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Recent Advances in Hybrid Seed Production of Cucurbits

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Abstract—*Cucurbit crops are mostly produced for their immature or mature fruits. Hybrid varieties play a vital role in increasing vegetable production due to their high yield potential, early maturing, superior quality, disease and pest resistance attributes. Techniques of hybrid seed production in cucurbits through hand emasculation and hand pollination, hand emasculation and pollination by insect, use of genetic male sterility system, use of gynoecious sex form and modification of sex expression. Recently use of markers gene to simply the procedure of identification & hybrid seed production by utilizing glabrous seedling markers in muskmelon which was controlled by single recessive gene. This could eliminate the tedium method of identification of male sterile plant and keep down the cost of hybrid seed production. Alternatively, extensive studies on plant growth regulators for large-scale hybrid seed production in many cucurbits should be undertaken.*

1. INTRODUCTION

The trend of F1 hybrid seed usage in vegetable is increasing globally in term of species, cultivars and volume of seed used. Availability of cost effective mechanism/method to produce large-scale F_1 seeds utilizing selected parental lines is an important factor, which ultimately determines the commercial viability of the hybrid varieties. In vegetables, hybrid seeds can be developed through manual emasculation (in case of hermaphrodite crops) followed by manual pollination of emasculated flowers or pistilate flowers (in case of monoecious crops with separate staminate and pistilate flowers) & seed production of commercial hybrids (large quantity of seeds for cultivation) based on such methods is economically feasible only in **cucurbits and few other vegetables**, in which a large number of F1 seeds are obtained from one manually pollinated crossed fruits. Ever since (since 1930s) the discovery of male sterility and self-incompatibility mechanisms and their proposed utilization in **hybrid seed production**, several mechanisms and methods have been evolved for the development of experimental and commercial hybrids [7].

Cucurbits are vegetable crops belonging to family Cucurbitaceae, which primarily comprised species consumed as food worldwide. The family consists of about 118 genera and 825 species. Although most of them originated in Old World, many species originated in the New World and at least seven genera in both hemispheres. There is tremendous genetic diversity within the family, and the range of adaptation for cucurbit species includes tropical and subtropical regions, arid deserts, and temperate regions. The genetic diversity in cucurbits extends to both vegetative and reproductive characteristics and considerable range in the monoploid (x) chromosome number including 7 (*Cucumis sativus*), 11 (*Citrullus* spp., *Momordica* spp., *Lagenaria* spp., *Sechium* spp., and *Trichosanthes* spp.), 12 (*Benincasa hispida*, *Coccinia cordifolia*, *Cucumis* spp. other than *C. sativus*, and *Praecitrullus fistulosus*), 13 (*Luffa* spp.), and 20 (*Cucurbita* spp.). In India, a number of major and minor cucurbits are cultivated (Tab. 1) in several commercial cropping systems and also as popular kitchen garden crops. Hybrids are most common in summer squash, bootlegourd, bitter gourd, cucumber, melon, and watermelon. Cucurbits share about 5.6 % of the total vegetable production of India.

Table 1: Commonly grown major cucurbits in India

English name	Scientific name	Origin	Chr. No.($2n$)
Cucumber	*Cucumis sativus*	India	14
Bitter gourd	*Momordica charantia*	Indo-Burma	22
Bottle gourd	*Lagenaria siceraria*	Ethiopia	22
Watermelon	*Citrullus lanatus*	Tropical Africa	22
Melon	*Cucumis melo*	Tropical Africa	24
Long/serpent melon	*Cucumis melo* var. *Flexuosus*	India	24
Snapmelon	*Cucumis melo* var. *Momordica*	India	24
Ridge gourd	*Luffa acutangula*	India	26
Sponge gourd	*Luffa cylindrica*	India	26
Pumpkin	*Cucurbita moschata*	Peru and Mexico	40
Summer Squash	*Cucurbita pepo*	Peru and Mexico	40

Winter Squash	*Cucurbita maxima*	Peru and Mexico	40
Ash gourd	*Benincasa hispida*	South East Asia	24
Pointed gourd	*Trichosanthes dioica*	India	22
Ivy or scarlet gourd	*Coccinia cordifolia* (syn. *C. indica*)	India	24
Round melon	*Praecitrullus fistulosuos*	Indo-Burma	24
Sweet gourd	*Momordica cochinchinensis*	South East Asia	28

2. SIGNIFICANCE OF HYBRID SEED PRODUCTION IN CUCURBITS

Hybrid plants result from crosses between two different varieties or inbreds. Hybrids are often superior over non hybrid varieties in vigour, yield, uniformity, as well as in other characters. And this is the main reason for their agricultural value. There are various factors which favours adoption of hybrid cultivars -

2.1 Easy identification of male & female flower

In hybrid seed production major problem for breeders is to separate male and female reproductive organ. But in case of cucurbits we can easily identify female due to presence of swollen hypogynous overy.

