four megaspores. The nucleus of the functional megaspore divides mitotically three times to produce eight nuclei, one of which eventually becomes the egg. The female gametophyte is an eight-nucleate, seven-celled structure, known as embryo sac. Cells present in embryo sac are synergids (two), egg cell (one), antipodal cells (three) and free nuclei (two), also called as polar nuclei. These are termed as polar nuclei because they originate from opposite ends of the embryo sac.

2.3. Pollination

After flower dehiscence (opening), anthers release pollen grains, which are disseminated to stigma tip. This transfer of pollen grains from the anthers (stamens) to the stigma of a flower is termed as pollination, and need a vector or pollination agent. The pollen from anthers can be transferred to the stigma of the same flower or another flower on the same plant, *i.e.,* self-pollination (autogamy), or to the stigma of a flower on another plant, *i.e.,* cross-pollination. The pollinating agents can be biotic or abiotic; insects, mammals and birds are biotic pollination agent, while wind and water are abiotic pollinating agents. Gravity also plays role in pollination in some species and is dependent on the flower structure. Certain features of flower make it fit for one or another mechanism of pollination. Species in which pollination is done by insects (insect-pollinated crops), known as entomophilous, have flowers with bright coloured petals *i.e.* are showy. A simple white flower for human beings could be of glowing blue-green colour for bees because they are able to see ultraviolet light. Other insects might detect dots, rings, and lines on the same flower marking out welcoming landing strips that our vision cannot perceive. Flowers of some species also have nectaries (sugary food) or scent and use them as lure to attract the insects. Sometimes, shape and appearance of flower is also a source of attraction for insects. The mirror orchid (*Ophrys speculum*) produces a flower that looks remarkably like the female of specie of large wasp or bee common to the area where it grows. Examples of insect pollinated crops are citrus, mango, apple, grapes, fig, cocoa, custard apple, carrot, radish, turnip, cabbage, cauliflower, cucurbits, onion, okra,

pepper, snapdragon, campanula, petunia, impatiens, primula, arum lily, day lily (*Hemerocallis*), foxglove (*Digitalis stewartii*), velvet plant (*Verbascum spp.*), and celosia.

Botanists estimate that only around10%-12% of the total species of flowering plants are wind pollinated, also termed as anemophilous plants. Wind pollinated flowers are not showy, usually green in colour, are of small size and lack nectaries and scent. Such flowers produce abundant pollen, which are smaller in size, light weight and have less nutritive value than the pollen of entomophilous plants. Stigmas are large and protruded to maximize the chances of catching pollen from air. Insects will gather pollen from anemophilous flowers when pollen from entomophilous flowers is scarce. Small solitary bees often visit the small and inconspicuous flowers of many species of grass, while the larger bumblebees and honeybees can be seen gathering pollen from corn tassels. Examples of anemophilous plants are grasses, spinach, beetroot (*Beta vulgaris* L. ssp. *Esculenta*), Swiss chard, *Amaranthus spp*., and trees such as date-palm, pecans, pistachio nuts, willow (*Salix*), birch (*Betula*), hazel (*Corylus*), beech (*Fagus*), and oak (*Quercus*). Pollination in conifers (gymnosperms) is also mediated by wind.

Plants in which birds help in pollination, produce red and yellow flowers, are termed as ornithophilous. Plants, in which bats (mammals) facilitate pollination, are termed as chiropterophilous, produce flowers of large size with strong fruit pedicels and strong scent as well as nectar.

2.3.1. Mechanisms of Pollination

On the basis of pollination mechanisms, plants have been grouped into two mating systems *i.e.* self-pollinated or cross-pollinated. As mentioned earlier, self-pollinated species usually accept pollen from the anthers of the same flower (autogamy) and therefore, flowers must be bisexual. Cross-pollinated species generally allow pollen from different sources to germinate on the stigma tip, finally resulting in fertilization and development of the zygote. Cross-pollination ensures variability in new generations of offspring. In nature, species show varying degrees of cross-pollination, ranging from lack of cross-pollination to complete cross-pollination.

Self-pollination: Self-pollination refers to the transfer of pollen from anthers to the stigma of the same flower. Sometimes, this term also encompasses the transfer of pollen to another flower on the same plant and thus demands any vector (wind or insects). Complete self-pollination is reported in a very few species. The percentage of self-pollination is influenced by factors such as wind, temperature, the populations and type of insects. Certain natural mechanisms promote or ensure self-pollination. Some floral structures favour selfing *e.g.* legumes such as peas. In some species, the stigma is closely surrounded by anthers and thus support selfing *e.g.* tomato. In lettuce, pollen is sticky but no insect pollination has been observed. Its pollen cannot be easily carried by wind, although some cross-pollination has been reported (George 2009). Some flowers fail to open before dehiscence of anthers and receptivity of stigma, and/or open when pollination has completed and these floral parts are not functional. This condition is referred to as cleistogamy. This type of pollination is

prevalent in peas, beans, lettuce, peanut, *Viola*, commelina, subularia, jewel weed (*Impatiens capensis*). Mostly these flowers are bisexual, small in size, inconspicuous, colourless and do not secrete nectar. All cleistogamous plants can also produce chasmogamous flowers. Chasmogamy is the condition in which pollination takes place after flower opening. Cleistogamy is dependent on environmental conditions as well as availability of pollinators. Plants mostly produce cleistogamous flowers when environment is harsh, otherwise chasmogamous flowers are produced in moist and high-light conditions *e.g.* jewel weed. This trait (cleistogamy) has many advantages in breeding (fixation and pure lines breeding) and seed production, especially in the field of crucifers, genetically modified organism (GMO) programmes to reduce the risks of cross-pollinations. The cleistogamous trait is known to be controlled by a single gene (*clgl*), and can be transferred by breeding methods such as back-crosses or haploidization. Self-pollination has not been observed in most woody plant species except some such as peaches (*Prunus*). Other examples of self-pollinated plants are linum, chicory and endive.

Cross-pollination: The transfer of pollen from anthers of flower of one plant to the stigma tip of flower on another plant by any vector is termed as cross-pollination. Cross-pollination is desirable both for population and individuals in a population because it increases heterozygosity (for evolutionary adaptation within the population) as well as plant vigour. There are certain mechanisms which promote cross-pollination. For example, if flowers of a self-pollinated plant open before self-pollination (in the bud stage), the chances of cross-pollination are increased and degree of crossing would be dependent on population of insects or wind speed. The most effective mechanism that induces cross-pollination is the dioecy, in which male and female flowers are borne on separate plants, *e.g.*, spinach, asparagus, holy (Ilex), hemp, date-palm and pistachio. Another mechanism, which is less stringent, is monoecy, *i.e.,* the condition in which both male and female flowers are borne on the same plant. Both, self- and cross- pollination is possible because plant can receive pollen from their own male flowers as well as male flowers borne on other plants. Examples of monoecious plants are cucurbits, sweet corn, walnut and oak.

Cross-pollination is also possible in plants with hermaphrodite flowers due to dichogamy *i.e.* maturity of male and female parts at different times, for example in *Beta vulgaris* (Lundqvist et al. 1973). Dichogamy is of two types, namely protandry and protogyny. Protandry refers to the situation in which the stamens mature and anthers dehisce before the pistil (stigma) is mature and receptive. It has been reported in carrot, parsnip, onion, leek, dianthus, campanula, impatiens, and sisal (*Agave sisalana*). In protogyny, stigma becomes receptive earlier than the maturity of stamens and release of pollen grains *e.g.* chilli, tea, avocado, and walnut. Dichogamy does not ensure cross-pollination but increases its chances. Moreover, dichogamous species if does not receive foreign pollen revert to self-pollination as observed in Campanula. Sometimes, the pollen from the same flower cannot fertilize and set seed, a condition known as self-incompatibility, and thus demands cross-pollination, *e.g.,* Brussels sprout and velvet plant (*Verbascum phoeniceum*). Sometimes, pollen is sterile, *e.g.,* potato, and cannot fertilize and therefore, cross-pollination is mandatory in such species.

Cross-pollination is also favoured in self-compatible species in some conditions which favour heterostyly *i.e.* differential length of stamens and stigma tip, for example in tomato due to flowering at high temperature. Other examples of plants exhibiting heterostyly are primrose, egg-plant and asparagus. Self-pollination in naturally cross-pollinated plants for consecutive generations increase homozygosity in the population but, sometimes cause decline in plant size, vigour, and productivity, referred to inbreeding depression.

Often cross pollinated crops: Some crops fall in the intermediate category, because they have genetic feature between self and cross pollinated crops. So, they exhibit 5% to 40% cross pollination. *P. coccineus* (runner bean) is a self-fertile plant, but some insect activity is required to 'trip' the stigma (*i.e.* rupture the stigmatic surface slightly), which cause cross pollination, especially early in the flowering season when up to 40% cross pollination is possible. Broad bean (*Vicia faba*) is also self-fertile, but up to 30% crossing is possible because of insect activity (Poulson 1975). Winged Bean (*Psophocarpus tetragonobolus*), also termed as Asparagus or Goa Bean, is partly self-pollinated and partly cross-pollinated. *Capsicum annuum* and *C. frutescens* are generally self-pollinated, but some cross pollination can occur between cultivars of the two species or among cultivars of the same specie. The degree of cross pollination in *Capsicum spp*. depends on populations of pollinating insects and timing of anther dehiscence. Prior to dehiscence of anthers, the stigmas are receptive to pollen transmitted from other plants. While, anthers continue to dehisce and release pollen up to 2–3 days after opening of individual flowers. Other examples of often cross pollinated are pigeon pea, tobacco and okra.

2.3.2. Fertilization

When compatible pollen lands on a receptive stigma, a pollen tube grows down the style to the micropylar end of the embryo sac and penetrates the sac to release two sperms or male gametes. One of these sperms unites with the egg cell, a process known as fertilization. The other sperm cell unites with the two polar nuclei to form a triple fusion nucleus. The simultaneous occurrence of two fusion events in the embryo sac is termed as double fertilization.

2.4. Barriers in Reproduction

Sexual incompatibility is the genetically controlled trait that restrict either the germination of pollen grain on the stigma or if germinated suppresses tube growth down the style in plant with the same incompatibility alleles. Incompatibility of various types has been reported in literature in various crops, which cause problem in selfing and/or crossing to produce inbreds and hybrids. Incompatibility can be self or cross in nature, each is discussed in detail in this chapter.

