Hybrid Seed Production Technology

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Abstract

Hybrid technology, harnessing the advantage of heterosis between two diverse genotypes to achieve maximum hybrid vigour, is widely recognized and commercially used for crop variety improvement both in field and vegetable crops. Hybrids can be developed using appropriate technology, irrespective of the mating and pollination system in the plant species. Production of hybrid seed depends on plant, pollinator and environmental factors, which influence it individually or in interactive ways. Hence, an understanding of these components is important to undertake hybrid seed production of a given crop species. The basic requirements for hybrid seed production at a commercial scale are (a) a unisexual flower or a bisexual flower with sterile pollen in anther or self-incompatible flower/plant; or pistillateness; or large conspicuous bisexual flowers for easy emasculation of flowers in plants to be used as the female parent and (b) abundant pollen production, dispersal and its easy transfer from the male parent to the female parent for satisfactory seed setting. These are dependent on floral biology, flower features, mode of pollination and reproduction of the crop species. Agronomic crop management with scientific insights is equally important for successful hybrid seed production. These are discussed in this chapter with appropriate examples.

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1 Introduction

Meeting the food demand of an ever-increasing population is the primary objective of agriculture. Hence, it is the constant endeavour of plant breeders to breed varieties that yield high under diverse agro-climatic conditions. A large number of hybrid varieties of the field crops, vegetables and flowers with higher productivity and other desirable traits are developed adopting appropriate selection and crossing methods of required genotypes. The F_1 hybrids, thus developed, are superior to their parental lines for productivity, quality and/or adaptability in diverse situations. Hence, there is a growing demand for hybrid seeds by farmers globally.

In simplest terms, the 'F_1 hybrid' is defined as the first filial generation of offspring of distinctly different parental types and refers to a plant cultivar derived by crossing two diverse parental lines/cultivars, each of which is an inbred line and is near homozygous. Crossing between two such genetically divergent but compatible parental lines produces hybrid seeds by employing controlled pollination. Hybrid seeds are heterozygotes in their genetic constitution and highly uniform in morphological features. The divergence between the parental lines results in better heterosis, whereas the homozygosity of the parental lines ensures a phenotypically uniform F_1 population. The basic principles of hybrid seed production are similar to those of an open-pollinated (OP) variety in terms of selection of the site and growing season, source of seed, cultivation methods, etc. However, special care is needed in terms of isolation, pollination techniques and manipulation of growing conditions for better seed yield and maintenance of the parental lines.

The primary factors that control hybrid seed production are the plant system, mainly the floral biology, mating type, pollination system and its underlying mechanism, and agronomic conditions like soil, season, irrigation, fertilizer, chemical, planting system, harvesting, etc. (Virmani 1994). All these factors determine the proper requirements of various inputs at an appropriate time for successful hybrid seed production. These principal factors or conditions can be considered as principles in hybrid seed production. In this chapter, we discuss those principles with associated practices.

Hybrid seed production depends on the method/system used for development of hybrid and pollination control mechanisms that govern the various activities in determining the isolation distance, planting ratio, synchronization of flowering, rouging and supplementary pollination. These are discussed below:

A hybrid is produced by crossing two genetically diverse parents. Pollen from male parent (pollen parent) pollinates and sets seed in female (seed parent) parent to produce hybrid seed. The development of a hybrid in a cross-pollinated crop is easier and more economical than that in a self-pollinated crop due to the higher outcrossing percentage in the former.

In nature, to create genetic variability and wider adaptation to different environmental conditions, the flowering plants have adopted various mechanisms for cross-pollination. Hermaphrodite flowers have both male and female reproductive organ in a single flower whereas the presence of unisexual/imperfect flowers favours out-crossing, which results in genetic heterogeneity and show wider adaptations (Frankel and Galun 1977). Flowering plants have evolved various mechanisms to favour cross-pollination. These are as follows:

1. Dicliny: The plants are unisexual.
   (a) Monoecious: The male and female flowers/inflorescence are borne on different nodes of the same plant e.g., cucurbits, maize, castor etc.
   (b) Dioecious: The male and female flower/inflorescence is borne on different plants. Field crops belonging to this class with hybrids are limited (e.g., spinach).
2. Dichogamy: The anther dehiscence and stigma receptivity occur at a different time that favours cross-pollination. Non-synchronization of male and female flowers may vary from one to a few days.
   (a) Protoandry: The anthers dehisce before the stigma becomes receptive, e.g. maize, castor, sunflower.
   (b) Protogyny: The stigma becomes receptive before the dehiscence of anther, e.g. pearl millet, Indian mustard, onion, cauliflower, etc.
3. Herkogamy: The stigma is covered with a waxy layer which does not become receptive until the waxy membrane is removed by honeybees resulting in cross-pollination, e.g. lucerne and alfa-alfa.
4. Heterostyly: The flowers have styles of different lengths (long, medium, short, pseudo-short), wherein the stylar length determines the outcrossing percentage, e.g. brinjal (Fig. 1).
5. Male sterility: Absence/atrophy/misformed/malformed male sex organ (stamen) or absence of functional pollen grains in a complete flower that does not allow self-fertilization.
6. Self-incompatibility: Failure of pollens to fertilize the ovule of the same flower, or that of other flowers on the same plant, e.g. *Nicotiana* and *Brassica.*

2 Genetic Principles in Hybrid Seed Production

Hybrid seed production requires a female plant in which viable male gametes are absent naturally or removed by artificial means. Hand emasculation is done to make a plant devoid of pollen so that it can be used as a female parent. Another simple way to use a female line for hybrid seed production is to identify or create a line that is incapable to produce viable pollen. This is called a male sterile line.

Fig. 1 Flower types in brinjal (long, medium and short style; from bottom to top)

2.1 Male Sterility

Male sterility prevents self-pollination, facilitates cross-pollination and promotes heterozygosity. Male sterility is exploited in agricultural crop plants for hybrid seed production. Male sterility is of three types: genetic, cytoplasm and cytoplasmic-genetic male sterility.

2.1.1 Genetic Male Sterility (GMS)

Male sterility is controlled by mutations in nuclear genes that affect stamen and pollen development. It can be controlled either by dominant/recessive genes. A male sterile line is maintained by crossing a male sterile line with a heterozygous male fertile line. Genic male sterility-based hybrids are available in safflower and pigeon pea. A GMS line (A-line) is maintained by backcrossing with the heterozygous B-lines (maintainer line). The A-line (seed parent) has 50% fertile and 50% male sterile plants. In hybrid seed production plot using GMS system therefore, it is required to rouge out 50% male-fertile plants. Seed and seedling markers that are closely linked to male sterility genes in the A-line can help to identify off-types and remove those male fertile plants from the field before flowering. In vegetable crops, GMS has been commercially used for hybrid production in muskmelon.

2.1.2 Cytoplasmic Male Sterility (CMS)

Cytoplasmic male sterility (CMS) is governed by extra-nuclear genes mainly present in the mitochondrial genome. These show non-Mendelian inheritance pattern and are under the regulation of cytoplasmic factors. In the majority of the cole crops in

Fig. 2 Flowers in CMS (**a**) and male fertile (**b**) parent in cauliflower

Brassicace family, cytoplasmic male sterility has been used commercially for hybrid seed production (Fig. 2).

2.1.3 Cytoplasmic-Genetic Male Sterility (CGMS)

Male sterility is controlled by an extra-nuclear genome and often nuclear genes restore fertility in the hybrid plants. The male sterility is controlled by both the nuclear and cytoplasmic genes. In cytoplasmic-genetic male sterility, restoration of female fertility is undertaken using restorer lines carrying nuclear restorer genes in the crop. The male sterile line is maintained by crossing with a maintainer line that has the same genome as that of the MS line but carries normal (N) fertile cytoplasm. The fertility restoration is done by fertility restorer genes (Rf). The Rf genes do not have any expression of their own unless the sterile cytoplasm is present. The Rf genes are required to restore fertility in S cytoplasm which causes sterility. Thus N cytoplasm is always fertile and sterile cytoplasm with Rf-- gene produces male fertile plants; while S cytoplasm with rfrf genes produces only male-sterile plants. Another feature of these systems is that Rf mutations (i.e. the mutations to rf or no fertility restoration) are frequent, so N cytoplasm with Rfrf is the best for stable fertility. Cytoplasmic-genetic male sterility systems are widely exploited in both field and vegetable crop plants for hybrid development due to the convenience to control the sterility expression by manipulating the nuclear gene–cytoplasm combinations in any selected genotype. Incorporation of these systems for induction of sterility evades the need of emasculation thus facilitating the production of hybrid seed under natural conditions.

2.2 Self-Incompatibility

Self-incompatibility (SI) is a mechanism that prevents self-fertilization through recognition of self (own) pollen on stigma on the flower in the same plant or that of other plants of the same genotype. But pollen from other plants carried by wind, insects and other vectors deposit on stigma of such flowers and set seeds. Therefore, self-incompatibility prevents self-fertilization and facilitate cross-fertilization. SI is observed in both hermaphrodite and homomorphic flowers. The self-incompatibility response is genetically controlled by one or more multi-allelic loci and relies on a series of complex cellular interactions between the self-incompatible pollen and pistil.

Besides the use of male sterility and self-incompatibility systems, the following methods/systems are also followed for effective hybrid seed production in both field and vegetable crops.