2.2. Large size of flower

Generally all cucurbits have large size of flowers so that hand pollination, emasculation, pollen collection become easy.

2.3 Relatively low number of seed required for establishment

Less no of seed required as compared to open pollinated seed. In case of gourds 5 to 7 kg seeds are required for cultivation in 1 acre while using hybrid seed, seed rate is lesser only 5 kg is enough for 1 acre.

2.4 Seed yield

Seed yield per unit area is a product of the multiplication of three components; the number of fruits per unit area, the number of seeds per fruit and the mean weight of the individual seed. Due to larger fruit size, we are getting more number of seeds per fruit as compare to other fruit vegetables. In many species of cucurbits , including cucumber (*Cucumis sativus* L.) [5, 13,21], melon (*Cucumis melo* L.) and squash (*Cucurbita* spp.) [14] the dominant component is the number of fruit per unit area. In a wide range of conditions maximal seed yield in cucurbits is positively correlated with fruit number per unit area and more fruits of smaller size produce more seeds than fewer fruits of larger size. In other words, and by example, two melon fruits of 1000 g each will always produced greater seed yield than one fruit of 2000 g [15]. Seed yield in all cucurbit crops grown in the field is greatly affected by environmental conditions out of the grower's control such as light intensity or temperature, but also by cultural practices including irrigation and fertilization management as well as pest and disease control.

2.5 Modification of sex form

Monoecious cucurbitaceous plants have imbalance sex ratio of male-female flowers that causes lower fruit yield. By the application of PGR we can modify the sex expression of cucurbits and used in HSP. Exogenous application of growth regulators may shift the sex expression towards femaleness by increasing the production of female flower and suppressing that of male flower in cucurbitaceous plants. Plant growth regulators have positive effect on the production of early flowering and yield. Growth regulators can decrease male and female flower ratio and increasing the number of fruits per plant and individual fruit weight as well as increase the total yield.

2.6 Cost-efficient hybrid seed production

In producing hybrid seed, the two varieties are grown in proximity for effective pollination; one variety serves as seed parent and the other as pollen parent. Natural pollination is brought about by either wind, insects, or other animals. In the past, crossing two varieties on a large scale was a very difficult task because the plants of many crop species bear both male and female reproductive structures, *i.e.*, they are capable of self pollination as well as cross pollination. Therefore, in order to prevent self pollination and obtain pure hybrid seed, it was necessary to emasculate the seed parent by hand, a costly operation which usually requires a large number of workers. Emasculation in this case refers to a physical removal of functional male reproductive structures from the seed parent. Different systems were devised which do away with the problem of emasculation and thus facilitate the commercial production of hybrid seed. Some effective systems are based on the use of male sterile plants, or self incompatible plants, or female plants as seed parents. An important feature of these systems is that they enable their male sterile, self incompatible, and female plants to reproduce their kind in practical ways.

3. HYBRID DEVELOPMENT IN CUCURBITS

The first F1 hybrid of watermelon was developed in 1930. The first F1 hybrid in public sector is in Bottlegourd (Pusa Meghdoot, Pusa Manjari) released by IARI, New delhi. Hybrid cultivars are commercialized in selected cucurbits, which express desirable heterosis for yield. In the case of melon, watermelon, cucumber, bottle gourd, several hybrid cultivars have been developed [7].

Table 2 : Cucurbit hybrids identified for general cultivation [8]

	Hybrid	
Crop	**National level**	**State level**
Muskmelon	Pusa Rasraj	Punjab Hybrid-1, MHY-3, MHL-10,DMH-4
Watermelon	Arka Jyoti	RHRWH-12
Cucumber	PCUCH-1, Hybrid No.-1, PCUCH-3	Pusa Sanyog, AAUC-1, AAUC-2
Bottle gourd	NDBH-4, PBOG-1, PBOG-2	Pusa Manjari, Pusa Hybrid-2, Kashi Bahar
Bitter gourd	Pusa Hybrid 1 & 2, NBGH-167	

4. METHOD OF HYBRID SEED PRODUCTION

Hybrid is produced by crossing between two genetically dissimilar parents. Pollen from male parent (Pollen parent) will pollinate, fertilize and set seeds in female (seed parent) to produce F1 hybrid seeds. For production of a hybrid crossing between two parents is important, the crossing process will results in heterosis. Three steps involved in F1 hybrid seed production of cucurbits are –

1. Development of inbred line and their production: The inbred lines developed through exploiting inbreeding depression and fixation of the desired traits in them. The seed of the developed lines are produced in isolation or by hand pollination.

