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Sahil
Department of Genetics and Plant Breeding, School of Agriculture, Lovely Professional University, Jalandhar, Punjab, India

Rajitha Nair
Department of Genetics and Plant Breeding, School of Agriculture, Lovely Professional University, Jalandhar, Punjab, India

Sanjeev Kumar
Department of Genetics and Plant Breeding, School of Agriculture, Lovely Professional University, Jalandhar, Punjab, India

Corresponding Author:
Sanjeev Kumar
Department of Genetics and Plant Breeding, School of Agriculture, Lovely Professional University, Jalandhar, Punjab, India

Innovation in hybrid seed production of vegetable crops: A review

Sahil, Rajitha Nair and Sanjeev Kumar

Abstract
Accessibility of profitable method to deliver huge scope F1 seeds chosen parental lines is a significant factor, which in the long run decides the trading viability of the hybrid genotypes. In Vegetable crops, albeit hybrid varieties can be produced through various methods like hand emasculation for hermaphrodite crops, accompanied by pollination of emasculated flowers and various other breeding methods, *viz*, male sterility, self-incompatibility, Chemical Hybridizing Agent (CHA). Commercial hybrid seed production based on these mechanisms is economically viable almost in all vegetables especially in tomato, brinjal, cucurbits, sweet pepper, etc in which a huge number of F1 generation seeds are produced from a single cross-pollinated fruit. Different approaches are advanced for the improvement of marketable and experimental hybrids. Therefore, these methods can play an important role in producing effective amount of vegetable seeds which will ameliorate the total productivity, production.

Keywords: Emasculation, CHA, male sterility, hybrids, seed production

Introduction
India ranks second as the producer of vegetable with the production of 187474.313 million ton (NHB, 2018-19) [29] but as contrasted with China we are even now lagging in productivity and production. The increase in productivity in this country is because of the wide coverage of paramount area under hybrid vegetables. The prime cause for less productivity in India due to the limited obtainability of good-quality of F_1 hybrid seeds which are newly released. So as to expand productivity, the germplasm accessibility of newly released hybrids in a lower rate is rudimentary. Therefore, to feed the increasing population there is a requisite to increase the production (18.58 MT/ha, by National Horticulture Board, 2019).

Hence, hybrid genotypes play an important role to increasing total productivity and production due to their early maturity, better quality, high yield potential, and resistance to disease and insect. The expeditious rise in productivity can be reached by the use of good-quality of germplasm and broad genetic base inbred or hybrid vigour. In addition to the use of government policies/ schemes enhanced vegetable production technologies. The main reason behind reduction in productivity of hybrid vegetables and low commercialization in India is may be because of the insufficiency of high-quality genotype or seed of new released hybrid variety. One more reason could be expensive hybrid vegetable seed like tomato, capsicum, chilli, muskmelon, cucumber, cauliflower, cabbage etc.

Production of F1 hybrid varieties in vegetable crops
The first hybrid variety of bottle gourd, 'Pusa Meghdoot' was developed in 1971 from Indian Agricultural Research Institute in India. First hybrid Bharat in capsicum and Karnataka in tomato was developed from the private sector by Indo-American Hybrid Seed Company. The significant accentuation was needed to the improvement of crossover (improved lines and hybrids) and their testing by the All India Coordinated Improvement Project (AICRP) on vegetables during mid-1980. Many F1 hybrid seeds are developed by the Government are in demand among farmers or small holders and these genotypes are multiplied by SSC at state level and NSC at national level.

Breeding methods in vegetables
The seed production in vegetable crops is based on proficiency and knowledge of breeding methods like cross-pollination, self-pollination & often cross-pollination, life cycle (Perennial, biennial & annual), sex form like hermaphrodite, dioecious & monoecious and compatibility like self-incompatible, compatible or self-fertile of these vegetables (Table no-1).

Table 1: List of Vegetable crop, life cycle, breeding methods, compatibility and sex form