2.3 Emasculation and Pollination

The male flowers are pinched off in the female lines a day before anthesis, or the stamens are manually removed from a bisexual flower in a female plant before flower opening (anthesis). This system is feasible when the male and female parts of a single flower or plants are separate. This is practised in bisexual perfect flowers where the androecium could be removed easily. In the female parent, the anther column is removed from a bisexual flower, the process called emasculation, and pollen of desired male line is dusted manually on the stigma of the emasculated flower in female parent to facilitate pollination and fertilization. This technique is commercially feasible in crops which have large, conspicuous flowers, easy removal of stamens/anthers, high seed set rate per pollination, low seed rate/ha and higher cost of hybrid seed, e.g. cotton, tomato, brinjal, chilli, melon, etc. This technique requires trained labour for emasculation, pinching, bagging, pollen collection and pollination adding to the cost of hybrid seed. It is, therefore, vital to know the floral biology, flowering time, crop morphology, and synchronization of flowering in the parental lines to plan emasculation and pollination in seed parent. The male and female rows are grown in recommended row ratios (male:female) or blocks. The fruit set on female lines is harvested for hybrid seed extraction.

2.4 Use of Gynoecious Sex Form

The gynoecious sex form has been commercially exploited for hybrid seed production in cucurbits. For hybrid seed production of cucumber, sponge gourd, bitter gourd and musk melon, the female and male rows are planted in a specific row ratio of 4:1 in the northern states of India under favourable climatic conditions to achieve high seed yield. The female parent bears only pistillate flowers and pollination is accomplished by insects (honeybee and wasp). To ensure good fruit, seed set and seed recovery, a sufficient population of the honeybee is maintained at the boundary of seed production plots. The male parent line is maintained by selfing (mixed pollination) and rouging out undesirable plants before contamination may take place. The female lines, i.e. gynoecious lines are maintained by inducing the staminate flowers with the application of silver nitrate (200 ppm) at two to four true leaf stages and followed by selfing. The weather conditions at seed production

Fig. 3 Pistillate flowers in a gynoecious line of cucumber

location play an important role, as the gynoecious lines are unstable under high temperature and long photoperiod conditions (Hormuzdi and More 1989). For this reason, the gynoecious cucumber did not become popular in tropical countries. However, a few true-breeding tropical gynoecious lines in cucumber and musk-melon have been developed (Fig. 3). These homozygous gynoecious lines are maintained by applying GA_3 at 1500 ppm or silver nitrate at 200-300 ppm or sodium thiosulphate at 400 ppm to induce staminate flowers at two and four true leaf stage. Homozygous lines are planted in strict field isolation. The gynoecious lines are crossed with a monoecious male parent to produce the F_1 hybrid.

2.5 Use of Chemicals and Growth Regulators

The hybrid seed can be produced by inducing femaleness and maleness with the application of chemicals and growth regulators. Spraying of etherel (2-choloro-ethyl-phosphonic acid) at 200–300 ppm at two and four true leaf stages and flowering is effective in inducing the pistillate flowers successively in the first few nodes on the female parent in bottle gourd, pumpkin, and squash, which are employed in hybrid seed production.

2.5.1 Sex Modification through Hormones and Chemicals

Though the sex expression in dioecious and monoecious plants is genetically determined, it can be modified to a considerable extent by environmental and introduced factors such as mineral nutrition, photoperiod, temperature and phytohormones. Amongst these, phytohormones are the most effective agents for sex modification and their role in the regulation of sex expression in flowering plants has been documented. The morphological differences in various sex types and their specific metabolic characteristics result from the possession of specific patterns of proteins, enzymes and other molecules. Modification of sex expression in cucurbits has been induced both by changing the environmental conditions and by applying treatments with growth regulators. Auxin treatment increases femaleness while gibberellins cause a shift towards maleness. Application of plant growth regulators is reported to alter sex expression and flower sequence in cucurbits when applied at the two to four true leaf stages, the critical stage at which a particular sex type can be suppressed or encouraged (Hossain et al. 2006).

Chemicals inducing femaleness:

- Auxins – Naphthaleneacetic acid (NAA), Etherel, Ethephon, Maleic hydrazide. Cytokinins, Brassinosteroids.

Chemicals inducing maleness:

- Silver nitrate ($AgNO_3$), Abscisic acid (ABA), Gibberellic acid (GA_3), Thioporpinic acid, Phthalimide, Paclobutrazol, etc.

In cucumber, $AgNO_3$ was found to be a potent inhibitor of ethylene action leading to femaleness. It should be sprayed when two to three true leaves are fully expanded. Gibberellic acid spray leads to excessive elongation and weakening of plants and there will be an increased number of malformed male flowers with less pollen. In gynoecious cucumber, there will be an increased number of male flowers on the vine when sprayed with silver nitrate or gibberellic acid, which made it possible for the multiplication of gynoecious lines in hybrid seed production. The sex ratio could be increased by the application of plant growth regulators like etherel or ethephon, gibberellic acid, naphthalene acetic acid and maleic hydrazide (Shailendra et al. 2015; Shiva et al. 2019).

2.6 Manipulation of Environment for Sex Modification in Hybrid Seed Production

The environment has a strong influence on sex modification. The role of environmental conditions in hybrid seed production and maintenance of the parental lines are described below.

2.6.1 Rice

The expression of male sterility and its restoration in rice is influenced by environment-sensitive genic male sterility (EGMS). It has been further classified into photosensitive and thermosensitive genic male sterility genes. The hybrids developed using these systems are called two-line hybrids, as no maintainer is required for the multiplication of the female line. The EGMS lines are multiplied by growing these in a season or a location in which the flowering period coincides with the required sterility/fertility change. For example, temperature-sensitive genic male sterility (TGMS) lines change to fertility at lower temperatures and the most ideal regime to induce a higher level of fertility is 27/21 °C. Therefore, in such cases, the TGMS lines need to be planted in such a way that the crop is at a fertility-inducing stage (say 5–20 days after planting) when favourable temperatures are prevailing. Similarly, the sterility-inducing stage coincides with a photoperiod of more than 13.75 h and temperatures above 32/34 °C. The hybrid seed production following this system depends on the critical fertility-inducing factors and their duration.

2.6.2 Castor

Castor is a monoecious plant species with staminate flowers and pistillate flowers located at different positions in a raceme (Fig. 4a). There are genotypes with a predominantly higher proportion of pistillate flowers governed by both genetic and environmental conditions (Fig. 4b). Low temperature promotes pistillate plants in particular genotype that reverts to monoecism with an increase in temperature and higher order of branches. Maximum female sex expression is seen when the daily temperature during raceme formation and development is less than 30 °C. It is also very high at the early growth stages of the female line in a higher soil nutrient condition. Female lines are multiplied at higher temperature condition that induces temperature-sensitive interspersed staminate flowers (ISF) and hybrid seed production is taken up at relatively low-temperature condition in which the ISF is not formed in a pistillate line. Therefore, two-line hybrid seed production has been possible in castor.

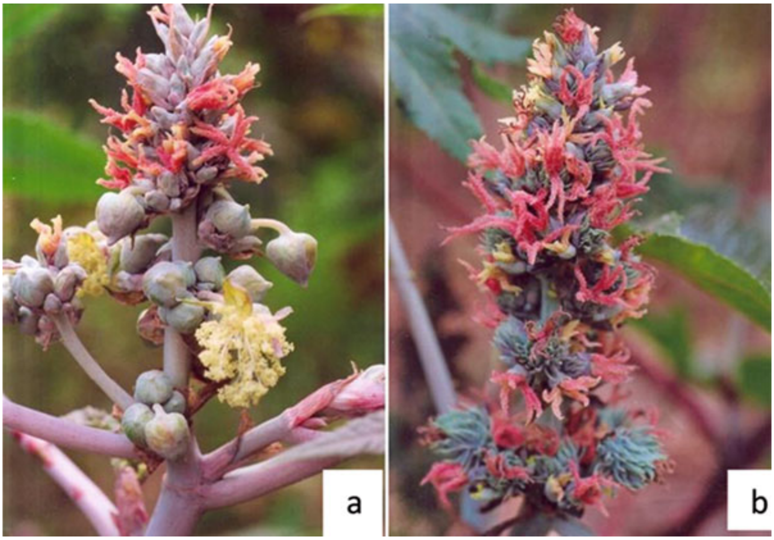

Fig. 4 Sex types in castor (**a** monoecious; **b** pistillate)

Male sex expression of several plant species is favoured at high temperatures and female sex expression at low temperatures. In tomatoes, male-sterile mutants develop male-sterile flowers at a temperature of above 30 °C and normal flowers at lower temperatures. In Brussels sprouts, low temperature affects the development of the androecium. In onion, the male-sterile plant produces viable pollen above 20 °C. In cucumber, high temperature and long day length (>14 h) favour male flowers.