2.4.1. Self-incompatibility

Self-incompatibility is an important agricultural trait. It is based on the ability of the pistil to recognize the presence of pollen from the same flower or plant so as to inhibit

its germination or subsequent development, allowing only genetically unrelated pollen to germinate, fertilize and set seed. This condition prevails even when both pollen and ovule are normal and viable. Self-incompatibility is due to genetically controlled physiological mechanism on stigmatic tip and stylar tissues or can be due to morphological variation in flowers in a population. There are two different systems of SI *viz.*, heteromorphic and homomorphic.

Heteromorphic system: In this system, stigma and anthers of flowers are of relatively different lengths, *i.e.,* heterostyly. It is believed that heteromorphic incompatibility is always sporophytic in nature. Based on relative positions of style and anthers, flowers can have distyly or tristyly.

In distyly, two different flower types are borne on different plants of the population namely, pin type (having long styles with short anthers) and thrum type (short style with long anthers) controlled by *ss* and *Ss* alleles, respectively. It is governed by a complex of six differentiating characters which are genetically controlled *viz.*, style length, stigmatic surface, stylar incompatibility, pollen incompatibility, pollen size and anther size. Pollen can germinate in a cross of pin × thrum and fertilize the egg cell but pin × pin or thrum × thrum cross is not fruitful. Distyly has been reported in Primula, Forsythia, Oxalis, Silia and *Turnera ulmifolia*.

Tristyly is a condition in which three different types of flowers, based on relative length of styles, are produced on different plants of a population. Tristyly is governed by two genes, each with two alleles (Ss and Mm); the double dominants have the shortest style, while double recessives have the longest styles. *Lythrum salicaria* is a tristylous herb in which stigma tip is located inside the corolla tube (in the short-styled flowers), but stigma comes outside the corolla in the mid- and long-styled flowers. Tristyly is also reported in other species such as brinjal and *Pontederia sagitatta*.

Other examples of plants with heteromorphic incompatibility system are narcissus, daffodils (*N. pseudonarcissus*), purple loosestrife (*L. salicaria*), and oxalis (*Oxalis spp.*). Besides above mentioned species, self-incompatibility is also reported in flowering annuals such as *Phlox drummondii*, *Borago officinalis*, and *Gilia achilleifolia*.

Homomorphic system: In this system, there is no morphological difference among flowers of all mating types in the population rather incompatibility is due to multiple allelic series. This type of incompatibility system is further categorized into two groups, the gametophytic and the sporophytic system.

Gametophytic System is also termed as oppositional factor system which is more wide spread than sporophytic incompatibility. In this system, ability of the pollen to function (formation of pollen tube) is determined by its own genotype and not by the parent plant. This incompatibility system is found in species having binucleate pollen that possess only one generative nucleus in the pollen tube. Inhibition in this system occurs during pollen tube growth. A series of alleles at a single locus (*e.g.,* S_a, S_b,..., S_n) or alleles at two loci in some species, control homomorphic incompatibility system. The alleles of the incompatibility gene(s) act individually in the style without dominance. The pistil is diploid containing two incompatibility alleles (*e.g.*, *S*1*S*3,

*S*3*S*4). Reactions occur if identical alleles in both pollen and style are present. Only heterozygotes for *S* alleles can produce pollen tube in this system. Examples are tomato, potato, wild relatives of petunia (*Petunia* × *hybrida*), lily (all *Lilium* species except *Lilium formosanum*), gaura (*Gaura lindheimeri*), evening primrose (*Oenothera* spp.), heliconia (*Heliconia*), Trifolium, and Nicotiana. Plants with single *S* locus cannot perpetuate by selfing and therefore, are virtually heterozygous at this locus.

The incompatibility characteristics of the pollen in sporophytic incompatibility are determined by the plant (sporophyte) that produces it. It occurs in species such as broccoli, radish, and kale. It is common in species with trinucleate pollen grains *i.e.* those having two generative nuclei. In this incompatibility system, inhibition occurs on the surface of stigma or during early pollen tube growth stage. The sporophytic system is under the genetic control of a single locus *S* that has multiple alleles. This incompatibility system is complex because it may have dominance (determined by the pollen parent) or individual action both in pollen and the style. Incompatible pollen may be unable to germinate on the stigmatic surface. For instance, a S_aS_b plant in which S_a is dominant over S_b, will produce pollen that will virtually behave like S_a. S_a pollen tube will not successfully pass through S_a style but can grow through S_b style. Hence, homozygotes of *S* alleles are possible. Sporophytic self-incompatibility has been reported in beetroot (*Beta vulgaris*), *Brassica oleracea* (Acephala, Botrytis, Gemmifera gongylodes, and Italica groups), radish (*Raphanus sativus*), sweet potato (*Ipomoea batatas*), ageratum (*Ageratum houstonianum*), chrysanthemum (*D.* × *grandiflora*), zinnia (*Zinnia elegans* and *Z. angustifolia*), marigold (*T. patula* and *T. erecta*), gerbera (*Gerbera jamesonii*), Dame's rocket (*H. matronalis*), dahlia (*Dahlia* × *hybrida*), and alyssum (*L. maritima*).

Physiologically, SI can be expressed either as i) inability of pollen to imbibe and germinate on stigmatic tip (broccoli) and is linked with cutinase enzyme system, ii) deposition of callose in the stylar region and/or slow pollen tube growth that delays delivery of sperm nuclei in the embryo sac well in time for fertilization to take place (tobacco) or iii) incompatibility reaction after fertilization (*Gesteria*); a rare mechanism.

Methods to overcome self-incompatibility: Self-incompatibility can be brokendown temporarily by some measures or techniques, which are listed below:

a) The removal of the stigma surface.
b) Application of electric shock (100 V) at the time of pollination, *e.g.,* in Brussels sprouts and white cabbage.
c) Early or bud pollination, *e.g.,* in crucifers. It is done usually 3 to 4 days before actual anthesis time because inhibitory proteins have still not formed.
d) Lowering the temperature (to slow down the development of the inhibitory substance).
e) Use of irradiated (0.1 krd to 2 krd) pollen.
f) Application of NAA or IAA on flower.

g) Application of glutamic acid, folic acid and nicotinic acid @ 50 mg/L, *e.g.,* in radish.

h) Repeated pollination, *e.g.,* chicory.

i) Transfer of self-compatible alleles in self-incompatible plants.

j) High temperature (55 to 60°C) treatment of pistil, *e.g.,* in *Brassica oleracea*, *B. oleracea* var. gemmifera, and *Solanum peruvianum.*

Besides these, other methods are specific for one or other type of incompatibility. For example, use of mutagens such as EMS (Ethyl Methane Sulfonate), X-rays, and radioisotopes, *e.g.,* P^{32}. The breakdown of self-incompatibility using mutagens is easier to achieve in gametophytic systems than sporophytic systems. Moreover, gametophytic system of self-incompatibility can be broken down by increasing the ploidy level, *e.g.,* self-incompatible diploid pear becomes self-fruitful when chromosomes are doubled *i.e.* in autotetraploid pear. This higher ploidy level would allow the pollen grain to have two different alleles and thus the allelic interaction could cancel the incompatibility reaction. Allopolyploids of *Brassica oleracea, i.e., B. napus*, *B. juncea* and *B. carinata* are self-compatible. Similarly, autopolyploids in *Solanum peruvianum* are self-fertile. But, this change in ploidy level cannot break down the sporophytic incompatibility because of the existence of two different alleles in a diploid individual. These alleles may interact to produce the incompatibility effect and polyploidy only increases availability of these alleles. Doubling of chromosome in *B. rapa* and *Raphanus sativus* does not alter self-incompatibility reaction (Kalloo 1994).

Significance of self-incompatibility

Although self-incompatibility is a constraint in sexual reproduction of plants yet, this mechanism can be used as a tool to facilitate breeding by various methods.

1) Self-incompatibility promotes heterozygosity and thus crossing in self-incompatible plants can result in significant variability and then superior recombinants can be selected.

2) Self-incompatibility is very useful trait to produce commercial F_1 hybrids, synthetics and triploids in plants with sporophytic systems, because it eliminates the cumbersome and costly emasculation process. Compatible inbred lines are used as parents. High degree of self-incompatibility enhances the chances of hybrid seed production to great extent in economically important vegetables of Brassicaceae, including cabbage, broccoli and cauliflower (Hirai and Matsumoto 2007).

3) Gametophytic incompatibility occurs in vegetatively propagated species. The clones to be hybridized are planted in adjacent rows.

4) The blooming period of annual ornamental plants and vegetative phase in leafy and root vegetables can be prolonged by preventing seed production due to self-incompatibility.

5) Self-incompatibility demands mixed plantations of apple varieties that cause uneven quality. Development of stable mutants of *S*-alleles in these self-incompatible varieties would confer self-fruitfulness and would

decrease potential variability. It would permit the segregation of rare recessive phenotypes in this highly heterozygous material.

2.4.2. Male Sterility

The plants having non-functional anthers and/or pollen are termed as male sterile and this condition is known as male sterility. It may also be expressed as absence of pollen, severe paucity of pollen, development of abnormal stamens, absence of stamens, and/or failure of anther dehiscence. This trait can eliminate the need for emasculation, a labour demanding procedure in hybrid seed production. Some male sterility genes are located in the mitochondria. Male sterility can be divided in to three types, which are listed as under:

Induced male sterility: This type of male sterility is induced by certain chemicals (gametocides) when used at a particular growth stage of crop. These gametocides (*e.g.* ethrel) at appropriate dose (250 ppm) interfere with development of normal male gametophyte and manifest their effect in the form of aborted anthers. Other examples of these chemicals are Dalapon, Estrone, Ethephon, Hybrex, and Generis. High temperature induced floral sterility results in reduced seed set, fruit size and yield.

Functional male sterility: Sometimes pollen is fertile but the anthers fail to dehisce (to release pollen grains). In this way, pollination and following fertilization cannot take place. Prevention of anther dehiscence has been attributed to lack of stomium. This type of male sterility arises due to spontaneous mutations. Functional male sterility has been reported in tomato and brinjal.

True male sterility: This type of male sterility occurs if androecium of flower is absent, as in case of unisexual flowers in dioecious (spinach, asparagus, papaya, holly) and monoecious (cucurbits) plants or microspores of hermaphrodite flowers are abnormal or non-functional. This abnormality is due to developmental irregularities leading to abortion of pollen grain and is of three kind, *i.e.,* genetic male sterility, cytoplasmic male sterility (CMS) and cytoplasmic-genetic male sterility (CGMS).