Vegetable crop	Life cycle	Breeding methods	Compatibility	Sex form
Tomato, Brinjal, Lettuce, Bell pepper, Chilli, Cowpea, *Lablab purpureus*	Annual	Self-pollinating	Self-fertile	Hermaphrodite
Coriander, Palak, *Chenopodium*, Amaranth.	Annual	Highly Cross-Pollinating	Self-fertile	Hermaphrodite
Cabbage, Cauliflower, Broccoli, Knol-Khol, Radish, Brussels Sprouts, Turnip, Beet root	Biennial	Highly Cross-Pollinating	Self Incompatible	Hermaphrodite
Onion, Carrot, Celery	Biennial	Highly Cross-Pollinating	Self-fertile	Hermaphrodite
Artichoke	Perennial	Highly Cross-Pollinating	Self-fertile	Hermaphrodite
Bottle gourd, Cucumber, Luffa, Bitter gourd, Squash, Pumpkin, Round melon, Watermelon.	Annual	Highly Cross-Pollinating	Self-fertile	Monoecious
Muskmelon	Annual	Highly Cross-Pollinating	Self-fertile	Andromonoecious
Bitter gourd, Cucumber.	Annual	Highly Cross-Pollinating	Self-fertile	Gynoecious
Ivy gourd, Asparagus, Pointed gourd.	Perennial	Highly Cross-Pollinating	Self-fertile	Dioecious
Spinach	Annual	Highly Cross-Pollinating	Self-fertile	Dioecious

The Principles of Hybrid Seed Production in Vegetable Crop

1. Selection of area and season for seed production

The locations or area is an important factor in the production of hybrid seed to improve the yield and quality of seed. Vegetable crops are preferred to be sown in areas of dry season at the time of seed extraction and seed maturity. The main seed production areas for cucumber and muskmelon in India are: Jalana (Aurangabad) in Maharashtra, Bangalore in Karnataka, U.P., Haryana, Punjab, Nandyal Valley in A.P., etc.

2. Isolation Distance

Most of the vegetable plants are highly cross-pollinated; therefore, the distance of Isolation for certified and foundation seeds should be sustained according to the standard of seed production. Distance of Isolation of the plant should be maintained from the same varieties and their wild relatives, cultivated species to maintain the genetic purity for successful hybrid seed production.

A. Time Isolation: Seeds of different genotypes of the same plant can be produced at the similar place every year. Long season Cross-pollinated crops with two production cycles are isolated by time. For instance, seed production of early and mid-maturity category of cauliflower can be timely isolated.
B. Distance Isolation: Isolation distance for cross-pollinated genotypes is more than self-pollinated varieties. The Isolation distance also differs with the insect and wind pollination.

3. Rouging

Rouging is the elimination of off type plants which do not establish the correctness to the definite restraint of particular genotype. Thus, rouging is done to maintain the genetic purity. The cross compatible crops like cucurbits, Cole crops, onion, etc. show excessive morphological diversity compared to self-compatible crops like tomato, peas, fenugreek, etc. Thus, the genotypes of self-compatible crops are stable and uniform.

Rouging stages

A. Pre-flowering: Based on vegetative attributes like foliage morphology, plant growth, colour, etc. rouging is done in seed production field.
B. During flowering: The late and early genotypes can be simply recognized based on sex expression and curd maturity in cucurbits and cauliflower respectively, and flower opening time in solanaceous crops.
C. During fruit development: Genetic purity of developing fruit i.e., fruit size, shape, colour, ripen fruit colour is examined and based on it, rouging is performed.
D. During maturity: The crops showing early maturity in late varieties and vice versa are supposed to be rouged out.

4. Seed Standards

It alludes to the inspection of field, manner of harvesting, transporting, packaging and processing. The affinity of the lots cannot be persuaded without the proper records of seed certification agency. It is thus, mandatory that the agency of seed certification should put emphasis on standard for processing crops.

Techniques of hybrid seed production

Seed production of commercial hybrids of hermaphrodite vegetable is developed through the method of emasculation followed by pollination, which is performed manually. Large number of F1 seeds is produced through these methods. By the by, in these vegetable crops additionally, expense of F1 seed creation can be reduced, if basically relevant mechanisms to abstain from selfing and boost crossing is turn in the half breed seed production area. For instance, in tomato crop, decrease in labour use of partial hybrid vegetable seed production can be accomplished by the end of manual emasculation methods, as it constitutes to about forty percent of the total consumption (Yordanov, 1983) [45].

The functional type of male sterility system has been used for hybrid seed production in tomato cv. Pusa Divya under poly house condition (Manjunath, 2009) [26]. Ethephon is used to produce hybrid seed production of summer squash for inducing the natural pollination and use of staminate flower. Cryopreservation of pollen provides numerous merits to the cross-bred vegetable seed production. This technique can impart a continuous availability of pollen which is fertile and also permits additional pollinations for better setting of seed.

Based on the mechanism of pollination, flower structure and sex forms, following are the methods of hybrid seed production

1. Hand pollination & Manual emasculation
2. Emasculation & open – pollination
3. Hand pollination in the absence of emasculated flower
4. Open- pollination in the absence of emasculated flower.