3 Agronomic Principles of Hybrid Seed Production

3.1 Environmental Requirements

Optimum growing season, conditions, and location are critical in obtaining good yield and quality of hybrid seeds. The regions with abundant sunshine are preferred for seed production. Unless the parental lines are specifically sensitive to a particular temperature and photoperiod for flowering and male sterility expressions, such as rice and castor, the hybrid seed crops can be raised in conditions favourable for the species. Sunshine hours are kept in planning in case of photoperiod-sensitive crops like lettuce and spinach, which require long-day conditions for flowering and seed set. Some species, on the other hand, require a low temperature to promote flowering (vernalization). Many temperate vegetables like cabbage, cauliflower, beetroot, European type radish and carrot need vernalization. High rainfall areas are not suitable for hybrid seed production due to the adverse effect not only on pollination but also on seed viability and vigour, whereas excessive wind speed may hamper the activity of pollinators, carry wind-borne foreign pollen from long distances resulting in contamination and cause seed shattering.

3.2 Land Requirement

The field selected for raising a hybrid seed crop should be free from ‘volunteer’ plants. Volunteers mean the plants originating from the seed/plant material of the previous commercial or seed crop. In vegetables, volunteer plants are seen in spinach, tomato, etc. The land should be levelled with proper drainage and should have sufficient organic matter in it. The cultural operations of hybrid seed production are similar to the production of open-pollinated varieties.

3.3 Isolation Distance

The spatial separation of the plots of hybrid seed production from any kind of contaminant is a critical requirement. Appropriate distances are to be maintained from other hybrids of the same crop, same hybrid seed production field not conforming to genetic purity standard, other related species, which may cross-pollinate, and fields affected by designated diseases to prevent genetic and disease contamination. Proper isolation standards, as required for a given species, are followed at all stages of maintenance and seed production to maintain the genetic purity of the hybrid. Isolations can be maintained in terms of distance or time, or a combination of both. For time isolation between seed production plots, information on the number of days to flowering of the parental lines should be known, and the planting schedule must adhere to this information. Two fields of hybrids may be planted without maintaining safe spatial isolation, provided an appropriate time interval has been kept between the two sowings, so that the pollination in the first planted field would be over before flowering/pollination starts in the second field or vice versa. The off-types, i.e. very late or early flowering plants should be removed/ discarded from the field to ensure genetic purity.

The isolation distances for hybrid seed production are recommended based on the flowering behaviour of the crop, the movement pattern of its pollen and the medium of its dispersal viz., wind, insect, etc. Some examples of isolation distances in the field and vegetable crops as per the Indian seed regulation (Annonymous 2013) are given in Table 1.

The mode of pollination determines the isolation distance to be maintained. In the case of self-pollinated species the isolation distance is relatively less, but in the case of cross-pollinated species relatively longer distances are maintained from other varieties. The isolation distance also depends on the direction of insect flight or of winds, which have a direct influence on the pollen movement.

Isolation by time allows seeds of different varieties of the same crop to be produced in nearby fields. If the season is long enough to allow two production cycles, then two cross-compatible hybrids can be isolated by time. In certain cases, a barrier crop is raised between the fields of two cross-compatible fields to minimize contamination.

4 Stigma Receptivity

The stigma of the flower is the organ on which pollen lands to facilitate fertilization and consequently seed formation. Variations in the stigma morphology support pollen germination and tube growth in the compatible pollen. Longer stigma receptivity in cytoplasmic male sterile (CMS) lines is a desirable trait that favours higher hybrid seed yield. It is important both for manual or natural pollination by vectors like wind, insects, etc. Initiation of stigma receptivity is reported to be highly variable across the plant species and genotypes. Generally, stigma becomes receptive at the time of flower opening (anthesis). In the case of CMS, the protogynous and protandrous type stigma becomes receptive one or few days before anthesis and is extended by a few more days afterwards (Lloyd and Webb 1986). The duration of stigma receptivity is usually longer in CMS lines than in their fertile counterparts. The stigma receptivity in CMS lines of rice, for instance, lasts for up to 4 days (Gupta et al. 2015). In *Brassica* spp. CMS lines with *Moricandia* cytoplasm, the stigma receptivity was recorded up to 6 days after anthesis (Chakrabarty et al. 2007). In another study, stigma of CMS lines of *B. juncea* was receptive for 6–8 days after

Table 1 Minimum isolation requirements for foundation seed of the parental line and certified seed of hybrids of some field crops and vegetables

S. No.	Crop	Isolation distance (m) FS@	CS#	Remarks
1.	Rice	200	100	Barrier crops may be grown to further minimize the pollen flow
2.	Maize	400* 600** 400$	200* 300** 5***	*Same kernel colour and texture; same or different hybrid not conforming to varietal purity **Different kernel colour and texture ***with common male parent $Not conforming to varietal purity requirements
3.	Sorghum	300* 400**	200* 400** 5***	*Other variety; same and other hybrid not conforming to varietal purity **Johnson grass, forage sorghum ***with common male parent
4.	Pearl millet	1000*	200* 5**	*Other varieties; same and other hybrid not conforming to varietal purity requirements for certification **with common male parent
5.	Rapeseed mustard	200* 100**	50* 50**	*Self-compatible **Self-incompatible
6.	Castor	600	300	–
7.	Cotton	50* 5**	30* 5**	*Other varieties of same species; same variety not conforming to varietal purity **Other varieties of different species; blocks of parental lines of same hybrids
8.	Tomato	50	25	–
9.	Brinjal	300	150	–
10.	Cauliflower, cabbage, beetroot, radish, turnip	1600	1000	–
11.	Bottle gourd, muskmelon, watermelon, sponge gourd, bitter gourd, pumpkin	1000	500	–

@FS foundation seed of parental lines, *#CS* certified seed of hybrids

anthesis (Mankar et al. 2007). In protogynous lines of *B. juncea*, the duration of maximum stigma receptivity was reported to be up to 3 days after stigma protrusion (Chakrabarty et al. 2011), though the seed set was observed up to 10 days after stigma protrusion. In comparison to the CMS and protogyny systems, the open-pollinated varieties showed stigma receptivity up to 4 days after anthesis(Maity et al. 2019).

In rice, the stigma remained receptive for six to seven days without pollination, with its maximum receptivity up to three days after the opening of the spikelet. In pearl millet CMS line ‘Tift 23A’ higher seed set was observed when pollinated 2–3 days before anthesis than the open-pollination after anthesis (Burton 1966). In sorghum, stigma becomes receptive one to two days before blooming and unpollinated inflorescence remains receptive up to a week or more (Ayyangar and Rao 1931; Maundar and Sharp 1963). In pigeon pea, stigma becomes receptive 68 h before anthesis and continues for 20 h (Prasad et al. 1977). In castor, pistillate flower retains receptivity for five to six days. In safflower, stigma remains receptive for 72 h. However, the duration of stigma receptivity is much influenced by the climatic conditions during flowering.

Duration of stigma receptivity showed variation in Cucurbitaceae family. Stigma remained receptive in *Luffa* species from 6 h before to 12 h after flower opening (Singh 1957). Stigma was more receptive at 3–4 h after anthesis for pollination in melons (Tarbaeva 1960). Nandpuri and Brar (1966) observed that the maximum stigma receptivity prevailed 2 h before anthesis and 2–3 h after anthesis in the case of muskmelon. Stigma is receptive 36 h before anthesis and remained so until 60 h after anthesis (Nandpuri and Singh 1967) in bottle gourd. Nepi and Pacini (1993) reported that the stigma became receptive 1 day before anthesis and remained receptive for 2 days in *Cucurbita pepo*. Stigma remained receptive for 24 h before and after anthesis in bitter gourd (Miniraj et al. 1993).

5 Pollen Viability

Pollen maturation to its release and dispersal, germination on a receptive stigma and fertilization are essential steps to plan the timing for hybridization for successful hybrid seed production. The response of pollen during its mobile phase, i.e. after its release and detachment from the anther of the pollen parent, is also important as pollen passes through different environmental conditions. The duration of pollen viability varies greatly among crop species and varies from few minutes to hours (Stanley and Linskens 1974; Shivanna and Johri 1985; Barnabas and Kovacs 1997). In cereals (*Poaceae*) pollen grains lose viability within 20–30 min from dehiscence. In rice, for example, the pollen grain loses its viability within 10 min due to prevailing high temperature that leads to pollen desiccation. In some species of Solanaceae and Fabaceae, pollen viability is reported for up to several weeks. A favourable environment needs to be identified for hybrid seed production. Pollen viability/longevity depends on several climatic factors like temperature, humidity, etc. as well as pollen vigour that might be influenced by the nutrition and disease status of the plant. Low atmospheric humidity (0 ± 40%) is favourable for longer pollen viability. In maize, pollen grain remains viable for a few minutes after its dehiscence. Pollen in sorghum remains viable for three to six hours in anther and the viability gets over within 20 min after its detachment from anther. In cotton, pollen remains viable for up to 24 h. However, pollen collected from younger buds showed viability for 44 h or more. Similarly, castor pollen remains viable for one day after anthesis.

Knowledge of both stigma receptivity and duration of pollen viability is important for successful hybrid seed production both in open fields and under protected conditions. If pollen viability can be prolonged with proper drying under vacuum and storing in a sealed container at 4–5 °C, the pollen parent may not be grown each year and stored pollen under controlled conditions could be used for hybrid seed production.

6 Pollination Control

Pollen dispersal and its transfer to the seed parent are very important steps in hybrid seed production. Usually, the two parental lines used for hybrid seed production are different in their morphological traits, including differential flowering time. The parental lines should flower simultaneously and follow similar flowering patterns so that there is effective pollination, followed by fertilization and seed set. The pollination/crossing is achieved either by manual operation or by allowing natural pollination by the wind and/or insects depending on the flower type and pollen characteristics.