Genetic male sterility: It is also termed as nuclear or genic male sterility. Genetic male sterility is expressed in the form of pistilloidy (pollen abortion) or abnormal anther development. It is governed by one recessive gene (ms) while, its counterpart, the Ms, is a dominant allele and results in normal anther and pollen development. Almost all diploid and polyploid plant species are supposed to have at least one male-sterility locus and in some cases may be more than one. It is widespread in plants, can occur in nature but mostly is induced artificially. It has been reported in tomato, brinjal, potato, sweet pepper, broad beans, lima bean, lettuce, cucumber, muskmelon, watermelon, summer squash, winter squash, broccoli, Brussels sprouts, cauliflower, and cabbage. Genetic male sterility is of limited value in hybrid seed production because expression of the gene can vary with the environment. So, it is difficult to eliminate the female population before either harvesting or sorting harvested seed. Moreover, a pure line of male-sterile (msms) plants cannot be maintained using

conventional breeding methods and can be regenerated by crossing those (msms) with a heterozygous (Msms) plant (Fig. 2.2).

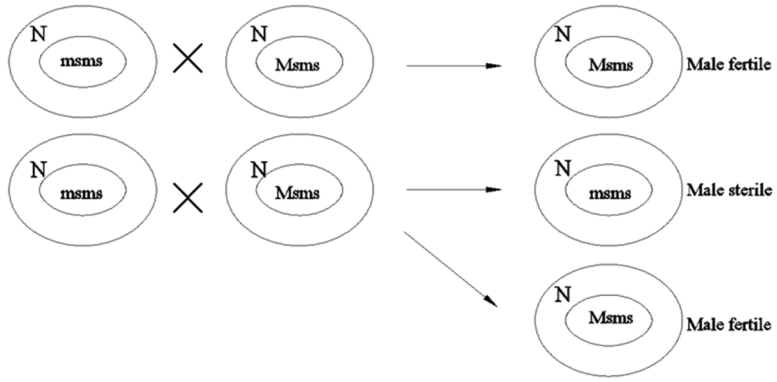

Fig. 2.2 Inheritance patterns of genetic male sterility. The gene Ms (male sterility) is dominant over ms allele.

Cytoplasmic male sterility (CMS): This type of male sterility is due to cytoplasmic DNA (genes of mitochondria) that causes malfunctioning of anthers or abortion of the microspores during development. A cytoplasm with sterility genes is described as sterile (S), while normal cytoplasm is designated as F (fertile) or N (normal). The sterile cytoplasm is dominant over the fertile one and therefore all the progeny of a cytoplasmic male sterile plant (♀) crossed with plant (♂) having fertile/normal cytoplasm will be male sterile (Fig. 2.3). It is because CMS trait is transferred only through the egg, while the male parent in the cross provides nucleus only. The CMS has been reported in corn, beet, carrot, onion, and pepper. This system can be exploited in breeding of ornamental species because all the offsprings are male-sterile and thus remain fruitless. These non-fruiting plants remain fresh and continue to bloom for a longer period, a demanded feature.

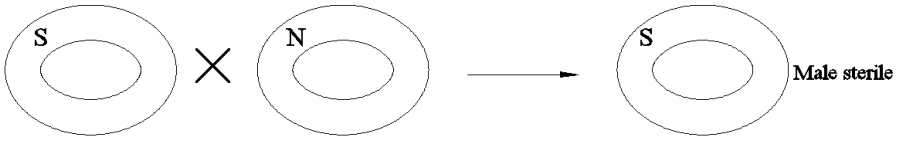

Fig. 2.3 Inheritance pattern of cytoplasmic male sterility. S= sterile; N= fertile.

Cytoplasmic-genetic male sterility (CGMS): This type of male sterility is due to complex interaction between nuclear and mitochondrial genes. Strictly speaking, in CGMS cytoplasm is sterile along with fertility non-restorer genes (*rf*).

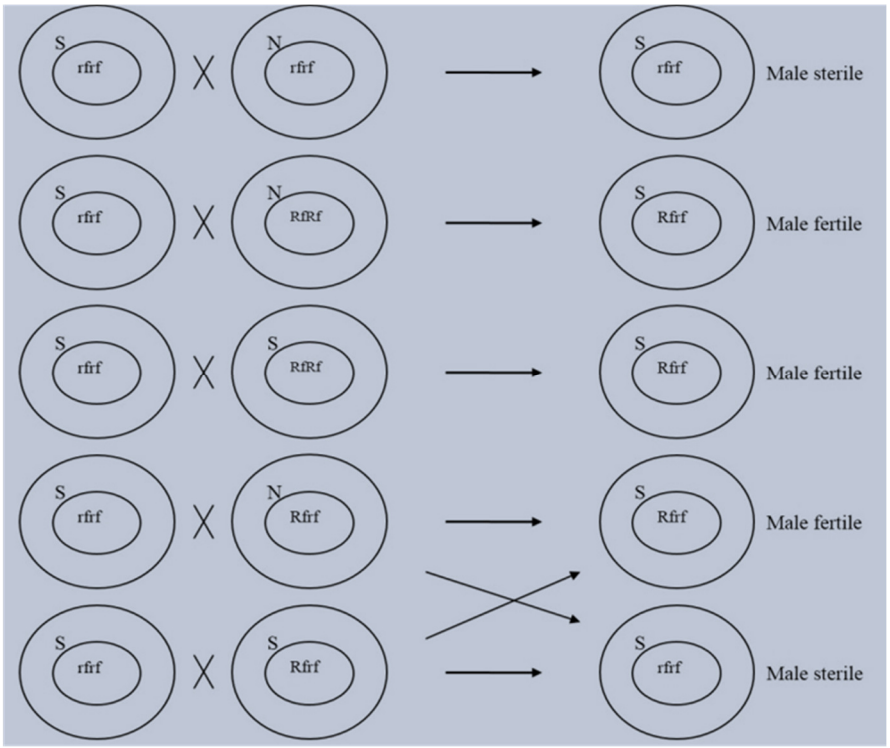

Fig. 2.4 Inheritance patterns of cytoplasmic-genetic male sterility. Rf is dominant over rf and can restore fertility. N denotes normal (fertile) and S denotes sterile cytoplasm.

If fertility restorer genes (*Rf*), which are dominant over sterile (S) cytoplasm and *rf*, are present in the nucleus, plant will be fertile and produce normal pollens in normal anthers (Fig. 2.4). As with CMS, cytoplasmic factor is transferred only by female parent in the cross.

Three line (A, B, C) system is followed when breeder wants to utilize CGMS for hybrid seed production. Line A is male sterile line (Sms ms); Line B is sterility (S) maintainer (Nms ms), present in commercial cultivars, while Line C is a pollen parent (NMs Ms). The maintainer line can be identified by crossing it with the male sterile line. If the cross of a plant (supposed to be maintainer) with a male sterile plant produces all male sterile plants in F_1, then it is a maintainer line.

Sms ms × Nms ms → Sms ms
(Male sterile) (Maintainer?) (Male sterile)

Maintainer line can be produced by backcross method through following steps (Fig. 2.5).

1) F_1 [SMs ms obtained from cross male sterile (Sms ms) × male fertile (NMs Ms)] is backcrossed with male fertile line (NMs Ms).
2) From the resulting progeny, heterozygous male fertile (NMs ms) plants are selected by test cross with male sterile plants, which are selfed.
3) From selfed progeny, maintainer (Nms ms) is identified by test cross with male sterile plants (Sms ms).

CGMS is widely used in hybrid seed production of many crops because it is the easiest and practical method of producing hybrid seed on large scale. It has been observed in onion, carrot, radish, Brassica crops (cabbage, cauliflower and Brussels sprouts), beet, and corn.

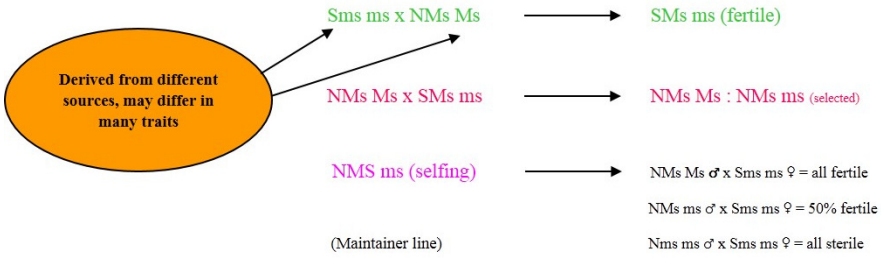

Fig. 2.5 Procedure of development and evaluation of maintainer line.

Exploitation of male sterility in plant breeding: Demand for hybrids is very high in the present era and a big task for breeder is the development of inbred lines so that the company can earn by keeping the parents secret. Male sterility has been used primarily as a tool by the plant breeders to keep the parental secret. Besides this, male sterility also eliminates the need to emasculate self-pollinated crops in hybridization program, which otherwise is cumbersome and time consuming. Male sterile cultivars are used as female parents in a cross without any need to emasculate the flowers.

2.5. Breeding Methods for Horticultural Crops

2.5.1. Plant Introduction

It is one of the most important methods of crop improvement. It involves exploration, collection, purification, conservation, evaluation, storage and utilization. Wild species, land races, old and modern cultivars can be collected through exploration in the native areas as well as from local resources and germplasm banks. Institute of Agri-Biotechnology and Genetic Resources at National Agricultural Research Centre, Islamabad, also has a wide range of germplasm of various crops and provide to breeders on demand. The introduced material must be subjected to quarantine measures and should be grown in isolation to check the presence of pathogens or insect-pests. Collected material need to be purified to achieve homozygosity and

homogeneity before use and then must be kept in pure form. Introduced material can be released as a variety, if it performs better than the existing varieties, or can be used in breeding program. Several fruit vegetables crops have been introduced in Pakistan and released as varieties.

2.5.2. Selection

It is the art of identifying and picking desirable variants in a population. Selection is the primary strategy in crop improvement programs. Variability is mandatory for selection to be practiced but selection cannot create variability. Plant breeders perform selection at different stages, starting from selection of genotypes from a population to selection of a plant or a group of plants obtained by crossing of different genotypes. Different types of selection methods used by breeders are discussed below.

Pure line selection

It involves identification and isolation of single best plant progeny for the development of a new variety. This method is commonly employed in self-pollinated crops to develop pure line varieties, while inbred lines can be developed by this method in cross-pollinated crops. After three years of selection, multi-location yield trials are conducted for two to three years and later on (usually during 7^{th} year), seed is multiplied for handing over to seed-production agencies.