Hand emasculation & Hand pollination

Hermaphrodite crops such as tomato, chilli, brinjal, pea, okra; French bean, cow pea and country bean and andromonoecious type of muskmelon are normally produced by this method. Normally, the emasculation of flower (bisexual) is done in

afternoon and pollination is carried next day early in the morning when anthesis occurs. Pollens are generally gathered by tapping the anthers carefully and pollination is achieved by dipping the stigma in gathered pollens. The method comprises of five steps, they are (Nishi, 1967) [30].

- Emasculation
- Bagging
- Pollination and bagging
- Tagging

The seedless triploid varieties of watermelons are F1 hybrids created by manual pollination of tetraploid female with diploid male parent planted in alternative rows (Kihare, 1951) [18].

Emasculation and Open pollination

Use of dicliny

a. Monoecy: hybrid seed of cucurbits are mostly produced through this method. The female and male parents are grown in conjunction in alternate rows in isolated plot (Singh *et al*., 1988; Swarup, 1991; Dev, 1999) [34, 42, 35, 6]. After every three to four row of female parent one row of male parent are sown (Kushwaha and Pandey, 1998 and Hazra and Som, 1999) [19, 8]. Before the opening of female flowers, the buds of male flowers are removed and this is continuously done for five weeks. After five weeks of pinching off the male flower to provide sufficient crossed fruit set, later the male flowering is stopped by cutting off the increasing point of the vine. The fruits collected from the male parent are selfed seeds of the male variety and the fruits of the female variety are the hybrid seeds. This method is known as cross blocking method. For e.g.: production of hybrid seeds from andromonoecious muskmelon.

b. Dioecy: a pair of chromosome X and Y controls the mechanism of dioecious sex form. The heterozygous XY is male and the homozygous XX is female. If this system id exact, all progeny will segregate into female and male in 1:1 (Bemis and Wilson; 1953) [1]. This method is mostly used in pointed gourd, spine gourd, spinach, asparagus and cassava. In this method female and male appear in almost equal proportion upon sowing open-pollinated varieties. Male plants of wanted variety are grown by the side for pollination. It is allowed to get naturally pollinated by wind. Hybrid seeds are those seeds which are developed inside the female parents (Singh *et al* 1988; Kumar and Dhaliwa1, 199O Chaudhari, 1992: Chaudhary. 1993; Hazra and Som, 1999) [34, 42, 20, 4, 5, 8].

c. Gynomonoecious: Peterson and Weigle (1958) suggested the use of gynomonoecious line in cucumber for hybrid seed production. They experimented by crossing and back crossing the Korean variety “Shogoin" with the picking variety “Wisconsin SMR 18" to instigate a gynomonoecious line to produce a high percentage of gynoecious plants (Peterson, 1960) [32]. The result of the percentage of gynoecious crops in different gynomonoecious line was found to show variability.

Hand pollination in the absence of emasculated flower

Use of dicliny

a. Monoecy: This method is applied to vegetables like pumpkins, melons, squash, gourds and cucumber. In this technique the female flowers and male flowers are bagged separately a day before opening and in the next morning it is artificially pollinated followed by bagging for 4-5 days. The expense of hybrid seeds is not so high because the single pollination produces large number of seeds. 10% of the population should be used to provide pollen. Hybrid seed production of muskmelon cannot be achieved by this method because F_1 cross with round fruits cannot be produced as the fruits of monoecious musk melon are elongated (Kumar and Dhaliwal, 1990; Sandha and Lal; 1999) [20, 40]. Thus, Pusa Rasraj, the hybrid of muskmelon was not accepted commercially.

b. Through Male sterility: Onion crop renders one of the scarce instances of early identification of male sterility (Jones and Emsweller, 1936) [13], its inheritance in the characteristics of progeny and utilization in hybrid seed production of vegetables (Jones and Clarke, 1943) [12]. These male sterile crops were either artificially incite through mutagenesis or isolated in natural population (Kaul, 1988) [17]. Now, male sterility has been also advanced through the techniques of genetic engineering (Williams *et al*., 1997; Kumar *et al*., 2000) [4, 46] and protoplast fusion (Pelletier *et al.*, 1995) [31].

Male sterility differentiated into two different categories, Kaul (1988) [17]. Genetic (induced or spontaneous) and Non-genetic (induced) male sterility. On the basis of phenotypic characters, genetic male sterility (GMS) has been differentiated into three groups i.e., functional, structural and sporogeneous. Likewise, non-genetic male sterility is differentiated into physiological, ecological and chemical male sterility. Additionally, on the basis of genotype, genetic male sterility (GMS) was classified as: Cytoplasmic Male Sterility (CMS), Genic Male Sterility (GMS), and Cytoplasmic Genetic Male Ssterility (CGMS), (Kaul, 1988) [17].