7 Synchronization of Flowering

Failure to achieve proper synchronization of flowering between the parental lines is the most commonly encountered problem in hybrid seed production, resulting in very poor or no seed set. Hence, synchronization of flowering of the parental lines is a prerequisite for successful hybrid seed production. This is because the seed set on the female parent depends on the availability of viable pollen supplied by the male parent during the flowering period, while the stigma is receptive. Synchronization of flowering means that the seed parent and pollen parent flowers simultaneously. In general, parental lines of most of the hybrids across the crop species differ in their growth duration and consequently flowering. Therefore, it is essential to determine the flowering behaviour in terms of its initiation, peak flowering, termination and thereby flowering duration to take up an appropriate seeding/sowing plan. This helps achieve synchronized flowering between the parental lines involved in hybrid seed production. It is observed that the seed parent should flower a day or two earlier than the male parent but not vice versa as the seed parent, particularly with the cytoplasmic male sterility, protogyny, and protandry systems, shows longer stigma receptivity duration. The pollen parent usually flowers profusely but terminates quickly, particularly in plants with non-branching and determinate growth habits. Knowledge about the flowering pattern of the parental lines/combinations in particular agro-ecological and seasonal conditions help in planning the sowing of the parental lines for hybrid seed production. Achieving synchronized flowering through proper seeding intervals, staggered sowing, seed treatment, spraying of chemicals, fertilizers and other agronomic interventions are possible only with prior knowledge of the flowering behaviour of the parental lines for hybrid seed production. The seed parent with CMS, protogyny, and protandry systems having an extended flowering period, with insufficient pollen availability will result in a low seed set. To supply an adequate quantity of pollen during the flowering of the seed parent, staggered sowing may be followed in the pollen parent at an interval of 3–5 days.

Following are some measures practised for achieving synchronization of flowering during hybrid seed production of different crops:

7.1 Rice

1. Staggered sowing: In general, the parental lines involved in hybrid development differ in respect of flowering time and duration due to their growth and development. Therefore, it is necessary to determine the seeding intervals between the parental lines and adopt staggered sowing to ensure synchronized flowering.
2. Fertilizer application: Young panicle development is compared by observing the primordium development of the parental lines 30 days after transplanting. The primordium development stages are indicators of the flowering of parental lines (Yuan 1985). Following this principle, it has been suggested that during the first three stages of panicle differentiation, the early parent is applied with a quick release of nitrogenous fertilizer (spray of urea at 2% delays flowering of the early parent) or spray the later developing parent with potassium di-hydrogen phosphate at 1.5%. This adjusts developmental differences up to 4 to 5 days.
3. Water management: In case a difference in the development of panicles is observed during later stages draining water from the field will delay early parent panicle development while higher standing water will speed panicle development to the late parent (Feng 1984; Xu and Li 1988). Restorer lines are more sensitive to water management.

7.2 Sorghum

1. Staggered sowings depending on plant growth, development, and flowering time and duration in the parental lines need to adopted (Murthy et al. 1994).
2. The advancing parent needs to be sprayed with 500 mg of Maleic hydrazide in 1 L of water, 45 days after sowing.
3. Urea (1%) solution can be sprayed on late parent (House 1985; Kannababu et al. 2002).
4. One irrigation may be skipped for the advancing flowering.
5. Spraying CCC (Chloro Chlorine Chloride) at 300 ppm for delaying flowering.

7.3 Pearl Millet

1. Staggered sowing of parental line.
2. Urea solution (1%) can be sprayed on late parental line.
3. Jerking of early parent delays the flowering.
4. Additional nitrogen fertilization or foliar spray to the late parent (Govila and Singhal 2003).

7.4 Sunflower

1. There should not be more than 3–4 days difference in the flowering to avoid staggered sowing problems.
2. Seed treatment with simple hydration of the female parent and with GA_3 at 50 ppm to the male parent for 18 h before sowing and spraying urea (1%) thrice on alternate days at the button formation stage to the male parent resulted in a good degree of synchrony in flowering between the parental lines in the hybrid, namely, KBSH-1 and TCSH-1 under North Indian conditions (Chakrabarty et al. 2005; Chakrabarty 2008).

7.5 Cauliflower

1. Staggered sowing of parental line.
   (a) Application of GA_3 at 100 ppm and IAA at 50 ppm thrice (at the initiation of bolting, 7 days after the first spray, and at the bud initiation stage) was found effective in achieving synchronization of flowering between the parental lines of cauliflower hybrids (Personal communication, S. K. Chakrabarty, unpublished).

8 Planting Ratio

Hybrid seed production depends on the seed set on the female plants out of the total area planted for hybrid seed production. Therefore, a higher hybrid seed yield is expected with a higher proportion of female (seed parent) population, in terms of rows/blocks. But it, in turn, depends on the pollen produced and supplied by the male parent population/rows. Assuming a good pollen producer, it is also critical to have information about its pollen dispersal ability and pollen viability till it lands on the stigma for effective pollination and fertilization. Therefore, the planting ratio/row ratio is one of the important factors that determine the hybrid seed yield. To estimate the optimum row/planting ratio systematic experiments are conducted on each crop and with different parental combinations. The role of pollinators and other vectors like wind play important roles in determining the planting ratio. The distance between the seed parent and pollen parent also becomes an important factor in

Table 2 Recommended planting/row ratio for hybrid seed production

Crop	Pollen parent:seed parent	References
Rice	2:8;2:10	Viraktathamath et al. 2003
Maize	1:2; 1:3; 1:4 or 2:4; 2:6; 2:8	Mac Robert et al. 2014; Sharma et al. 2020
Sorghum and pearl millet	2:6	Singhal and Rana 2003; Govila and Singhal 2003
Cotton	1:4; 1:5	Lather and Singh 1997
Sunflower	1:3; 2:6 or block planting of the male and female parent	KempeGowda and Kallappa 1992; Ranganatha et al. 2003
Pigeon pea	1:3	Tikle et al. 2014
Rapeseed and mustard	2:8	Maity et al. 2012
Castor	1:3;1:4	Chakrabarty 2003
Cucumber, sponge gourd, pumpkin	1:3; 1:4	Sharma et al. 2004
Tomato, brinjal, Chilli	1:3; block planting of the male parent	–

hybrid seed production plots, particularly in a predominantly self-pollinated crop like rice as natural wind is helpful to a great extent in increasing seed set. Planting geometry is also important in wind-pollinated hybrid seed production plots. Planting the seed/pollen parent in rows perpendicular to the wind direction is the principle in case of wind as pollen vector.

Lal and Singh (1990) observed that 2:1 (female:male) ratio was optimum for hybrid seed production in muskmelon. Soto et al. (1995) compared manual pollination and open pollination for the production of the parental lines with a female:male ratio of 3:1, 6:1 and 9:1 in cucumber and they reported that 6:1 ratio was economic for hybrid seed production. Kushwaha and Pandey (1998) recommended a planting ratio of 4:1 for hybrid seed production in bottle gourd. Sharma et al. (2004) reported a 3:1 ratio for hybrid seed production of cucumber. Satish Kumar (2005) reported that there was no significant difference among planting ratios compared for seed yield and its attributes in bitter gourd hybrid seed production. Recommended planting/row ratio for hybrid seed production is given in Table 2. Hybrid seed production plots in maize and cauliflower are shown in Figs. 5 and 6, respectively.

9 Supplementary Pollination

Supplementary pollination is a method to ensure adequate pollination of the seed parent by physical or mechanical methods or by maintaining an abundant vector population. It is achieved by different practices as per the flowering behaviour and flower morphology of the crop. For instance, it can be performed manually by rubbing the capitulum of the sunflower with desired pollens; shaking or jerking the rice inflorescence (panicle) with bamboo sticks or pulling a rope through the

Fig. 5 Field layout in hybrid maize seed production

Fig. 6 Seed production of CMS-based cauliflower hybrid with a planting ratio 1:3 (M: F)

rows of the male parent. Supplementary pollination has to be done for 7–10 days during flowering. Time, duration and frequency of supplementary pollinations are important for higher seed settings in the seed parent. In rice, for example, the first supplementary pollination is performed in the morning during the initiation of spikelet opening. This process is then repeated 4–5 times in a day at an interval of

30 min till the completion of flowering in both the parental lines. In the case of cross-pollinated crops, insects are the primary pollinators. In such cases, supplementary pollination by honey bees or bumblebees results in higher hybrid seed set and yield. Keeping beehives in hybrid seed production plots coinciding with flowering is recommended for realizing higher quantity and better quality of hybrid seed in sunflower, rapeseed, etc.

Vegetable crops viz., tomato, garden pea, fenugreek and cowpea are primarily self-pollinated, whereas, other vegetable crops like onion, carrot, cole crops, cucurbits and *Brassica* are cross-pollinated in nature. The natural insect population (honey bees/solitary/bumble bees, wasp, butterflies, moths, beetles, flies) is normally sufficient under open conditions to ensure satisfactory pollination, but with high plant populations maintained in seed production plots, there is a possibility that the natural insect population may be insufficient to ensure proper seed set. Therefore, the introduction of supplementary bee hives improves pollination and seed set. However, care must be taken to ensure that pest protection chemicals are not used in a way to harm useful pollinating insects. The spray of chemicals should be avoided at peak pollination period to support insect activity and should be done only in the late afternoon.