Mass and progeny selection

It is one of the oldest method in which individual plants are selected from a mixed population on phenotypic basis, their seeds are mixed (bulked) and used to grow the next generation. It can be practiced in both self- and cross-pollinated crops, but is more common in cross-pollinated crops. Varieties selected by this method show more stability and/or resistance to environmental stresses and diseases because of broader genetic base, although show less uniformity of the produce than the pure lines. Rouging is necessary to maintain high yield in varieties selected through this method. Selection is started using 50 to 1000 plants of a landrace or old unimproved variety; selection of individual plants and bulking is continued for six years using a standard check variety for comparison. During 7^{th} to 8^{th} year, variety is named and released.

Progeny selection is commonly used in cross- and often cross-pollinated crops and is a little bit different from mass selection. Progenies of selected plants (10-50 off-springs) are grown and performance of progeny is tested. Seed of plants, whose performance is better, are bulked to produce the next generation. Variety can be released by this method in 8 to 10 years.

Recurrent selection

It is a modified form of progeny selection, mostly involved in cross-pollinated plants, that involves reselection of inbreeding plants generation after generation. It is a cyclic selection to increase frequency of desirable alleles for a specific character in a population and thus results in population improvement. The selected plants are

selfed, which are then intermated in all possible combinations. The seed is then bulked and another cycle of selection is performed. The developed population can be used to produce homozygous inbreds (for hybrid varieties) or for production of synthetic varieties. Three types of selection *viz.,* simple recurrent selection, recurrent selection for general combining ability (GCA), and recurrent selection for specific combining ability (SCA) can be performed depending on the objectives *i.e.* either breeder wants to develop hybrid or synthetic variety

Clonal selection

In this selection method, superior clones of asexually propagated plants are selected from a mixed population. It is usually practiced in garlic, sweet potato, ginger, turmeric, banana, citrus, apple, pear, peaches, apricots, etc. Clonal selection is performed in crops with reduced or no flowering and fruit set, as well as those exhibiting apomixis and inbreeding depression. Crops showing high level of heterozygosity *e.g.* citrus, mango, apple, pears and ornamental plants, are usually propagated asexually and variants are selected as clones. Different clones are compared and the best performing clone (s) are selected.

A wide range of clones are selected from different geographical areas and/or different sources. Superior clones are selected after plantation during first year and planted separately during second year for evaluation on the basis of morphological traits. During third year, superior clones are selected with the standard variety. The selected superior clones are grown in replicated yield trials during fourth year. On the basis of performance in fourth year, the best clones are selected, multiplied and released.

2.5.3 Hybridization

Genetic variability is very important in breeding of horticultural crops and can be generated by hybridization. After hybridization, segregating progenies and advancing population are handled, and desired segregants are selected by various breeding methods, employing various selection procedures. These methods are pedigree, bulk, single seed descent, and can be used alone or two methods can be combined. A brief description of these methods is as under.

Pedigree method

It is widely used for the improvement of self-pollinated vegetable crops. Both parents, used for hybridization, are well adapted and have desirable characters. This method is employed for development of new varieties and inbred lines in self-pollinated and cross-pollinated crops, respectively. Proper record of the ancestry of each selected plant(s) in each progeny is maintained and therefore, named as pedigree method. Usually, 14-15 years are required for varietal development and release by this method.

Bulk method

This method is also known as mass or population method of breeding. After hybridization, segregating population of self-pollinated vegetable crops is grown in bulk plot (F_1 to F_5) with or without selfing. Superior plants are selected in F_6 and later generations, as in pedigree method. Preliminary yield trial is conducted in 8^{th}

year, followed by multi-locational yield trials for two to three years. The material developed through bulk method, must be tested in such an environment that favor the selection of desirable genotype(s). Crosses involving low × high yielding parents showed increase in yield over the generations in this method but, not with high × high yielding parents. This method is less expensive, convenient and provides more chances of selection of outstanding segregants than pedigree method. However, it does not reveal the mode of inheritance of oligogenic characters.

Single seed descent method (SSD)

It is a modified form of bulk method of breeding in which single seed is randomly selected from each plant in F_2 and later generations. The selected seed is mixed (bulked) and sown to have next generation. Bulking is practiced till F_5 to achieve homozygosity. Large number (400 to 500) of plants is selected in F_6 and progeny of each individual plant is grown separately in rows. Superior progenies are selected till F_8 and submitted for multi-locational yield trials to select the best progeny that is released. This method is simple because large number of crosses can be evaluated without complex record keeping as in pedigree method. The material can be advanced till F_5 generation in greenhouse, where two to three generations can be grown each year, to shorten the breeding time. But, many superior plants may be lost in this method as well as identity of superior F_2 plants cannot be maintained. SSD is good to handle large population of tomato, eggplant, pepper and cucurbits and performs well for low heritable characters.

Backcross method

This method involves recurrent backcrossing of F_1 with one of the parents, known as recurrent (recipient) parent, to transfer a specific trait to a variety that was lacking it. Progeny of each backcross is again crossed with the recurrent parent. Other parent, which has a desirable trait and is used only once, is known as donor parent. The cycle of backcrossing is repeated for 5 to 6 generations with selection of plants having desirable trait in each generation, which are used for backcrossing. The genotype of original variety is restored except the character (trait) donated by the donor parent. Backcrossing is used in both self- and cross-pollinated vegetable crops. The specific trait to be transferred to the recipient parent should be highly heritable. Backcross breeding methodology is dependent on the character (trait) under observation, *i.e.,* whether controlled by dominant or recessive gene. If trait under study is controlled by a dominant gene, F_1 can be backcrossed with recurrent parent in each cycle of backcross and so the desired trait can be incorporated in 10 years. But, in case of a trait controlled by a recessive gene, F_1 is selfed and homozygous recessive plants are identified by growing the F_2 plants in specific stress condition or on the basis of specific trait *e.g.* fruit shape. Therefore, 12 to 14 years are required for incorporation of such recessively controlled traits in the cultivated varieties. The time period can be shortened by growing more than one generation in greenhouse and using marker assisted breeding (See section 3.5.3 for detail). Breeders have used this method to transfer disease (fungal, bacterial and viral) resistance, earliness (precocious lines), morphological and quality traits of fruit. Double backcross and backcross pedigree methods have also been used in vegetables.

2.6. Heterosis

In hybridization between different cultivars within specie or in interspecific and intergeneric crosses, progenies normally show more biomass development, higher rate of plant growth and fertility. This phenomenon is referred as heterosis, which was described by Charles Darwin in 1876. There are two models being commonly used to describe heterosis. In dominance model, the recessive alleles present at different loci are complimented in the hybrid. The overdominance model suggests that the induced vigour in the hybrid is the outcome of interaction of different alleles. Dominance model is relatively more popular than the over-dominance model (Charlesworth and Willis 2009). According to dominance model, the hybrid parent may contain more number of genes than either parent as one parent may have copies of genes missing in the other parent. However, only this factor may not be responsible for the whole heterotic response since related cultivars having slight genetic variations may also show some heterosis. While, interspecific and intergeneric hybrids offer higher heterotic response compared with intraspecific crosses for example hybrids of radish and cabbage (Karpechenko 1927), hybrid of wild tomato (*Solanum pennellii*) and cultivated tomato (*Solanum lycopersicum*; Eshed and Zamir 1995). It can be concluded that heterosis increases with increase in the phylogenetic distance between the parental stocks hence interspecific crosses show more heterosis compared with intraspecific crosses. In population genetics, only genotypes which are reproductively perfect and have dominant alleles will produce higher biomass and prevail during the evolutionary process compared with recessive detrimentals, which are usually eliminated when homozygous.

At cell level, no dramatic increase in the cell size was observed rather cell proliferation rate could be higher in hybrids compared with their parents. Hence, heterosis appears differently across different crosses and plant tissues. In hybrids, flowering time is the most commonly affected trait depending upon the specie. Progression to flowering could be either slowed down with prolonged vegetative growth or enhanced with higher biomass and fertility. These heterotic effects might be regulated by alteration of circadian rhythms and metabolic profiles (Fievet et al. 2010; Birchler et al. 2010). Studying gene expression patterns using genomic methods is a commonly used technology. In hybrids, gene expression was presumed to be additive compared with the parents. However, evidences have been reported for both additive and non-additive gene expression behaviours (Paschold et al. 2010; Riddle et al. 2010). The parents having higher genetic variation normally show divergent gene expression patterns (Birchler et al. 2010) leading to positive or negative phenotypic effects; however, the hybrids usually show positive heterosis. This suggests that there is ambiguity about gene expression behaviours either they are correlated, caused or could be used for predicting heterotic responses. Hence, further studies are desired by comparing the gene expression patterns in the parents and the hybrids to underpin relationship of gene expression and heterosis.

It is a general perception that continuous inbreeding may cause inbreeding depression, which leads to loss in yield and heterosis. However, it has been observed that removal of recessive deterimentals during inbreeding did not affect heterotic responses. There was no decrease in heterosis magnitude due to enhanced frequency

of superior alleles in the inbred lines (Birchler et al. 2010). Polyploids also exhibit progressive heterosis and maximization of diverse genomes in polyploidization markedly enhances the magnitude of heterosis. Exceptional heterosis has been reported in interploid crossing of intrageneric allotetraploids in potato (*Solanum tuberosum*). In autotetraploidy, the heterosis exhibited by single cross hybrids of AABB and CCDD will be significantly less compared with a double cross hybrid having ABCD genomes from different parents suggesting further exploration of heterotic response in hyperploids with diverse allelic patterns. Further, allelic dosage also impacts the heterosis magnitude in polyploids and plants with AABB genome could be more vigorous than either AAB or ABB (Goral et al. 2005; Birchler et al. 2010). Hence, for heterosis among strains, the parents shall be homozygous at different loci affecting a trait.

2.7. Plant Genetic Resources

Horticultural crops are heterogeneous group of plants including trees, shrubs, vines and herbs. These range from tropical to sub-arctic zone and sea level to high mountains. Fruit crops are generally highly heterozygous in nature; hence, these are mostly propagated by asexual means. Change in the public attitude towards genetic resources enhanced their significance. In 1980s, US Supreme Court termed germplasm as a common heritage and allowed to patent living material including crop cultivars and established methodologies etc.