GMS is manipulated for hybrid seed production of muskmelon, for example Punjab hybrid-1. Both lines are grown-up in ratio of 4:1. Although, to sustain the better crop diversity in rows of female, it is advised that parent genotype should be grown with double seed rate than actual seed rate. It is suggested that female variety seedling are generally grown in polythene bags and to ignore the fertile plants in the female rows. The variety with male sterility is sustained in heterozygous condition by making a cross with maintainer line by maintaining a proper distance of isolation. Amidst the genetic emasculation, Cytoplasmic genetic male sterility (CGMS) and Genetic male sterility (GMS) are utilized in production of improved varieties of chillies. Manipulating these male sterile genotypes, hybrid varieties like Arka meghana, Kashi surkh, Arka harita, etc were recognized for commercial use. Various chemicals like ethrel, GA3, Ethephon, etc. are used in the production of improved or hybrid seed production.

Open-pollination in the absence of emasculated flower

This method is widely used in cross-pollinated vegetable crops in which either pollination results in a small quantity of seeds or the emasculation is difficult. Here benefit is taken of multiple allelic system of self-incompatibility is common in broccoli, cauliflower, cabbage and brussels sprout, kale and radish and male sterility in carrot, onion, beet, etc.

Use of male sterility

Male sterility is used in different vegetable crops. It may be

Cytoplasmic Male Sterility (CMS), Genetic Male Sterility (GMS) or Cytoplasmic Genetic Male Sterility (CGMS). Use of male sterility involves the steps, (a) recognition of male sterility, (b) relocation of male sterility attributes into the desirable genotype by repeated back crossing, (c) extension of male sterile lines by developing maintainer line (d) production of hybrid seeds by manual pollination.

Table 2: List of various commonly used techniques of hybrid seed production

Crops	Mechanism
Tomato, Okra, Brinjal, Sweet pepper, Hot pepper	HE+ HP
Cucurbits	Pinching of staminate flowers + HP
Capsicum, Tomato	MS + HP
cauliflower, Cabbage, Carrot, Muskmelon, Onion, Radish, capsicum	MS+ NP
Cucumber, muskmelon	Gynoecism + NP
Cole crop	SI+ NP
Cucurbits	Removal/ pinching of staminate flowers* + NP
Summer squash, Winter squash etc.	PGR & pinching of male flowers* + NP
Chilli	GMS + BP
Baby corn, Sweet corn	Detasseling + WP

HE: Hand emasculation; HP: hand pollination; NP: natural pollination; BP: Bee pollination; WP: Wind pollination; SI: Self-incompatibility; PGR: plant growth regulator; MS: Male Sterility GMS: Genetic Male Sterility

Varieties with high proportion of pistilate flowers are beneficial for hybrid seed production of vegetables.

Table 3: List of various CHAs used in the production of hybrid seeds of vegetable

Chemicals applied	Vegetable crops	Observations
CCC inhibitors by Gibberellin	Tomato	Discerning impeded the growth of stamen (Rastogi and Singh, 1988) [34, 42]
GA3	Tomato	Tomato plants treated with GA3 gave rise to distinct nstamens and pistils (split) (Chandra Sekhar and Sawhney, 1990) [3].
GA3	Pepper	Caused inhibition of pollen development (Sawhney, 1981) [41]
GA	Cole crops	It reported favourable for further use (Van Der Meer and Van Dam, 1979) [43]
ABA (Abscisic acid)	Tomato	Selectively impeded the growth of stamen (Rastogi and Singh, 1988) [34, 42]
NAA	Tomato	Persuade male sterility (McRae, 1985) [25]
NAA & IAA	Cucumber	Increased the growth of pistillate flowers (Laiback & Kribben, 1950)
FW-450 (Mendok)	Tomato	reported promising for further use (Moore, 1959) [28]
Ethephon	Brinjal	selective male sterility was induced(McRae, 1985) [25]
Dalapon	Tomato	male sterility was induced (Brauer, 1959) [2]
Dalapon	Pea	Induced male sterility (Brauer, 1959) [2]
TIBA Triiodo benzoic acid	Tomato	Induced some male sterility (Rehm, 1952) [39]