10 Roguing

A quality seed must be free from any genetic or physical admixtures. To ensure high genetic and physical purity, it is important to have proper isolation and regular removal of off-types and volunteer plants from the female and male/restorer parental lines at all growth stages. Rouging is the removal of undesirable plants from seed production plots, which helps prevent further genetic contamination from off-types (plants of the same species but different genotypes) by cross-pollination. The undesirable plants may be volunteer plants from the previous crop, off-types produced by the out-crossing with contaminants and admixtures during the process of harvesting, threshing, packing and handling, or the presence of ‘pollen shedders’ a term used for male fertile plants in the seed parent population.

Rogues or off-types may occur in a crop due to any of the following reasons.

- The diversity of the morphological types within a crop may be wide. This tendency is greater in predominantly cross-pollinated (e.g. cauliflower, cabbage, cucurbits and onion) than self-pollinated (e.g. peas, tomato, fenugreek) crops.
- Some plants may display deviation from the normal type due to developmental variation.
- Volunteer plants may arise from vegetative pieces or dormant seed of the previous crop grown in the same field.

The timely and careful removal of all off-type plants in both female and male lines ensures the high purity of hybrid seed. Plants having seed-transmittable diseases, in either the female or male parent population, are also to be removed at the early stage to maintain good seed health.

Vegetable varieties often show genetic variability in morphological traits after growing over several generations. It is, therefore, necessary to exert control and keep the natural variation within acceptable limits by inspecting the crops at different growth stages and removing individual plants which do not conform to the defined limits of that variety.

It is always easier to conduct intensive rouging in breeder seed plots than in large commercial seed production plots. Hence, maintaining the highest genetic purity of the parental line seed during their multiplication at the pre-basic, breeder (equivalent to basic of OECD) and foundation (equivalent to Certified 1 of OECD) seed stage is as important as the following roguing in the hybrid seed production plots.

Variety description based on morphological characteristics like leaf shape, flower colour, fruit shape and colour generally form the basis for rouging, though some of these like leaf colour, plant height, and earliness of flower initiation are known to be influenced by environmental conditions.

11 Harvesting, Threshing and Seed Extraction

The best time for harvesting seed crops is at a stage when the highest yield of the best quality seed can be obtained. The seed of various field and vegetable crops are extracted from dry seed heads, dry fruits or from fruits (as in many vegetables) in which the seeds are wet at the time of extraction. Harvesting of the male parent should be done before the female (seed) parent. Threshing can be done by hand or by machines. Care is taken while transporting material from the field to the threshing floor. Both the trolley and the threshing floor are cleaned of any seed/ plant parts to avoid genetic admixture or contamination by weed seeds at this stage. Threshing machines should also be properly cleaned to avoid any admixture.

12 Seed Drying

At the time of maturity, the seed contains higher moisture content than the optimum for better germination and storability. Seeds from pulpy fruits like tomato, watermelon, muskmelon, cucumber and brinjal have very high moisture content at harvest and absorb more during wet extraction. Seeds of vegetables like onion, carrot, *Brassica*, etc. have relatively low moisture at harvest, but in drier climates may be prone to shattering loss. Sometimes, the seed may also have high moisture content due to adverse climatic conditions such as pre-harvest showers. Therefore, to reduce seed moisture to the optimum level, threshed and cleaned seeds are dried before storage. For ambient storage, seed moisture should be kept under 9–12% and for sealed packaging and storage it should be 6–8%. Natural and artificial methods of forced drying may be used for this purpose.

The hybrid seed production technologies in maize, castor and cotton under open field conditions and that of tomato and bitter gourd under protected conditions are described here.

13 Hybrid Seed Production in Maize (*Zea mays* L.)

Maize hybrids are derived from inbreds that are resulted from repeated inbreeding of specific maize population. Hybrids have early plant maturity, better disease resistance, food processing and nutritional quality. Based on the number and type of parents involved in a cross, maize hybrids can be classified as (i) single-cross, (ii) three-way, (iii) double-cross, and (iv) top-cross. Crossing between two inbred lines results in a single-cross hybrid, whereas crossing a single-cross hybrid with inbred line results in a three-way hybrid. Double-cross hybrid is derived by crossing two single-cross hybrids. To produce a top-cross hybrid, an inbred is crossed with an open-pollinated variety. Among all types of hybrids, single-cross hybrids are inherently more heterotic and uniform than any other form of hybrid.

For the production of the quality seed of a single cross maize hybrid, the respective parental lines are multiplied in isolation in the breeder and foundation seed stage and the single cross is produced in the certified seed production stage. In a hybrid seed production plot, male and female parents of foundation seed are planted at consecutive rows in the seed production plot. Depending on the pollen dispersal capacity of the pollen parent and the nature of the seed parent, the ratio of male and female rows can vary considerably. The tassel of the female plant is removed before initiation of pollen shedding so that the pollen of the male parent is available in the seed production plot. Detasseling of the female is necessary to avoid ‘female-selfing’. Mechanical removal of tassels from the female parent before dehiscence is called detasseling. Tassels can be removed manually or mechanically. In a CMS-based hybrid system, detasseling is not required as female lines possess male-sterile cytoplasm.

The management of both the parents is important and requires adequate attention during seed production. The key factors that determine a successful quality hybrid seed production are as follows:

- Purity of the female and male parents.
- The ratio of female to male parents in the seed production field.
- Timely removal of off-types, diseased and rogues to prevent contamination.
- Detasseling of the female parent at an appropriate time.
- Synchrony of silking in female and pollen shedding in male parents.
- Careful and separate harvesting from the female and male parent rows to avoid mechanical seed mixtures.

13.1 Selection of Area

The area for seed production should have a similar climatic condition (temperature and photoperiod) as that of the area where the variety is intended to be recommended for cultivation. The area should have mild and dry weather with abundant sunshine. During seed production especially flowering, temperature, R.H., and wind velocity should range between 25 and 30 °C, 60–70%, and 2–4 km/h, respectively. Rain-free season (post rainy) with good irrigation facility is recommended for seed production as it favours better seed set and lesser incidence of diseases and insect problems. Areas prone to excess rains, high humidity, extreme temperature, strong winds and hail storm should be avoided.

13.2 Field Selection

The field selected for seed production should be well-drained, levelled with fertile soil preferably sandy loam to loamy soils as maize is very sensitive to water logging and drought. In addition, should be away from commercial crop and related crop species, free from pest and disease incidence, volunteer plants, weed seeds, off-types, diseased plants, soil-borne pathogens. The field should not have maize in the previous season.

13.3 Isolation

Maize is a cross-pollinated crop and proper isolation distance from other maize fields is required to produce genetically pure seed. Following measures are generally taken to maintain purity during seed production. Seed plots must be temporaly or spatially isolated to avoid contamination during the flowering by wind-borne pollen from neighbouring fields. Temporal isolation is achieved when pollen shedding of the male parent and silking of the female parent is either early or later (15 days) from the flowering of other maize fields in the vicinity. The minimum standard for isolating maize seed production fields is often established by national seed regulatory agencies taking account of factors like pollen count, wind speed and direction, pollen dispersal, and insect activity. Minimum isolation distance ranges from 200 m to 600 m.

13.3.1 Spatial Isolation

Pollen from other sources is excluded by maintaining physical distance of the seed production plot from the contaminating fields. Isolation distance of 400 m is the minimum seed certification standards for maize seed production. Component isolation required for different types of hybrids may vary from 5 to 7 depending on the number of parent involved in the hybrid (Table 3).

Table 3 Number of isolations required for hybrid maize seed production

S. No.	Type	Combinations	Parents	Breeder seed	Foundation seed	Certified seed	Total
1.	Single-cross	Inbred x inbred	2	2	2	1	5
2.	Three-way	F_1 × inbred	3	3	2	1	6
3.	Double-cross	F_1 × F_1	4	4	2	1	7
4.	Top-cross	Inbred × Var.	2	2	2	1	5
5.	Double top cross	F_1 × Var.	3	3	2	1	6

13.3.2 Temporal Isolation

In circumstances where spatial isolation is not possible, the same can be achieved through adjusting sowing time between two plots differing by at least 40 days difference.

13.3.3 Border Rows

Distances less than 200 m can be modified by planting border rows. Border rows must be planted with seed used for planting male rows in the seed field. Seeds saved from male rows of the previous production of the same cross cannot be used for planting border rows, or for planting within the isolation distance.

The isolation distance is maintained at 300 m if the kernel colour or texture of the contaminating maize is different from that of the seed parent, or if the contaminating field is planted with sweet or popcorn. In this case, modification of isolation distance by planting border rows will not be permitted.

Mandatory distances in the case of spatial isolation can be reduced to not less than 100 m in the case of:

- Planting of border rows of the pollen parent adjacent to the production plot (max. 10 rows).
- Presence of natural obstacles (forests, dense hedges, dams) of sufficient height, width and vegetation to shield from pollen flight.
- Isolation strips of the pollen parent or pollen-sterile corn planted between production and commercial fields.