2.7.1. Significance of Germplasm

Any genetic material that could be used to develop new cultivars or plant material that could be used for further propagation is termed as germplasm including seeds, leaf, stem, pollen, or cultured cells. Germplasm may also include intraspecific hereditary stocks used by the breeders to develop new cultivars. Any heterogeneous genetic entity formed by different genotypes and has ability to survive across different environments may also be used as germplasm. Adverse climate changes and modernization of horticultural practices have led to rapid extinction of valuable germplasm during the process of domestication (Fig. 2.6). During domestication, artificial selection was made for useful traits in plants. Their seeds or vegetative plant materials were retained for onward plant multiplication. Primitive cultivars and wild relatives could play a significant role in crop improvement, particularly in resistance against biotic and abiotic stresses. Compared with cash crops, fewer efforts have been made to save horticultural crops' wild relatives. Attempts were started to save the local fruit cultivars in living collections in Romania, Russia, China and Asia Minor in late 80's. Most of the material was saved and less desired breeding lines were discarded that could have been retained for future crop improvement program against changing climate and abiotic stress factors.

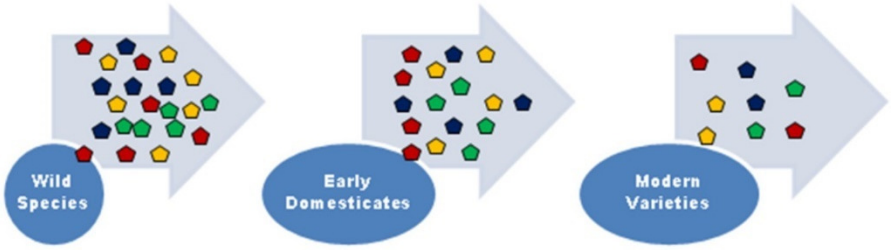

Fig. 2.6 Loss of wild gene pool during the process of selection and domestication over time.

2.7.2. Centers of Origin and Genetic Diversity

Several classifications of centers of genetic diversity are available in the literature. Centers of origin may also be termed as centers of diversity if evolutionary forces are highly active there. Several centers of diversity could be available for a single or multiple crops and therefore, may not be regarded as centers of origin. Frequent introgressive hybridization could affect the frequency of different genes at various centers of diversity. Hence, different centers of diversity could be equally important depending upon the nature and frequency of variation available there. Vavilov (1951) classified 8 major centers for crop origin in the world having more than 7000 apples and 2500 pear cultivars (Table 2.1). However, only few of these valuable genetic resources exist now, which signifies the dire need for plant germplasm conservation.

Table 2.1 Centers of origin of ancestors of cultivated fruits and vegetables.

S. No.	Crops		Regions of Origin	References
	Fruits	Vegetables		
1	Most temperate fruits	Beet, lettuce, okra	Northern hemisphere of old world	De Candolle (1885), Vavilov (1930)
2	Peach		Central China	Hedrick (1917)
3	Wild sp. of *Malus* and *Pyrus*	Turnip and relatives, radish, peas	Europe, Mediterranean Turkestan, China and Japan	Vavilov (1951), Zagaja (1977)
5	Most *Citrus* sp., mango	Cucurbits, turnip and relatives, black pepper, eggplant, spinach	South East Asia	Swingle and Reece (1967), Naik (1949)
6	Fig	Cabbage, yams, onion and allies, carrot	Asia Minor	Storey (1975)
7	Strawberry, blueberry, raspberry, blackberry, cranberry, elderberry avocado, pineapple, nuts	Sweet potato, peppers, tomato	North America	Darrow (1966), Bergh (1975), Cobley (1956)

2.7.3. Collection, Conservation and Utilization of Plant Genetic Resources

Working collections of crop plants have been maintained including potentially useful material as commercial cultivars, breeding parents and plant material screened for specific objectives. Rest of the material was usually discarded due to lack of space, facilities and budgets for plant maintenance. These plant collections were poor representations of the wild relatives, which may contain different ecotypes that need to be conserved for conserving genetic variation available in each wild species. Wild relatives are valuable resource of genetic variability in economically important traits like pest and disease resistance, winter hardiness, drought tolerance, vigour of growth and productivity. Most of today's commercial fruit crops have been developed based upon narrow genetic base *e.g.*, Kinnow, orange, peach, avocado and several other fruits. Use of wild germplasm in the breeding programs was started in mid-19th century with impressive outcomes. Germplasm conservation could be more economical by prioritizing and concentrating on the hotspots with more wild germplasm and higher extinction rate like Asia Minor and China. Some important plant genetic resource institutes working in different countries are listed in Table 2.2.

Germplasm conservation is important to minimize genetic erosion, which occurs mainly due to the introduction of new and highly uniform cultivars, loss of traditional farmer's cultivars and monocropping. Further, there are chances of development of new diseases or insect-pests in future. Loss of genetic diversity leads to the genetic vulnerability of the plant material to such stress. Highly uniform crops have been more vulnerable to insect pest and environmental hazards due to certain genetic change. There are several reasons for germplasm collections including developing understanding about the origin of crops, germplasm exchange at national and international levels, and due to lack of available diversity in existing *ex situ* collections. The technical and logistic planning shall be made before time and local people should be involved to ensure speedy location of the target species. Germplasm could be conserved by collecting wild species and primitive land races. Rich biodiversity provides raw material for breeding and biotechnology applications for crop improvement. Biodiverse plant material should be properly identified, documented, evaluated, and maintained for exchange and utilization.

Plant genetic resources can be divided into five categories *viz.*, i) Advanced varieties and bred varieties; ii) genetic stocks, *i.e.* lines that carry particular mutations, cytogenetic rearrangements, or linkage markers; iii) bulk populations or composite crosses developed from crosses from a wide variety of cultivars; iv) Folk varieties of landraces used in pre-scientific agriculture; and v) wild progenitors. Collections are usually biased, based on the phenotypic expressions. Hence, random sampling of plant material including both seed and vegetative parts is recommended.

Table 2.2 List of important plant genetic resource and conservation centers and databases.

S. No.	Genetic Resource and Conservation Centers/Databases	Crops	Links	Country
	Bioversity International (Formerly IPGRI)	All	http://www.bioversityinternational.org/	Italy
	Centre for Genetic Resources (CGN)	Apple and leafy vegetables	http://documents.plant.wur.nl/cgn	The Netherlands
	CATIE	Guava, jack fruit and litchi	http://catie.ac.cr/en/colecciones-y-bancos-de-germoplasma/otros-bancos-de-germoplasma	Costa Rica
	California Rare Fruit Growers	Acerola, bael fruit, cranberry	http://www.crfg.org/	USA
	Northwest Berry and Grape Infonet, Oregon	Grapes and berries	http://berrygrape.org/	USA
	CRA, Consiglio per La Ricerca e La Sperimentazioni In Agricoltura	Citrus, other fruits, vegetables	http://sito.entecra.it/portale/cra_dati_istituto.php?id=209	Italy
	Chinese Crop Germplasm Information System (CGRIS)	Fruit and vegetable crops	http://icgr.caas.net.cn/cgris_english.html	China
	The Online European Minor Fruit Tree Species Database – EMFTS Database	Minor fruits	http://www.ueresgen29.unifi.it/netdbase/db1.htm	European Union
	USDA Germplasm Collection Center	Tropical fruits	http://www.ars.usda.gov/Main	USA
	Center for Plant Conservation		http://www.centerforplantconservation.org/	USA
	The James Hutton Institute Commonwealth Potato Collection	Potato	http://germinate.scri.ac.uk/germinate_cpc/app/index.pl	UK
	International Potato Center (IPC)	Potato	http://www.cipotato.org/	Peru
	The World Vegetable Center (AVRDC)	Vegetables	http://avrdc.org/	Taiwan
	Tomato Genetics Resource Center (TGRC)	Tomato	http://tgrc.ucdavis.edu/	USA

Systems for germplasm conservation

There are different systems of germplasm conservation. Some important systems include cold storage of scions, cryo-preservation of tissues, pollen and seed storage. In cold storage, scions of different fruit plants could be stored for about one year at 0°C. In grapes, scions could be stored up to four years at 1°C to -5°C. In deciduous fruit crops, there is little need to store scions. Tissue culture is promising for propagation, maintenance and preservation of the germplasm using limited resources and space. For example, meristem culture of thousands of citrus and grape cultivars can be stored in an area of 3-4 m^2. Strawberry plantlets could be stored up to 6 years.

There are two major techniques of tissue culture based plant preservation including, i) slow growth techniques, and ii) cryopreservation. Slow growth techniques involve lowering the growth temperature and light intensity thus reducing the plant growth and metabolism. Different media supplements including osmotic inhibitors and growth retardants could be used for tissue dehydration. By using such techniques, plant material could be stored for medium term *i.e.*, 1-5 years. Storage of plant tissues at ultra-low temperatures is called cryopreservation. This technique completely stops cell division and metabolic processes. The material could be stored for longer duration. Cryopreservation also offers advantages including virus elimination using cryotherapy, *e.g.*, banana streak virus (BSV). It provides solution for safe and long-term storage of transformation competent cell suspension. However, cryopreservation is limited by post-cryopreservation viability of the plant material which could be resolved by careful dissection of tiny meristem for preservation. Plant propagation following conservation through tissue culture may have some disadvantages. In fruit crops like apple, micropropagated plants or scion cultivars grafted on micropropagated rootstocks have shown a delayed bearing behaviour with reduced yield (Zimmerman and Miller 1991; Czynczyk et al. 2007). Such response has not been found in micropropagated guava plants rather *in vitro* propagated plants showed higher number of fruit setting and development compared with the control plants (personal observations of corresponding author). Genetic stability in micropropagated plant material is another dilemma. There are reports of somaclonal variants and polyploids that need to be verified using genetic markers. Several risk factors may also mimic the utilization of tissue culture facilities for long term germplasm storage, particularly in the developing countries with limited resources. These factors include power failure, fire, flooding and other natural disasters. It is therefore strongly recommended to use cryopreservation facility as a germplasm backup system and field plantations should be made in parallel to ensure the germplasm security. Maintenance of mature and bearing material could also help breeders to use pollens for further genetic and breeding related studies. Conclusively, plant germplasm collections are like "pharmacies filled with miracle drugs without labels" (Witt 1985) that need to be characterized and uncover important genes present there for development of biotic and abiotic stress resistant cultivars.

2.8. Mutation Breeding

2.8.1. Mutagenesis and Types of Mutation

Mutation breeding involves utilization of mutations for crop improvement. Any sudden heritable change in an organism at gene or chromosome level is termed as mutation. Simple genetic recombinations are not called as mutation. Breeders are usually more interested in genes or chromosomal mutations; however, cytoplasmic mutations also occur particularly in fruit plants or asexually propagated crops. Mutations provide basis for genetic variation in a population. Treating a biological material with a mutagen to induce mutations is termed as mutagenesis. Mainly there are three types of mutations according to the point of origin or occurrence.