Protected cultivation for hybrid seed production

The absence of adequate isolation, disease, insects and virus free surrounding in the production of true to type seeds for commercial raising are the main difficulties in improved vegetable seed or variety production. Juxtaposed to open field, protected cultivation can collect excessive yield of seeds with better quality (Jat and Tomar, 2015) [33]. The most destructive problem for quality vegetable seed production is due to viral disease and insect vectors, and if these pest and diseases are monitored by protected surroundings will reduce the use of insecticides, herbicides, etc. The seed production is effectuated by the change in climatic conditions. The attack of mottle mosaic virus disease and other destructive pests is high during monsoon season and there is no better management to this. So, growing crop under net house could control all these destruction by sheltering the plant from unfavourable climatic condition and various pests. It also gives an alternative for off season and quality seed production. This net house is competent for production of hybrid seeds of chilli, okra, tomato, Bell pepper, cucurbits and brinjal as contrasted to open field condition (Jat *et al.*, 2015; Jat *et al.*, 2016) [9, 10]. Production of hybrid seeds of indeterminate varieties and hybrids of sweet pepper, cherry tomato, parthenocarpic cucumber and bitter gourd can be grown in greenhouse. As compared to the open field cultivation, seed yield of such crops can be three to four times more (Kalyanrao *et al*., 2012; Kaddi., 2014; Jat *et al.*, 2017) [14, 11]. Comparably, greenhouse with natural ventilation may also require for hybrid or improved seed production of vegetables, while the yield of crop is generally two to three times inflated above the field condition, but the expense of production of seed is only one third of the seed formed under partial temperature-controlled greenhouse. (Kalyanrao *et al.*, 2014; Singh and Tomar, 2015) [9].

The benefits of hybrid seed production of vegetables in in protected condition

1. Higher plant population can be sustained.
2. In cross-pollinated vegetables, isolation distance can be reduced.
3. Seed quality and seed yield is increased.
4. Issue of synchronization of flowering can be reduced.
5. No need to emasculate female parents because there are no insect pollinators.
6. Hand pollination can be easily performed under protected condition.
7. Seed vigour and seed viability expanded through proper nutrient management.

8. Plants do not get damaged by un-seasonal rains.

Extraction of Seed

Two main techniques of seed extraction

1. Wet method for seed extraction: wet method technique is used for brinjal, cucumber, tomato, watermelon, muskmelon, bitter gourd, ash gourd, etc. There are two methods under these techniques, *viz*, fermentation method, alkali method and acid method.
2. Dry method for seed extraction: The matured vegetables are harvested and kept under sun for two to three days. Then the seeds are removed and kept under sunlight in 8:00-11:00 am and 2:00 – 5:00 pm to decrease the moisture content. These methods are used in sponge gourd, ridge gourd, okra, chilli, etc.

Genetic purity testing

Grow out test (GOT) is mostly used to assess the genetic purity of a germplasm or seed. It includes the juxtaposition of morphological characteristics of crops initiated from sample of seed, through that of reliable samples from the entire crops growth period but it is time engrossing, varies according to season and is laborious, which impedes seed certification. Thus, biochemical markers have been used in genetic purity evaluation of seeds, but these are limited in number, affected by crop stage, varieties of plant and environment. So, a proficient and an exact technique are needed for cost effective and expeditious seed testing for hybrids of vegetable. For testing the genetic purity by use of DNA marker into PCR mixture and we test the purity in a lot of seed built on the initiated differences among the genotypes at the level of classifications of nucleotide. Simple Sequence Repeat (SSR) markers have been extensively utilized for evaluating the purity of seed in different vegetable crops like chilli (Mongkolporn *et al*., 2004) [27], tomato and cabbage (Liu *et al*., 2007) [22, 24], cauliflower (Zhao *et al*., 2012) [37], melon (Liu *et al*., 2006) [23], bunching onion (Tsukazaki *et al*., 2006) [36], and squash (Ferriol *et al*., 2003). DNA marker are play an important implement for genotypes recognition and to improve the seed quality in various plants as it saves time, provides precise result and labour consumption is less.

Conclusions

Many mechanisms have not been used for the development of commercial hybrid varieties. The cytoplasmic male sterility (CMS) is mostly used method for the production of hybrid or improved seeds in vegetables, but, in many other vegetables, it has not been used because of the absence of sterile cytoplasm. The exploitation of GMS is limited to only rare plants, because of the issue related with the multiplication of seed of male sterile varieties. In the presence of expeditious technologies of biotechnology, it may be expected that transgenic male sterility methods will be rapidly used in future; especially vegetables with cytoplasmic male sterility (CMS) line are unavailable. Progressing in use of expedient molecular markers may give suitable cost-effective selection tactics to discard 50% male fertile sister crop at seedling stage, which may provide the way to use monogenic recessive male sterile lines in various vegetable crops. Alternately, phenotypic restoration of nuclear male sterile line can be attained utilizing certain chemicals to overcome the issue of maintenance, as already experimented in tomato. Thus, various techniques like these can improve the production of hybrid vegetable seed production.

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