Differential blooming dates are permitted for modifying isolation distances, provided five per cent or more of the plants in the seed parent do not have receptive silks when more than 0.50 per cent of plants in the field, within the prescribed isolation distance, are shedding pollen.

13.4 Use of Border Rows

Isolation is facilitated through the use of border rows, which are the rows of male parents planted around the borders of the seed production plots. The higher the number of border rows, the lesser the chance of contamination from undesirable pollen and better pollination efficiency due to the availability of desirable pollen of the male parent.

(i) The minimum number of border rows to be planted for modifying isolation distances less than 200 m is determined by the size of the seed field and its distances from the contaminant.
(ii) Border rows are sown in the seed field adjacent to it, but in no case separated by more than 5 m from the seed field.
(iii) Border rows must be sown at the same time with that of the male parent in the seed production field.
(iv) Border rows should be planted on all the sides of seed production field.
(v) Seed fields having diagonal exposure to contaminating fields should be planted with border rows in both directions of exposure.
(vi) If two hybrid seed fields, with different pollinator parents, are within the isolation distance of one another, border rows are necessary for each of them to avoid contamination of the respective seed parent.

13.5 Planting Pattern

Hybrids are produced by controlled mating among genetically distinct parents. Achieving high seed yield requires manipulation of the physical location of the male and female parents. Planting patterns for hybrid depend principally on the pollen-shedding ability of the male parent which determines the amount of pollen that is available and nicking or synchronization of flowering of the male and female parents (which determines the likelihood that pollen will be available when the silks have emerged from the female parents and are receptive). For a single cross hybrid, the ratio of female to male rows in the field is usually 3:1, whereas it may extend up to 8:2. The recommended planting patterns include 4:1 (four rows of the female parent to one row of the male parent), 4:2, 6:1, and solid female with inter-planted male.

13.6 Pollen Control

Pollen control is extremely important as it is vital for the production of a genetically pure seed crop. The popular pollen control methods are detasselling and the use of male sterility. Detasseling involves physically removing tassels from the female parents before it sheds pollen. Manual detasseling is achieved by grasping the stalk just below the tassel and removing the tassel with an upward jerk. In mechanical detasseling, cutting or pulling of the tassel is undertaken with machines which improves the efficiency as compared to the manual approach but a loss of 1–3 uppermost leaves in the former leads to a significant loss of seed yield. Detasseling should be initiated when the top 3–4 cm of the tassel are visible above the leaf whorl and continue every day until no more tassels are left. Regular monitoring before and after detasseling phase is essential for ensuring genetic purity.

13.6.1 Precautions During Detasseling

1. Grasp the complete tassel so that all pollen-bearing parts are fully removed.
2. Immature detasselling should be avoided. It may cause a few spikelets to be left in the leaf whorls, which may emerge and shed pollen. Also, the top leaves are likely to be pulled out, leading to a reduction in yield or attack of the disease.
3. Do not hold the tassel too low on the stalk to prevent pulling out of plant tops.
4. Once detasselling starts in a field it must be repeated daily in all weather. A fixed time should be observed every day. Be particular to start detasselling from the same side every day, in the case of a large field.
5. Mark all the male rows at both ends by driving long wooden markers in the ground, or by some other suitable means. The markers should be painted white.
6. Look out for suckers (tillers) on female plants and also for lodged or damaged plants in female rows, as they are likely to pass unnoticed during detasselling.
7. The detasseller should drop the tassels on the ground after removing them and not to carry them in hand, as this may involve the danger of contaminating receptive silks.
8. Put an experienced detasseller in charge of this operation. He should walk behind the other detassellers and check that no tassels are left in the female lines.

13.7 Flowering Manipulation

Achieving synchronization of flowering among parental lines, i.e. reaching the flowering stage simultaneously (a phenomenon known as nicking) is most important in hybrid seed production. For optimum seed setting the male parent should shed pollen when the first silks become receptive silks. The most common technique for achieving synchronization is split date planting of the male and female parents. Split date planting is made based on some combination of days to flowering, growth stages, and heat units accumulated from the date when the first parent was planted. As a precautionary measure, male parents may be planted on two or more dates to extend the pollen-shedding period. In case the gap of flowering of male and female parents is high (>5 days), an adjustment in the sowing dates will be required to ensure synchronization of flowering whereas where the gap is less than 5 days, foliar application of growth regulators, fertilizers or mechanical measures could be used.

13.8 Rogueing

Ideally, seed production plots should not have been planted with maize in the preceding season to prevent contamination by volunteer plants. In addition, all undesirable off-type plants showing variability in morphological characteristics (e.g. plant height; leaf shape and size; flowering habit; silk and ear characteristics; kernel shape size and colour) should be removed from the field. Start removing distinctly tall and extra vigorous plants at the knee-high stage. At the pre-flowering stage, rogue out off-type plants which are easily distinguishable based on plant characteristics such as leaf shape, size, plant height, etc. Continue rouging during the flowering stage to remove plants differing in tassel or silk character. Roguing for off-types and malformed plants should be completed before pollen shedding. Diseased plants affected by stalk rot should be rogued from time to time. At harvesting, off-textured or off-coloured ears are to be discarded. During hybrid seed production, four field inspections are undertaken, one before flowering and three during flowering.

14 Hybrid Seed Production in Castor (*Ricinus communis* L.)

Castor is an important non-edible oilseed crop possessing a unique fatty acid composition that is used for various industrial purposes, mainly as lubricants and innumerable derivatives for pharmaceutical use. India is a major castor-producing country with a phenomenal increase in area and productivity due to the commercial use of hybrids. The development of hybrids in castor is based on the pistillate mechanism. Some critical points in hybrid seed production in castor are given below:

14.1 Isolation

Castor is a cross-pollinating species with the wind as a primary source of pollen dispersal and transfer. The extent of cross-pollination mainly depends on the direction and velocity of the wind, and the proportion of female to male flowers on the raceme. Genotypes that produce mostly female or 100% female racemes easily get pollinated by foreign pollen from sources located as far as 1000 m distance. Besides the wind, insects like honey bees, butterflies, moths, etc. are also known to play a role in pollen dispersal that results in variable levels of cross-pollination leading to contamination of varieties and parental lines. Based on systematic research under AICRP on Oilseeds in ICAR, India the following isolation distances for different seed categories are recommended (Table 4).

Considering the requirement of long isolation distances, to produce genetically pure seed, it is ideal to take up hybrid seed production of castor in non-traditional areas and off-seasons to avoid contamination.

Table 4 Recommended isolation distances for different seed categories of castor hybrids/varieties

S. No.	Seed class	Isolation distance (meter)
Varieties and male parents of hybrids		
1.	Nucleus (equivalent to pre-basic of OECD) and breeder equivalent to basic of OECD)	1500
2.	Foundation (equivalent to Certified 1 of OECD)	1000
3.	Certified (equivalent to Certified 2 of OECD)	600
Female parents of commercial hybrids		
1.	Nucleus and breeder	2000
2.	Foundation	1500
3.	Certified hybrid seed	600

14.2 Season and Planting Condition

The time of planting and production season has a profound influence on sex expression in castor. While warm and rainy seasons with an average daily temperature of about 30 °C provide an ideal environment promoting male flowers for undertaking seed production of varieties, and multiplication of the male parents of hybrids, the mild winter season with an average temperature ranging between 15 and 25 °C during flowering is the most conducive for taking up hybrid/certified seed production as it favours the production of female flowers. In the case of varieties and male parents, such exposure to male promoting environment, i.e. rainy-summer season encourages good expression of plants bearing mostly male spikes which could be easily eliminated through timely rouging. Similarly, the female parents, which are environmentally sensitive, when raised in male-promoting environments preferably in summer season with an average daily temperature above 32 °C produce interspersed staminate flowers (ISF) which are crucial for the multiplication of the female parents.

14.3 Breeder/Foundation Seed Production of Female Parents

It is one of the most critical steps in hybrid castor seed production. The seed to be used to produce the breeder seed of female parents is the nucleus seed. The ideal season to undertake seed production of pistillate lines is summer (planting at about 25 °C during January's second fortnight). The following two methods have been adopted in India for producing breeder seed of female parents based on their pistillate nature (Ramchandram and Ranga Rao 1990).

14.3.1 Conventional Method

- As per the prevailing standards of Indian seed certification (Annonymous 2013), 20 to 25% of monoecious plants are allowed in seed production plots to ensure adequate pollen supply to pistillate plants.

- Prior to flower opening in primary racemes (at least 2–3 days before), all deviants that did not conform to the diagnostic characters, especially with respect to the node numbers (up to primary spike), nature of internodes, bloom and leaf characteristics are discarded.
- At the stage of flower opening in primary racemes, pistillate plants are identified conforming to the diagnostic morphological characteristics of the female parent and tagged distinctly at the base of primary racemes.
- All monoecists which exclusively bear male flowers beyond three whorls at the base of the spike are removed.
- Plants with interspersed staminate flowers, if any, should be retained subject to the condition that the retained plants fulfil all other prescribed standards.
- The numbers of female and male plants are counted in each row and the monoecious plants are removed if these are above the stipulated percentage.
- Tagged female plants are examined regularly for possible reversion to monoecism in secondary, tertiary and quaternary order racemes. Remove the tags as and when the female plant reverts to monoecism up to the fourth sequential order of the branches.
- On maturity, female plants bearing the tags are harvested and seeds from each picking are kept separately after proper drying, packing and labelling.