2. Testing of combining ability: The combining ability (gca/sca) is tested by line x tester or diallel cross method.

3. Production of F1 hybrid seed: Techniques have been developed and is variable for crop to crop. Techniques of hybrid seed production in cucurbits –

i. Hand emasculation and hand pollination.

ii. Hand emasculation and pollination by insect.

iii. Use of genetic male sterility system.

iv. Use of gynoecious sex form.

v. Hybrid seed production through modification of sex expression.

1. Hand emasculation and hand pollination

This technique is applicable for limited scale produciton, since lot of trained labour are required in pinching, pollen collection and hand pollition.

2. Hand emasculation and pollination by insect

The male flowers from female lines are pinched off day before of anthesis regularly, which honeybees and other insects (voluntary) uses as a pollinizer. The male and female are grown in alternate rows. The fruit set on female lines are of hybrid and harvested for seed extraction. The planting ratio varies within the crops e.g. summer squash 3:1 and 4:1 in muskmelon and cucumber but depend upon the population of bees in plot. The technique used in bottle gourd, pumpkin, muskmelon, cucumber, summer squash and bitter gourd for hybrid seed production.

3. Use of genetic male sterility system

Male sterlity system have been utilized for commercial hybrid production in muskmelon (Punjab Hyb.1). The genetic male sterlity in musk melon controlled by single recessive gene (msms). For hybrid seen production, the male sterile line used as female parent. Since genetic male sterile line is maintained in heterozygous forms, 50% fertile plants are to be removed at flowering. The other 50% having non-dehiscent empty anther are retained in female rows. The female and male are grown in 4:1 ratio. However, to maintain the good plant population in female rows it is suggested that seed parent should be sown with double seed rate. It is also advised that female line seedling should be raised in polythene bags and transplanted at flower appearance in order to avoid the fertile plants in female rows. The pollination is done by honey bees and 1 to 2 medium sizes hives are good enough to ensure the good pollination and fruit set at female row. The male sterile line is maintained in hetrozygous form by crossing with maintainer line under adequate isolation distance or under cover.

Table 3:Male sterile lines reported in cucurbits for HSP

Crop	Male sterile lines
Muskmelon	ms-1 [3]
	ms-2 [2]
	ms-3 [11]
	ms-4 [17]
	ms-5 [10]
	Only ms-1 is commercially utilized.
Watermelon	msg (male sterile mutant) [19]

4. Use of gynoecious sex form

The gynoecious sex form have been commercially exploited in hybrid seed production of cucumber (Pusa Sanyog) at IARI, R.S. Katrain and in muskmelon (MH10) at PAU, Ludhiana. For hybrid seed sproduction female and male rows are planted in 4:1 ratio. The female (seed parent) bear only female flowers and pollination is done by insect (honeybee). To ensure the good fruit and seed recovery, the sufficient population of honeybee 1 to 1½ colony of medium size has to be kept at the boundary of seed production plot to boost the amount of crossing. The parental lines i.e. male parent maintained by selfing (mixed pollination) and rouge out undesirable plants before contamination take place. The female lines *i.e.* gynoecious lines maintained by inducing the staminate flower through the sprays of silver nitrate 200 ppm at two to four true leaf stage and then selfing is carried out. It was observed that 10-11 male flowers appear per 100 nodes. The performance of gynoecious lines are unstable under high temperature and long photo period condtions because of their thermo-sepcific responses for gynoecious stability. That is why the gynoecious cucumber did not receive much attention in the tropical countries. However, few true breeding tropical gynoecious lines in cucumber and muskmelon have been developed at IARI. As a result of development of true breeding line, muskmelon hybrid Pusa Rasraj was developed. These homozygous gynoecious lines are maintained by using GA3, 1500 ppm or silver nitrate 200-300 ppm or sodium thio sulphate 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 monocious male parent to produce F1 hybrid.