Gene or point mutation involves a change at molecular or sub-microscopic level. Normally, change occurs in the nucleotide sequences of DNA molecules leading to the formation of a new type of amino acid, protein or prevents formation of a normal protein. This kind of genetic changes may be accompanied by the emergence of a new trait that may be inherited following Mendel's Laws. In case of polygenic traits, selection efficiency is low but mutation rate is quite high. Chromosomal mutations or aberrations involve splitting and subsequent changes in the chromosomal structure. Such changes could be characterized as deficiency leading to loss of a segment of the chromosome (deletion); doubling of the chromosome segment (duplication); rearrangement of genes in reverse order in a segment of the chromosome (inversion); and segmental exchange between different chromosomes (translocation). Genome mutations are changes that occur in set of the chromosomes and are less desired by the breeders. Breeders are more interested in gene mutations, because chromosomal rearrangements normally produce negative results like lowering fertility of the offspring. Mutants are of great value for breeders as sources of new useful characters. Mutagenesis may be instrumental in crossing of crops having small flowers like mango and other vegetable crops.

There are two main sources of mutations *viz.,* spontaneous and induced. Spontaneous mutations occur in natural populations without manual treatment at a rate as low as 1 per million. In fruit crops, most of the present day mutants are spontaneous in nature and have replaced the original cultivars in crops like apple, mango and citrus. Spontaneous mutations could be induced by cosmic radiations, extreme temperatures and ageing process. Buds remaining dormant for prolonged periods could be affected by higher endogenous metabolic accumulation. Rate of spontaneous mutations is quite low in nature; hence, it is desirable to enhance the existing rate of mutagenesis by artificial sources. Induced mutations are artificially caused using physical or chemical agents to improve one or two easily identifiable characters in a well adopted cultivar.

2.8.2. Characteristics of Mutations

Mutations are generally lethal and detrimental. Mutants may be sterile with low vigour, viability and decreased chlorophyll synthesis. Most of the mutations are recessive in nature; hence, for detection of any phenotypic changes it is important to treat heterozygous plant material. Most of the horticultural plants particularly fruit crops are vegetatively propagated and heterozygosity is more common in these crops. Strong phenotypes could be observed in mutants having deletions, duplications and recessive mutations in heterozygotes. Reverse and back mutations are less common compared with forward mutations. Normally, mutations revert to original when non-mutated cells or tissues take over the mutated ones.

2.8.3. Mutagens

Mutations could be induced by physical or chemical mutagens. Physical mutagens include different types of radiations including ultra violet (UV), electromagnetic and corpuscular radiations. Electromagnetic radiations include gamma rays, x-rays omitted from radioactive isotopes like ^{60}Co and ^{137}Cs; and corpuscular radiations

including thermal/slow neutrons and beta particles omitted from radioactive isotopes ^{32}P and ^{35}S. Slow and thermal neutrons are less influenced by variation in O_2 and temperature. These are more effective than other radiation sources. High density neutrons obtained from ^{32}P and ^{35}S isotopes may cause irreparable damage including chromosomal aberrations. Thermal neutrons could be applied as chemical solutions and induced in sap stream of trees during active growth. The plants could be treated as a whole or in parts. Special installations are required to irradiate whole plants/trees like Gamma fields, which are highly expensive to build and maintain. Small gamma chambers with controlled environmental conditions are more useful in treating vegetative parts. Seeds are most commonly used for irradiation compared to other plant parts since these are easy to handle and store for long terms. Seeds can be subjected to a variety of physical and chemical environments and can be soaked, heated or frozen.

Number of chemical mutagens is rising with the time; however, only a few mutagens belonging to alkylating agents are useful in practical mutation breeding. Frequently applied mutagens include diethylsulphate (DES), ethyl methane sulphonate (EMS), methyl methane sulphonate (MMS), isopropyl methane sulphonate (iPMS), azide and colchicine. Other toxic and carcinogenic mutagens include ethyleneimine (EI), N-nitroso, N-ethylurea (NEU), N-nitroso, N-methylurea (NMU) and 1,4-bisdiazoacetylbutane. These mutagens function via direct chemical action with the chromosomes. Safe and potential mutagens are alkylating sulphonates and sulphates including EMS, iPMS and DES. Alkyl group reacts with DNA and interferes with its synthesis. Alkylation involves replacement of H atom with the alkyl group (ethyl/methyl). These alkylating agents give point mutations and few chromosomal aberrations. Mutagenic compounds transferring one alkyl group are more efficient and useful mutagens compared with compounds transferring more alkyl groups. All alkylations never give rise to mutations since selection sieves among cells, *i.e.*, repair process, DNA transcription and competition determine whether an observable change will take place or not. Nitroso compounds like sodium azide are highly toxic and carcinogenic.

Another very effective chemical is known as colchicine. Colchicine induces polyploidy, aneuploidy and changes other than genome mutations. Application of colchicine is highly effective, inexpensive and versatile. Exposure to colchicine can be manipulated by its concentration in solution, duration of treatment, temperature and hydrogen ion (H^+) concentration. Colchicine causes mutations by chromosomal rearrangements and inhibits assembly of tubulin subunits in the spindle fibers. This halts chromosomal movement and accumulation at metaphase of mitosis. Chromatids separate but are not divided into separate cells thus chromosome number is doubled. For successful doubling, more number of small and actively growing meristems should be treated with colchicine. The easiest way of its application is germination of seeds in diluted solution of colchicine, rinsing with water after hours or days and plant seeds. This method is not applicable in vegetatively propagated species. Colchicine dose rate ranges from 0.1% to 0.9% depending upon the type of tissue and specie. Higher killing rate of plant tissues could be regulated by increasing exposure time to colchicine. Enhancing its dose rate will lead to more killing, resulting in more hyperploid tissue and vice versa. At least 50% killing of plant

tissues is desirable to have more rate of chromosomal doubling. Conventional mutation breeding procedure is explained in Fig. 2.7.

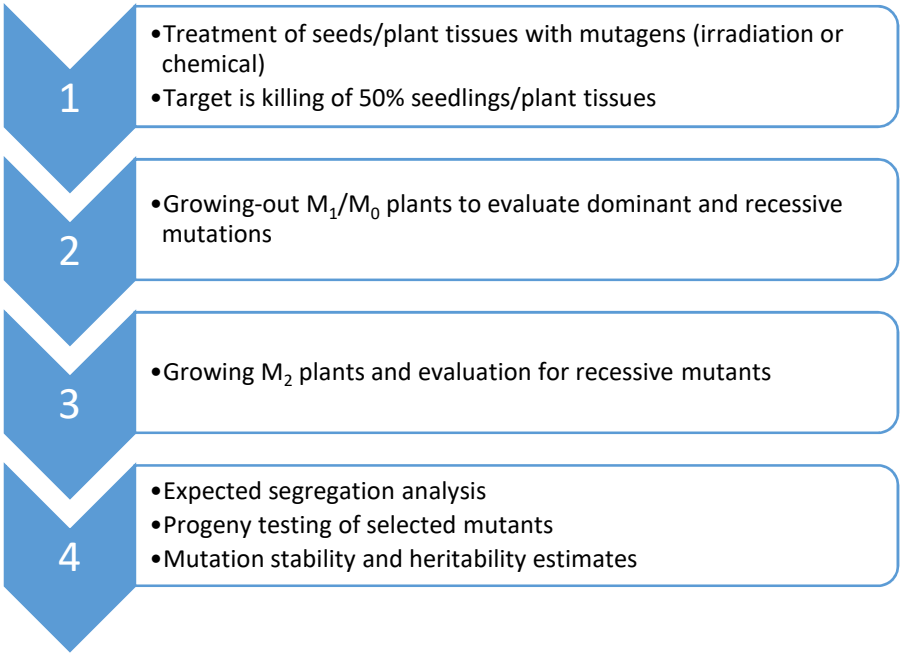

Fig. 2.7 Conventional mutation breeding procedure.

2.8.4. Applications of Mutation Breeding

Mutation breeding could be easily used to uncover and homogenize existing chimeras and make mutants stable; suppress incompatibility in crosses between distantly related species, to induce haploids; to produce sexuality in the apomicts; to induce variability in widely adapted specie that have already approached selection limit; to induce new characteristics like disease resistance, self-compatibility for which no known source of gene(s) is available for hybridization; and to produce specific mutation and preserve it using asexual propagation and break linkages with undesirable characteristics. A list of important fruit and vegetable crops in which different traits have been improved using different mutagenic agents has been provided in Table 2.3. Conclusively, mutation breeding has been supplementing classical breeding and never been its replacement. For success in mutation breeding usually large plant populations are required for treatment and screening with higher mutation rate in the desired trait. There is need for efficient methods of identifying mutations by phenotypic examination and simple tests.

Table 2.3 A list of selected mutants developed in horticultural crops with trait(s) of improvement.

S. No.	Crops	Mutagen	Trait(s) altered	References
Fruit Crops				
1	Apple	Gamma radiation	Skin colour	Brunner and Keppl (1991)
2	Ber	Gamma rays and *in vitro* culture	Valuable morphological changes	Zheng et al. (2009)
3	Guava	Gamma rays and *in vitro* culture	Low seeded	Zamir et al. (2009)
4	Mango	*in vitro* culture	Resistance against anthracnose	Litz (2009)
5	Pineapple	*In vitro* culture and gamma rays	Resistance against bacterial rot disease	Lapade (1995); Ibrahim et al. (2009)
6	Papaya	Gamma rays	Plant height (dwarf plants were obtained)	Chan (2009)
7	Pear	Gamma radiation	Disease resistance	Masuda et al. (1997)
8	Mandarin (*Citrus reticulata*) and Pummelo (*C. grandis*)	Gamma rays and *in vitro* culture	Seedlessness	Somsri et al. (2009)
9	Sweet Orange (*C. sinensis*)	Gamma rays	Resistance against Citrus canker	Belasque Jr. et al. (2009)
	Citrus (*C. suhuiensis*)	Gamma rays	Germination percentage increase,	Noor et al. (2009)
10	Grapefruit (C. paradisi L.)	X-rays	Flesh colour, seedlessness	Hensz (1991)
Vegetable Crops				
1	Potato	Ethyl Methane Sulfonate (EMS)	Improved starch	Muth et al. (2008)
2	Cabbage	EMS	Leaf	Himelblau et al. (2009)
3	Melon	EMS	Andromonoecy, delayed ripening	Boualem et al. (2008); Dahmani-Mardas et al. (2010); Gonzalez et al. (2011)
4	Tomato	EMS	Delayed ripening, reduced height; virus resistance; yellow fruits	Rigola et al. (2009); Piron et al. (2010); Gady et al. (2011)
5	Pea	EMS	Leaf and floral traits	Triques et al. (2007); Dalmais et al. (2008)

2.9. Ploidy Manipulation in Breeding

Eukaryotic organisms have typically two sets of chromosomes in a nucleus and are classified as diploids. Gametes are usually reduced through meiosis and have one set of chromosomes (haploid). Plants or cells having more than two sets of chromosomes in a nucleus are called as polyploids. For example, triploid has 3 sets of chromosomes, tetraploid 4 sets, pentaploid 5 sets and hexaploid = 6 sets etc. Polyploidy is common in nature. Long term evolutionary potential and flexibility of polyploids enables the organisms to avoid static genetic state as enforced by gene redundancy. Therefore, 50%-70% of the angiosperms have gone through the process of polyploidization during evolution. A list of horticultural crops with their ploidy status is provided in Table 2.4. Frequency of polyploidization is much higher (~1 in 100,000 plants) in flowering plants.