14.3.2 Modified Method

- Unlike in the conventional method, all monoecious plants are rogued out at least 2 to 3 days before the flowering begins in the primary raceme.
- Female plants are examined critically for various morphological characteristics, particularly the number of nodes up to primary raceme (most of the female flowers on primary raceme fail to set fruits due to the non-availability of pollen).
- A large number of interspersed late male flowers may appear on primary as well as subsequent order racemes in about 35 to 50% of the female populations which provide sufficient pollen for the late developed female flowers on the same raceme, or on later order racemes.
- Plants are examined regularly for any reversion to monoecism up to fourth order raceme and the off-types are rogued out. However, the pistillate plants reverting to monoecism in fifth sequential order onwards can be allowed in the population as supplementary source of pollen.
- Seed are collected from all female plants and kept as per the picking order after proper drying and labelling.

14.3.3 Other Precautions

- The number of ISF may vary from 1–2 to >10–15 male flowers per spike. These plants should be retained as pollen sources.
- However, the primary spikes with a highly ISF nature, i.e. 5–6 male flowers per whorl tend to revert to monoecious in the later orders which are to be closely observed.

- The majority of the primary spikes may not set seed due to the non-availability of pollen. However, the later orders or on the matured primary spikes itself interspersed male flowers appear and fertilize the female flowers.
- Observe the female plants for any revertants at any stage (secondary to pentenary). Remove such revertants.
- In case the number of revertant is high (>30%), those in the third or fourth order may only be removed and the seed may be harvested from the earlier orders only.
- In case the proportion of late revertant female plants with interspersed male and occasional bisexual flowers increases in the population, those may be allowed to remain.
- However, seeds should be collected only from all-female plants and picking-wise seed lots be kept separately.
- Seed from late-order revertant female plants should not be allowed to be mixed with those from all the female plants.

14.4 Certified Hybrid Seed Production

The cross-pollinated nature of castor with differential sex expression due to its high sensitivity to environmental factors (climate, nutrition, etc.) makes hybrid seed production complicated. In castor, pistillateness, a polygenically controlled character, is highly variable but can be managed to a large extent by various agronomic manipulations like sowing time, nutrition and irrigation. Hence, knowledge of crop's adaptation and following location-specific agronomic recommendations are prerequisites for obtaining higher seed yield.

The practices for successful hybrid seed production of castor in different situations are detailed below:

Genetic contamination should be avoided by strictly following the stipulated isolation distance (1000 m) and timely rouging. Sowing should be done towards the end of the monsoon season with an average temperature of about 25–30 °C depending on the location so that the emergence of the primary and secondary spikes coincide with the cool season. If delayed, the flowering period might experience higher temperatures resulting in ISF in female parents. A planting ratio of 3:1 or 4:1 (female:male) lines with two male rows as border rows all around the hybrid seed production plot is suggested.

Rouging is an essential activity in the certified hybrid seed production plots to keep the female parent plants completely pistillate and get these fertilized by the desirable pollen parent. The possible rouge plants in the female line are monoecious, pistillate with ISF, sex revertant and plants with hermaphrodite or bisexual flowers. The stages of rouging are as below:

First: Within 30 days before primary spike initiation, off-types are removed both in female and male lines based on morphological characteristics.

Second: At the time of primary spike initiation, monoecious and pistillate plants with ISF or hermaphrodite flowers in the female parent should be removed. In the male parent population, all morphological deviants should be removed. The male plants with male flowers in more than 2–3 whorls in the primary spike should be removed.

Third: At the time of secondary spike initiation, in addition to the above, revertant in the second order should also be removed. Deviants based on the spiny or non-spiny nature of capsules should be removed. Plants with ISF may increase with increasing temperatures. The removal of plants or spikes depends on the population size and the extent of ISF plants in the plot.

Fourth: At the time of tertiary spike initiation, early revertant, plants with ISF and hermaphrodites should be removed. This depends on the population size and extent of the revertant. If the number of revertant is high and the population size is low, only reverted spikes should be cut off and removed after harvesting the primary spike.

A seed yield of 10–12 q/ha and 8–10 q/ha in the male and female lines, respectively could be achieved depending upon the soil and growing conditions. An average yield of 12–15 q/ha of hybrid seed is achieved in a hybrid seed production plot with good management.

15 Hybrid Seed Production in Cotton

Hybrids are commercially cultivated in cotton on a large scale in India. Both intra- and interspecific cotton hybrids have been developed in India since 1970. The cultivation of conventional F_1 hybrids significantly increased the lint yield over the best open-pollinated varieties available in upland and Asiatic cotton (Tuteja et al. 2011). Despite adopting the emasculation and pollination technology for hybrid seed production, and raising its cost, hybrids are popular among farmers. Cotton hybrids cover more than 90% of a total of 12.2 mha area (Singh 2016).

Hybrid seed in cotton, including GM varieties, is produced either by manual emasculation and pollination technique in case of inter-varietal and inter-specific hybrids or only by pollination in case of male sterility-based hybrids.

Cotton is an often cross-pollinated crop. The average natural outcrossing is about 6%. Cotton pollen being heavy and sticky cross-pollination occurs only by insects, i.e. honey bees and bumblebees. The majority of the hybrids released in India are based on the inter-varietal crossing. Development of such hybrids involves three steps viz.: (i) growing of female and male parents in separate blocks nearby, (ii) emasculation of female parent plants and (iii) pollination of the female parent with the pollen from the male parent.

Hybrid seed production using male sterility eliminates emasculation since the pollens are sterile in female parents. However, pollination has to be done manually. In cotton, mainly two types of male sterility such as genetic male sterility and cytoplasmic genetic male sterility are used for seed production. Thus the cost of hybrid seed production can be reduced.

For hybrid cotton seed production, the soil should be well drained and medium to heavy deep. Maintenance of genetic purity for certified seed production of conventional or male sterility-based hybrids depends upon the use of safe isolation distance.

For hybrid seed production in cotton female and male parents are planted in the same field with 5 m isolation between parents and keeping a minimum distance of 30 m from other cotton crop in the area (Meshram 2002). The sowing dates of parental lines are adjusted in such a way that there is the synchronization of flowering between the female and male parents with continuous supply of pollen till the crossing season is over. Staggered planting of male parent is suggested depending on the date of flowering in male and female and pollen production in the male parent (Doddagondar 2006; Doddagondar et al. 2008).

15.1 Emasculation of the Female Parent

Flower buds that are likely to open the next day are chosen for emasculation in the early afternoon. Different emasculation methods can be adopted depending upon the flower types.

15.1.1 Doak Method or Thumb Nail Method

This is the most successful method used in hybrid seed production of tetraploid cotton with more than 40% seed set. The method involves the removal of the corolla along with anther sheath by giving a shallow cut at the base of the bud using the thumbnail and applying a jerk/twisting action (Doak 1934). Care should be taken so that the white membrane covering the ovary is not damaged that affects the boll setting. It should also be ensured at the time of emasculation that no anther sac remains attached at the base of the ovary causing selfing and genetic impurity in the hybrid seed lot. Emasculated flower buds are generally covered with tissue paper bags (9 cm × 7 cm) to prevent contamination from foreign pollen and marking the emasculated flowers.

However, this method is not suitable for hybrid seed production in diploid cotton in which the flower buds are small and the style is short and fragile rendering the method unsuitable.

15.1.2 Pinching off of the Top of Corolla

Also known as Surat method, this is useful for the emasculation of diploid flowers where the top portion of the flower bud is pinched off using the thumb and first fingernails so that the tip of the stigma gets slightly exposed, and the bud is lightly covered with mud. As the buds mature the stigmatic head extends sufficiently for pollination. In genotypes where stigma protrusion/exsertion is relatively low, the entire corolla is removed and mud is applied on the unopened anther sacs. Pollination is done on the following morning (Mehta et al. 1983).

15.1.3 Straw Tube/Copper Straw Method

In this method, the top of the corolla is pinched off and a piece of straw tube is inserted into the style to separate all anthers in anther column and leaving the tube in the same position till pollination is done on the next morning. However, during the process of emasculation more time is required.

15.1.4 Removal of Petals and Dusting off Anthers

Petals are removed by the thumbnail method, and the pollens are brushed off from the anthers by lightly touching and moving the thumb and first finger down and up along the staminal column. A light tapping at the flower pedicel dislodges any excess anthers sticking to the bracts. This method is useful in G. *Herbaceum* species in which anthers are of granule type, which can be removed easily and dropped to the ground.

15.2 Pollination

The emasculated buds are covered with red-coloured tissue bags for easy identification. The emasculated buds are pollinated the following day between 8 and 11 am (or longer in some cases) when the stigma receptivity is maximum. When the crossing is done during October-November (temperature ranges from 31 to 35 °C and RH ranges from 63 to 70%) the male flowers are collected and dried under the sun for a few hours for effective pollen dehiscence and dusting. The androecium with pollen is shaken gently or rubbed on the stigmatic surface. Sufficient pollen is to be used on the stigmatic lobes for proper development of the locules. The crossed flowers are again covered with a white-coloured tissue bag to distinguish them from emasculated buds awaiting cross-pollination. A thread is tied to the pedicel for the identification of crossed bolls. Fertilization occurs after 12–30 h of pollination and hence the crossed buds are kept covered for 3–4 days (Deshmukh et al. 1995).