Table 4 : Gynoecious lines reported in cucurbits for HSP

Crop	Gynoecious lines
Bittergourd	DBGy-201& DBGy-202 [1]
Muskmelon	Wisconsin 998 (WI 998) [16]
	86-104, 105 &118 [12]

Table 5. Commonly utilized mechanism/methods for developing commercial hybrids [6].

Mechanism	Commercially exploited crop
Bagging/protection of staminate & pistillate flowers + MP	Bottle gourd, pumpkin, watermelon
Pinching of staminate flowers +MP/NP	Bitter gourd, bottle gourd, pumpkin, watermelon
Gynoecism + NP	Cucumber, Muskmelon
PGR & pinching of staminate flowers + NP	Summer squash, winter squash
MP= manual pollination, NP= Natural pollination, PGR= plant growth regulator	

5. Hybrid seed production through chemical sex expression

The hybrid seed can also be produce in cucurbits by the application of chemicals for attaining the sex of cucurbits. Specific chemicals are known to induce femaleness and maleness as desired. The spraying of ethrel (2choloro ethyl phosphonic acid) 200-300 ppm at two and four true leaf stage and another at flowering is useful for inducing the pistilate flower successively in first few nodes on the female in bottle gourd, pumpkin and squash for F1 seed production. The row of male parent is grown side by the side of female and natural cross pollination is allowed. In the absence of insect, hand pollination is possible when two sexes are separate. Four to five fruit set at initial nodes are sufficient for hybrid seed. The complete suppression of male flowers in squash can be achieve at higher concentration of (400-500 ppm) of ethrel applied twice and has made hybrid seed production comparatively easier and nearly 56% of toal squash seed produced in USA is of F1 hybrid. The other chemicals like GA3, (10-25 ppm) in cucumber, MH(100 ppm), ethephone (600 ppm) in squash induces female flowers.

5. ROLE OF MARKER GENE IN HYBRID SEED PRODUCTION OF CUCURBITS”

Use of markers gene to simply the procedure of identification & hybrid seed production was reported by Foster [4] by utilizing glabrous seedling markers in muskmelon which was controlled by single recessive gene. This could eliminate the tedium method of identification of male sterile plant and keep down the cost of hybrid seed production.

In watermelon hybrid seed production can be facilitated by using male sterility coupled with a seedling marker. Some research was initiated to combine the *ms male sterility and dg* delayed-green seedling marker into watermelon and to develop Genic Male-sterile Watermelon Lines with Juvenile Albino Seedling Marker. Three chlorophyll-deficient seedling marker mutants have been described in watermelon [18, 21-22] - Spotted (*Sp*), Delayed green (*dg)* and Juvenile albino *(ja).*

- Spotted (*Sp) - is a dominant mutant that produces yellow* spots on the cotyledon, leaf, and fruit.
- Delayed green (*dg) is a recessive mutant that* produces yellow cotyledons and pale-green, new, true leaves that turn green as development progresses.
- Juvenile albino *(ja) is a recessive* mutant in which chlorophyll deficiency is expressed under short-day conditions.

Ideally, seedling markers that are useful for hybrid seed production should be controlled by a single recessive gene and incorporated into the seed parent because the abnormal phenotype(s) conferred by the recessive gene will not be expressed in the hybrid. Furthermore, off-types in the seed-parent, resulting from outcrosses during reproduction and mixes during seed handling, can be recognized before pollination.

Table 6 : Male sterile line with marker gene

Male sterile line with marker	
MSDG-1 and MSDG-2	Male sterile with delayed green seedling marker [21].
93JMSB-1, 93JMSB-1-1, and 93JMSF3-2	Juvenile albino seedling male sterile line [22].

6. CONCLUSION

In general, productivity is a major criteria to get maximum return but like other vegetable crops, quality and availability of the product during lean periods are also equally important to fetch better price in the markets. Therefore, development of hybrid/varieties with better adaptability under off-season should be undertaken. Further, in order to reduce cost of hybrid seeds it would be appropriate to utilize the available genetic mechanisms for hybrid seed production. In this regard, development of high frequency pistillate/gynoecious lines is advocated. Alternatively, extensive studies on plant growth regulators for large-scale hybrid seed production in many cucurbits should be undertaken. For developing multiple biotic stress resistant lines, validity of already available molecular markers with established linkage may be tested in order to examine their feasible use in breeding programme for development of parental lines.

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