Table 2.4 Ploidy status, reproduction and propagation in major fruit and vegetable crops.

S. No.	Common Name	Botanical Name	Ploidy		Mode*	Propagation
	Fruit Crops					
1	Apple	*Malus domestica*	Diploid	2x = 34	CP	Asexual
2	Avocado	*Persea americana*	Diploid	2x = 24	CP	Asexual
3	Banana	*Musa sapientum*	Natural Triploid	3x = 33	Parthenogenesis	Asexual
4	Citrus	*Citrus* sp.	Diploid	2x = 18	CP	Asexual
5	Coconut	*Cocos nucifera*	Diploid	2x = 32	CP	Sexual
6	Date	*Phoenix dactylifera*	Diploid	2x = 36	CP	Asexual
7	Falsa	*Grewia asiatica*	Diploid	2x = 36	SP	Sexual/ Asexual
8	Fig	*Ficus elastica*	Diploid	2x = 26	CP	Asexual
9	Grapes	*Vitis* sp.	Diploid	2x = 38	SP	Asexual
10	Guava	*Psidium guajava*	Diploid	2x = 22	SP, 5-10% CP	Sexual
11	Mango	*Mangifera indica*	Diploid	2x = 40	CP	Asexual
12	Olive	*Olea europea*	Diploid	2x = 46	CP	Asexual
13	Papaya	*Carica papaya*	Diploid	2x = 18	CP	Sexual
14	Pear	*Pyrus malus*	Diploid	2x = 34	CP	Asexual
15	Pineapple	*Ananas comosus*	Diploid	2x = 50	SP	Asexual
16	Raspberry	*Rubus* sp.	Diploid	2n = 14	SP	Asexual
17	Strawberry	*Fragaria ananassa*	Octaploid	8x = 56	CP	Asexual
	Vegetable Crops					
1	Black pepper	*Piper nigrum*	Diploid	2x = 48, 52, 104	SP	Sexual
2	Cabbage	*Brassica oleracea*	Diploid	2x = 18	CP	Sexual
3	Carrot	*Daucus carota*	Diploid	2x = 18	CP	Sexual
4	Cucumber	*Cucumis sativus*	Diploid	2x = 14	CP	Sexual
5	Egg plant	*Solanum melongena*	Diploid	2x = 24	SP	Sexual
6	Lettuce	*Lactuca sativa*	Diploid	2x = 18	SP	Sexual
7	Musk melon	*Cucumis melo*	Diploid	2x = 24	CP	Sexual
8	Okra	*Abelmoschus esculentus*	Amphidiploid	2x = 29, 36	SP, OCP	Sexual
9	Onion	*Allium cepa*	Diploid	2x = 16	CP	Sexual
10	Peas	*Pisum sativum*	Diploid	2x = 14	SP	Sexual
11	Pepper	*Capsicum* sp.	Diploid	2x = 24	SP	Sexual
12	Potato	*Solanum tuberosum*	Diploid, Natural tetraploid	2x = 24, 48, 72	CP	Asexual
13	Radish	*Raphanus sativus*	Diploid	2x = 18	CP	Sexual
14	Sugar beet	*Beta vulgaris*	Diploid	2x = 18	CP	Sexual
15	Summer squashes, pumpkins, marrow	*Cucurbita pepo*	Diploid	2x = 40	CP	Sexual
16	Sweet potato	*Ipomea batatas var batatas*	Diploid	2x = 90	CP	Asexual
17	Tomato	*Solanum lycopersicum*	Diploid	2x = 24	SP	Sexual
18	Turnips	*Brassica rapa*	Diploid	2x = 20	CP	Sexual
19	Water melon	*Citrullus lanatus*	Diploid	2x = 22	CP	Sexual

20	Winter squashes	*Cucurbita mixta, C. moschata*	Diploid	2x = 40	CP	Sexual

** Mode of reproduction and pollination i.e. self-pollinated (SP), cross-pollinated (CP) and often-cross pollinated.*

2.9.1. Mechanism of Polyploidization

There are several mechanisms proposed for spontaneous occurrence of polyploidization (Ramsey and Schemske 1998). Polyploids emerge from a mitotic or meiotic disorder, which leads to unreduced gamete formation, rarely found in nature. After fusion of unreduced gametes, triploid zygotes are formed that could be unstable and sterile due to uneven distribution of chromosomes in the gametes. The fusion of unreduced diploid and triploid gametes could further lead to develop polyploids including tetraploids, pentaploids etc. Autotetraploid apple and citrus have been reported in diploid populations (Ramsey and Schemske 1998; Usman et al. 2012), and autohexaploid in sugarbeet (Bingham 1968). The complexity in chromosomal pairing possibilities leads to deletion or addition of chromosomes in the gametes. Even-ploid zygotes will be more stable compared with odd-ploid zygotes. Autoploids usually arise from a mutation in the chromosomes, while alloploids are outcome of hybridization and subsequent mutation in the chromosomes. The other path of development of polyploids is simple somatic doubling during mitosis (Fig. 2.8). These mitotic aberrations have been reported in the meristematic tissue of the crop plants including tomato (Ramsey and Schemske 1998). This somatic doubling could be induced using mitotic inhibitors like colchicine as described in section 2.8.3. Autoploids can also be used for induction of seedlessness through interploid breeding (4x × 2x) as reported in citrus (Fatima et al. 2002; Usman et al. 2008). Another mechanism of polyploid formation is reported in orchids where several nuclei fertilize one egg and the phenomenon is termed as polyspermy (Ramsey and Schemske 1998).

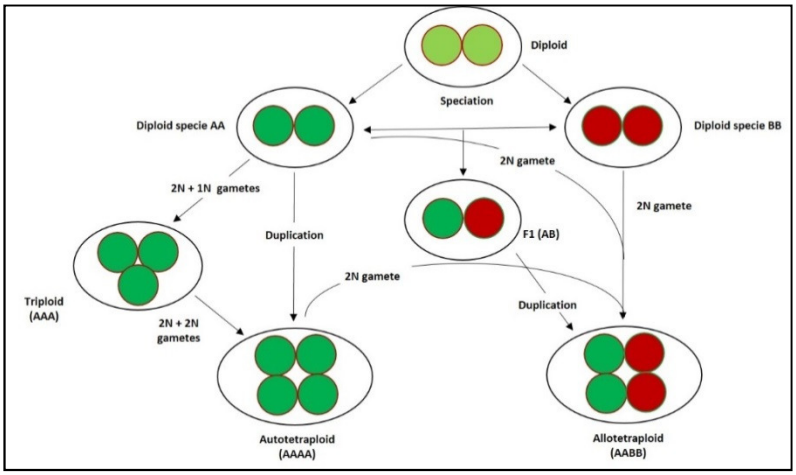

Fig. 2.8 Different pathways of polyploidization in plants. A and B represent different genome types, and N represent the gametic chromosome number.

Aneuploid is an organism or cell with other than an exact multiple of the haploid or basic number of chromosomes. These are usually formed due to meiotic irregularities including variation in chromosomal arrangements during meiotic pairing between ploidy types and defective pairing of the homologous chromosomes in F_1 hybrids due to divergence in structure and number of chromosomes. Aneuploids result from i) non-disjunction or non-separation of alike chromosomes during meiosis ii) chromosomal lagging or slow movement of one chromosome than other during anaphase and chromosome is lost. The haploid chromosome number may be like 4x-1, 4x-2, 2x-1 etc. iii) Irregular chromosomal distribution that usually occurs in odd ploids, *e.g.,* triploids and pentaploids. Multipolar mitosis may also lead to irregular distribution of chromosomes in anaphase resulting in cells with different aneuploid chromosome number. In aneuploids, gene product is altered in the affected chromosome, which may be decreased upto 50% in monosomics and increased up to 50% in trisomics resulting in imbalance in the biochemical and physiological pathways.

2.9.2. Types of Polyploids

Polyploids are further classified as euploid or aneuploid based on their chromosomal composition. Aneuploid may be hyperploid or hypoploid owing to an addition or deletion of the whole chromosome, respectively. Different types of aneuploids include: a) Monosomic (2n-1), missing a single chromosome; b) nullisomic (2n-2), missing both copies of a chromosome; c) double monosomic (2n-1-1), missing 2 non-homologous chromosomes; d) Trisomic (2n+1), having an extra chromosome; e) double trisomic (2n+2), having 2 non-homologous extra chromosomes; f) tetrasomic (2n+1+1), having one extra chromosome in two different chromosomes; and g) quasidiploid (2n+1-1), having one pair of a chromosome missing and extra chromosome is present in another pair. It is reported that 30%-40% of the progeny of autoploids may be aneuploids (Comai 2005).

Euploid is an organism or a cell having an exact multiple of the haploid chromosome number like 2n, 3n, 4n, 5n. These are homoploids in nature. Addition or deletion occurs as a whole set of chromosomes. Majority of the polyploids are euploid in nature and tetraploids are the most common class of euploids available. A list of important fruit and vegetable crops with their ploidy levels, mode of reproduction and propagation is provided in Table 2.4. Euploids are further classified in to autopolyploids and allopolyploids commonly called as autoploids and alloploids, respectively.