For an effective seed setting, the crossing programme should be continued for about 10–14 weeks after the initiation of flowering. Light and frequent irrigation during this period facilitates good boll development.

Besides a strict roguing both in the female and male parent plots, all un-emasculated buds and flowers in the female parents need to be removed to avoid any admixture in the resultant hybrid seeds.

Fully matured and completely opened bolls are to be picked and collected for seed. These are cleaned of any lint, washed, dried, cleaned and stored after tagging. Damaged/undeveloped/underdeveloped bolls should be discarded before sorting.

16 Hybrid Wheat Seed Production

Though, not commercially very popular, hybrid wheat varieties are in use in some parts of the world, specially in Europe and the UK. In India due to the low level of heterosis reported in wheat, hybrids are not yet in use. Conventionally, three-line hybrid wheat seed production is taken up using specific female-male line in a specified proportion and following recommended methods for proper growth of the parental lines, to synchronize flowering and effective pollination. These tend to increase the cost of production of hybrid seeds. In order to make hybrid wheat seed production easy and cost-effective, the blend hybrid seed production method has been proposed which maximizes hybrid seed production with cost reduction. A recent study has reported that a blending of 6 to 8% restorer line seeds with the seed parent seed increased the yield of mixed hybrid seed production with higher purity (Nie et al. 2021).

17 Hybrid Seed Production in Tomato under Polyhouse Conditions

Tomato seed production under open field conditions is often affected by various environmental stresses resulting in poor seed quality and quantity. Thus, seed production of tomato is preferred to be undertaken under protected conditions, i.e. low-cost poly house or climate-controlled poly house, which give higher seed yield and of better quality with lesser use of chemical pesticides.

17.1 Growing Seedling

Sowing of the parental line seeds is done at least 25–30 days before the transplanting. About one week before transplanting, the irrigation is withheld in the nursery to harden the seedlings.

17.2 Transplanting

The male and female lines are planted with 25–30 days old seedlings on ridges (in raised beds) 90 cm apart with the plant-to-plant spacing of 60 cm. A planting ratio of 4:1 for female:male plants are recommended.

17.3 Intercultural Operations

The parental lines should be stacked before flowering not only to protect the fruits from rotting but also to make more space to move between rows for emasculation and pollination operations. The seed plot is regularly weeded to keep it free from weeds. Irrigations at 4–5 days interval are sufficient. Flowering and fruiting are two critical stages of irrigation. The lower branches should be continuously pruned to encourage the proliferation of upper branches. Off-types and virus-infected plants must be removed before hybridization. As the hybrid fruits are developed on the female plants, sturdy stacking to withstand the fruit load should be given.

In the absence of a functional male sterile system, the hybrid tomato seed is produced by manual emasculation and pollination. For this, the crossing should be initiated at 40–45 days after transplanting.

17.4 Emasculation

The female flower must be pollinated by the pollen from the male line. To prevent self-pollination, the stamens from the flower buds of the female line are removed before they shed their pollen. Emasculation is initiated at about 50–60 days after sowing in the first cluster of 3–4 flowers, selected for crossing. Buds are forced-open with sharp-pointed forceps and anthers, which are fused to form a cone-like structure around the stigma, are removed by a vertical splitting of the cone. Emasculation in the bud stage is done in the evening hour.

17.5 Pollen Collection

The best time for pollen collection is during the late evenings before the pollen has been shed. The anther cones are removed from the flowers and are placed in suitable containers viz. Petri dish or cups. The anther cones are dried by sun-drying or by placing under a 100-W lamp overnight. The dried anther cones are taken in a cup with a lid of fine mesh and shaken to extract pollen. Pollen is collected in cups or rings and used for pollination. These pollens remain viable for at least one day.

17.6 Pollination

Emasculated flowers are generally pollinated the next day in the morning hours. The stigma is dipped into the pollen container or pollinated by touching the stigma with the tip of the index finger dipped in the pollen pool. Successful pollination is easily detected within one week by the enlargement of the pollinated fruit. After crossing operations are completed, any non-crossed flowers on the female plants are removed to avoid any chance of contamination from selfed fruits before harvest.

17.7 Harvesting

On an average 50 or more fruits are retained on medium fruited female parent plant. Tomato fruits ripen in about 50–60 days after pollination. Fruits are harvested after full maturity and collected in non-metallic containers, such as polythene bags, plastic buckets or crates.

17.8 Seed Extraction

The ripened fruits are crushed manually or by machines. The bags of crushed fruits are kept in big plastic containers for fermentation and for separating the gel mass embedded with the seeds. Seeds can be extracted from the fruit mass following fermentation, acid or alkali methods, washed thoroughly, cleaned of any debris, sieved and dried.

An average yield of 3 to 3.5 kg hybrid seed can be expected from an area of 100 sq m. polyhouse.

18 Hybrid Seed Production in Bitter Gourd under Insect-Proof Net House

Hybrid seed production of bitter gourd under the insect-proof net-house is a lucrative and environment-friendly technology, wherein the crop is vigorous, insect-free, and exhibits higher fruit and seed yield with better seed quality as compared to open field conditions. It also helps in reducing the cost of seed production and indiscriminate use of pesticides for insect and pest control. The essential steps in hybrid seed production technology of bitter gourd under net house conditions are given below:

Seedlings of the male and female parent lines are raised from genetically pure seed stocks and transplanted, maintaining a planting ratio of 3 female rows to 1 male row requiring a total of 30 female and 10 male plants for a 100 sq. m area. Inter-cultural practices are followed as recommended for the commercial crop at a given location and season.

Bitter gourd requires trailing to support the rapid growth of vine after planting. Staking reduces the fruit rot and diseases, ensures better pollination, facilitates easy harvesting, and gives higher seed yield. Bamboo poles, wooden stakes, PVC pipes, or similar materials could be used as trellis to support vine and keep the fruit and foliage off the ground. The trellis are either placed as an erect pole with horizontal support or in a dome-like structure. For seed production, vertical stakes are better than dome-shaped structures. The trellis should be 2.0–2.5 m high above the seed bed. The staking supports the climbing vines and lateral stems. Strings/ropes should be used to secure adjoining stakes. When the primary branch of the vine reaches the top of the trellis, all the lateral branches of the vine should be cut to promote pistillate flowers.

18.1 Flowering Behaviour

Bitter gourd is monoecious and bears staminate and pistillate flowers separately where the proportion of staminate flowers is very high as compared to pistillate flowers. The average ratio of staminate to pistillate flowers in monoecious lines varies from 12:1 to 9:1. To achieve higher yield, high sex ratio (higher pistillate flowers per vine) is desirable. Application of growth regulators like GA_3 at 50 ppm sprayed thrice at three leaf, tendril and bud initiation stage was found promising for higher induction of pistillate flowers, fruit set, seed yield and quality (Nagamani et al. 2015).

18.2 Pollination

Bitter gourd is a highly cross-pollinated crop. Female and male flowers are borne on different nodes of a vine. Hybrid seed production is done by manual hand pollination. For successful hybrid seed production, pollination is to be initiated one week after first female flower opening in a vine. All opened flowers and fruits are removed from female parent before pollination. Unopened female and male buds are covered with butter paper bag and cotton, respectively (to prevent contamination). Pollination is done next morning between 7 am and 12 noon. Pollen from male flower of male parent is manually dusted on stigma of female flower. Pollinated flowers are covered with butter paper bag to prevent contamination. Although stigma remains receptive for one day in spring-summer and more than one day in rainy season but pollination is undertaken between 7 am and 12noon on the day of anthesis.

Generally, 12–14 fruits/vines are retained for higher seed yield and quality. Ripe fruits are harvested when they turn orange in 25–30 days and 20–25 days after pollination in the spring-summer and monsoon seasons, respectively. Seeds should not be harvested from partially orange or burst fruits as it affects seed quality.

18.3 Seed Extraction

Seed extraction is done manually by opening the ripe fruits and removing the seeds from the fruits, followed by macerating to separate the red mucilaginous seed coat. Extracted seeds should be shade-dried to a moisture content of 6–8% before packaging.

An average seed yield of 2.5 kg is expected from an area of 100 sq. m. insect-proof net house.

19 Cauliflower

For very long, cauliflower hybrids used in commercial cultivation were based on self-incompatibility (SI) system. However, instability of SI system and problems associated with the multiplication of the parental lines using bud-pollination are its major drawback. Hence, CMS systems were identified and a large number of CMS-based hybrids are now available for commercial cultivation. The methods for hybrid seed production are specific to the system of male sterility and location of adaptation. ICAR-Indian Agricultural Research Institute has developed *Ogura* cytoplasm-based CMS lines for hybrid development in early, mid and late maturity groups of cauliflower and standardized the seed production technology of CMS based mid maturity cauliflower hybrid, Pusa Cauliflower Hybrid-3 which has recently been introduced for commercial seed production (Anonymous 2021).

Thus, it is evident from the above deliberations that hybrid seed production is a specialized activity, which requires a thorough understanding of the flowering behaviour, sex expression under varying environments and pollination dynamics of each crop before undertaking it.

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