Autopolyploids (autoploids) are polyploids having multiple copies of the basic set of chromosomes (x) from the same genome. Spontaneous autoploids have been recovered in many crops including triploids and tetraploids in citrus (Usman et al. 2006; Padoan et al. 2009; Fatima et al. 2010). Odd polyploids like 3x, 5x, 7x are usually sterile due to: a) unpaired chromosomes, b) non-availability of balanced gametes, and c) increased zygote death. Odd polyploids are used for the development of seedless fruit, *e.g.,* Kinnow mandarin. Even polyploids like 4x, 6x, 8x are often fertile owing to equal segregation of homologs during meiosis. Autoploids show a variety of effects on the phenotype, physiological and biochemical attributes of the

crop. In ornamentals, spontaneous doubling has been reported to increase the plant growth and vigour, for example in tulip and hyacinth (Acquaah 2007). Multiple advantages of polyploidization in nature have inspired breeders to manipulate ploidy for crop improvement leading to induced polyploidization through treatment of diploids with mitotic inhibitors like colchicine, oryzalline and dinitroaniles.

n = Haploid ploidy level

x = Basic chromosome number

In	Diploids	2n = 2x 1n = 1x
	Tetraploids	2n = 4x 1n = 2x
	Hexaploids	2n = 6x 1n = 3x

Allopolyploids (Alloploids) are polyploids developed with different type, origin and combinations of different genomes (Acquaah 2009; Chen 2010). Alloploidization occurs frequently in nature and causes speciation in angiosperms. Examples of natural alloploids are strawberry and blueberry (Chen 2010). Wide hybridization is performed between distantly related parents to associate economically important characters and to create allopolyploids. The hybridized genomes could be homeologous to each other. If only segments of the chromosomes differ in the combined genomes, then it is called as segmental alloploidy and the chromosomes are called homeologous chromosomes indicating ancestral homology (Acquaah 2009). Induced alloploidy have been used to reveal genes with novel functions (neo-functionalization) and complementing genes (sub-functionalization) among *Cucumis* species (Chen et al. 2007).

2.9.3. Significance of Polyploidization

Polyploids are highly significant from evolutionary point of view and many crop plants are polyploids including strawberry, banana and grapes. Polyploids offer following salient advantages:

i. Gigantism is the primary advantage of polyploids as the organs can be larger, including leaves, flowers, fruits and seeds leading to enhanced yield. This gigantism could be caused by more vigorous growth in polyploids compared with their diploid progenitors.

ii. Polyploids are more heterotic compared with their diploid progenitors. Heterosis could be fixed in hybrids by allopolyploidization. Intergenomic recombinations could be avoided in allopolyploids due to enforced pairing of homologous chromosomes and same level of heterozygosity could be maintained for several generations. Autopolyploid hybrids have more heterosis than diploid hybrids; whereas, autopolyploid inbreds show more inbreeding depression and may be smaller or similar in size compared with diploid inbreds. These responses, however, need further testing and wider studies. In random mating populations, each single locus has normally two alleles. Gene frequency of both alleles will be equal p=0.5, q=0.5. Frequency of heterozygosity = Total – freq. of homozygotes

Diploid = 1- (p^2+q^2) = 0.5
Tetraploid= 1- (p^4+q^4) = 0.875

iii. Gene redundancy is defined as masking of recessive alleles by dominant wild-type alleles in polyploids. It has the ability to alter gene function by changing the redundant copies of essential genes. Gene redundancy has buffering effect on recessive deleterious alleles thus reducing chances of occurrence of spontaneous mutations in polyploids. In alloploids, fixing of divergent pollens could be exploited at 1n stage of polyploids.

iv. Polyploidization may induce compatibility in self-incompatible crops thus allowing self-fertility and gain of asexual reproduction. In autoploids it could be induced by interallelic interaction in 2x pollens.

Polyploids may have following disadvantages:

i. **Alterations in cellular architecture and cell regulatory implication:** Increase in the genome content or chromatin enhances the cell volume by increasing about 1.6-fold in surface of the nuclear envelop. This is advantageous for cells having higher metabolic rate in bacteria to eukaryotes and could alter the cell regulatory mechanism. The endoreduplication of DNA in the nucleus leads to endopolyploid cells having genome content greater than the germ line. It usually results from cycles of DNA replication in the absence of mitosis. Endopolyploids could help by providing cells with different volumes during development of the organism. However, endoreduplicated diploid cells are not directly corresponding to the true polyploids. They are limited to allelic diversity present in the original zygote and maintain diploidy in the meristemtic cells. True polyploids contain higher number of chromosomes in all of the cells.

ii. **Changes in gene expression regulation**: In already optimized parental gene expression patterns any change could be deleterious. However, these changes in expression patterns could contribute to heterosis allowing better adaptation to the novel cellular and environmental conditions. Occurrence of the deleterious effects is more likely compared to the heterosis. Increase in copy number of all the chromosomes hypothetically shall equally affect all genes causing a uniform rise in the expression patterns. However, there are few classes of genes which don't show consistent gene expression patterns in polyploids. In such classes, genes may be consistently retained as duplicates or returned to singlets. The alternative patterns of gene losses and retention have been reported in Arabidopsis and families like Compositae (Blanc and Wolfe 2004; Buggs et al. 2012). The structural genes like proteosomal protein genes and ribosomal protein genes were retained as duplicates and gene expression regulatory factors like transcription factors and promotors were returned to singletons (Yoo et al. 2014). This explains how the transcription factors and promoters do not change in proportionate to increase in the ploidy. Polyploidization also changes structural relationship between some components of the cell and regulate progress in cell division via reversible gene expression regulation and epigenetic resetting. In autopolyploids, expression of most of the genes, *e.g.*, sucrose synthase is enhanced with increase in ploidy; however,

expression of a few other genes is inversely related to ploidy (Yoo et al. 2014). Hence, there is need to study gene expression patterns more widely across different ploidy plants with same genetic origin to enhance our understanding towards ploidy induced gene expression alterations.

iii. **Difficulties in cell divisions**: Three or more sets of chromosomes involved in meiosis may produce aneuoploids depending upon the type of polyploid. Autopolyploids potentially form multivalents including tetravalents and bivalents at metaphase I. Tetravalents are more likely to produce abnormal segregation of chromosomes leading to 3:1 or 2:1 and a laggard. Trivalents cannot give rise to balanced products in triploids. Random segregation of multiple chromosomes produces aneuploid gametes and zygote varies in viability depending upon species. Adaptation of bivalent pairing during the process of meiosis helps in stabilizing polyploids and avoids abnormal segregation. Mechanisms of normalization in meiosis in autotetraploids have an important role in adaptation and stabilization as 30%-40% of their progeny may be aneuoploid. In mitosis, spindle irregularities may create difficulties and little is known on mitotic stability in polyploid cells.

iv. **Epigenetic instability**: Polyploids have been more successful during the evolutionary process and show better adoption to the cold and dry conditions compared with their diploid progenitors. Most frequently herbaceous perennials are most successful in cold and dry conditions. Gigantism present in polyploids has been achieved during evolution; hence, they are relatively more successful in cold and dry conditions compared with diploids.

Polyploids are more heterozygous as compared with diploids. Random mating of tetraploid has more probability of being heterozygous at any given locus. Unique capacity of having 3 or more alleles at the same locus is called polyallelic loci. Absence of such loci leads to make polyploids relatively more inbred with reduced vigour and fertility. Simple somatic doubling is more common in plants. Potential of polyploids is not fully explored in autotetraploids. Autopolyploidization does not enhance heterozygosity, rather leads towards inbred plant development and higher genetic redundancy. Hence, for success in ploidy manipulation, simple somatic doubling is less desirable as autopolyploids developed through this method will not be successful for longer periods.

2.10. Molecular Breeding Tools

Crop improvement programs are based on breeding many selected parents randomly or combinations, followed by repeated stringent selections and backcrossing for obtaining homogeneity and trait fixation. The success of these programs is handicapped in fruit plants by long juvenile phase. Accelerated selection at juvenile stage can be done for simply inherited traits which are not related to reproductive growth. Complete evaluation of the fruit trees is only possible at bearing stage. Conventional methodologies to accelerate breeding and induce early flowering in tree crops include grafting of seedling scions on early flowering or dwarfing rootstocks, extension in the growing season with artificial light and temperature

conditions, ringing or girdling of trunks, bark scoring, root or shoot pruning, fertilization, foliar spray of urea, use of plant growth retardants like daminozide and paclobutrazol, and water stress. With the advent of molecular breeding techniques and our understanding towards cytogenetics, molecular biology and omics tools, the acceleration of tree breeding programs has become possible. Molecular breeding tools, including a wide variety of molecular markers and high through-put genome sequencing, have been quite effective for selection, characterization of genetic diversity in a population and for improvement in different crops. Use of molecular markers has enhanced breeding efficiency manifolds in different crops compared with phenotypic selection alone. These markers have been more useful to enhance genetic gain where phenotype is highly influenced by the environment. Millions of bites of available genomic information enable to discover beneficial alleles followed by cloning and characterization for quantitative trait loci (QTLs) using molecular markers. Multiple traits could be modified simultaneously by using selection indices that consider multiple characters and selection efficiency could be enhanced.

Back crossing, pedigree breeding and recurrent selections are common breeding methods used for crop improvement. Genetic improvement is measured by genetic gain (ΔG), which can be enhanced by increasing heterozygosity in populations, selection intensity and heritability. Transgenic approaches help plant scientists to create novel genetic diversity beyond boundaries of species and genera, and can exploit organisms across kingdom. The available genome maps provide more accurate estimates of the number of loci, effects of different alleles and gene expressions controlling or regulating different traits of interest including quantitative traits. Biotechnology further facilitates molecular stacking of transgenes controlling a single trait or a complex of traits in to a single locus by transgenic event(s) in to the nuclear genome or the organelle genome including mitochondria or chloroplast. Useful available genetic diversity can be developed in to constructs and favourable genes could be concentrated in desired elite cultivars. Functional genomics allows population profiling for RNA, protein and metabolites for the characters which are associated with elite traits. Marker assisted backcrossing could be used for the transfer of transgene to the elite cultivars. Endogenous genes and the environmental factors may influence the transgenic modifications; hence, forward breeding may be required for optimization of the transgene expression and the genetic back ground (Mumm 2007). Conclusively, adoption of molecular plant breeding techniques is rising in horticultural crops, particularly tree fruits, and reduction in juvenility is one of the key objectives. A better understanding of the recent developments in molecular techniques and an integrated approach of main components of molecular breeding and conventional breeding tools could be the road to success in crop improvement programs. A detailed discussion of the biotech tools is provided in the upcoming chapters.

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