

Genetic Mechanisms for Hybrid Seed Production in Vegetable Crops

A K Joshi

Regional Horticulture Research and Training Station

Dhaulakuan, Sirmour, HP.

Contact: 94184 52348, joshi.ajayram@gmail.com

Introduction:

Heterosis/hybrid vigour, whereby, F_1 falls outside the range of parents with respect to some character (s). This advantage in vegetables was first reported in cucumber in 1916 by Hayes and Jones, and thereafter it has been exploited in other vegetables like tomato, bell pepper, hot pepper, brinjal, cucumber, muskmelon, watermelon, bottle gourd, pumpkin, onion, carrot, cabbage, cauliflower etc.

Heterosis is generally manifested in the following ways.

- High yield in terms of large size, weight or number of fruits.
- Uniformity in shape, size, color and maturity.
- Early maturity
- More number of roots, bulbs, heads, curd knobs and sprouts per units area.
- Resistance to biotic and abiotic factors.

Bases of heterosis:

Genetic, physiological and biochemical causes were assigned earlier for the manifestation of heterosis. In contrast, heterosis has been established as genetic phenomenon, which may involve:

- i) Dominance *i.e* accumulation of favourable dominant genes distributed among parents.
- ii) Overdominance *i.e* favourable interactions between two alleles at the same locus.
- iii) Epistasis *i.e* complementary interaction of additive, dominance or recessive genes at different loci.

Once the dispersion of genes among the parents has been established, the heterosis breeding approach can be applied where over-dominance or over-

interaction is observed. However, in self-pollinated crops where dispersion of genes with partial to complete dominance, epistasis, coupled with over-dominance at some loci is observed, improvement of parental lines and then development of F₁ hybrid should be the basic approach. In case of cross-pollinated crops, if such dispersion arises, development of improved lines through inter population improvement and then developing the F₁ hybrid, should be the need based approach.

Mechanisms for hybrid seed production:

Once the hybrid is developed, commercial seed production becomes the mandate. Seed production is easier in those crops where monoecious, dioecious, self- incompatible and male sterile conditions are present. Various techniques can be used for hybrid seed development.

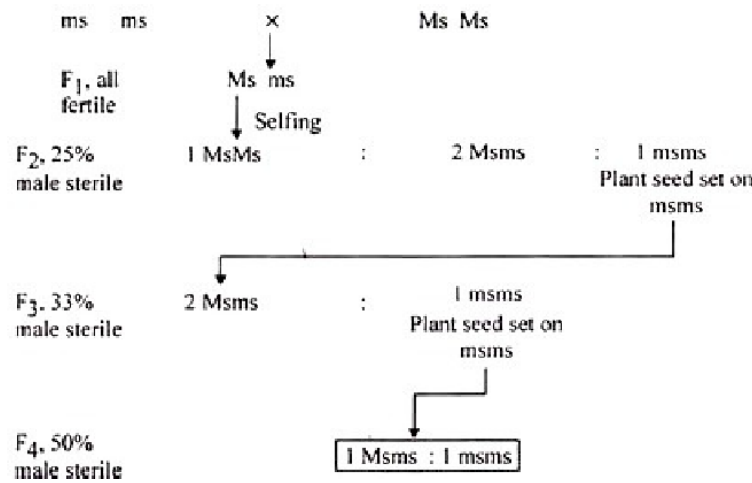
- i) Both hand emasculation + hand pollination: Okra, tomato, eggplant, hot and sweet pepper, owing to hermaphrodite nature of the flower.
- ii) Removal of staminate flowers+ hand pollination: Cucurbits (squashes, bitter and bottle gourd).
- iii) No emasculation + hand pollination : Cucurbits owing to monoecious nature of blooming.
- iv) Hand emasculation + wind pollination: Dioecious vegetables like spinach and asparagus.
- v) Hand emasculation + insect pollination: Tomato where emasculation can be done with ease and stigma receptivity is observed for longer duration of the day.
- vi) Self-emasculating mechanisms+ free insect pollination:
 - Using self-incompatibility *e.g.* turnip, radish, cabbage, Brussels, broccoli, cauliflower and chinese cabbage.
 - Using male sterility *e.g.* GMS in muskmelon, tomato, hot and sweet peppers and CMS in onion, cabbage, carrot, hot pepper and cauliflower.
 - Using gynocicism *e.g.* cucumber.
 - Using gametocide like GA₁/GA₇(onion), maleic hydrazide (tomato, capsicum and onion) sodium 2,3-dichloroisobutyrate (tomato).

Various Mechanisms for Hybrid Seed Production

Genetic Male Sterility:

The male sterility gene usually is recessive and the male sterility may be due to pollen abortion, failure of anther dehiscence, anther abortion, pistillody of the anthers or several other possible causes. Through a combination of crossing, selfing and collecting seed set on sterile plants, a population can be created which segregates in 1:1 ratio for *Msms*.

***Msms* individuals as given below:**

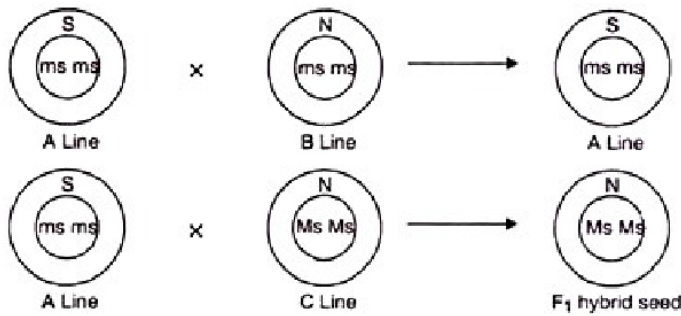


Now, onwards the seed set on *msms* will always segregate into 1 fertile: 1 sterile. This stock seed will be used as the female parent in hybrid seed production. However, 50% male fertile plants will have to be rogued from the seed production rows prior to pollen shedding.

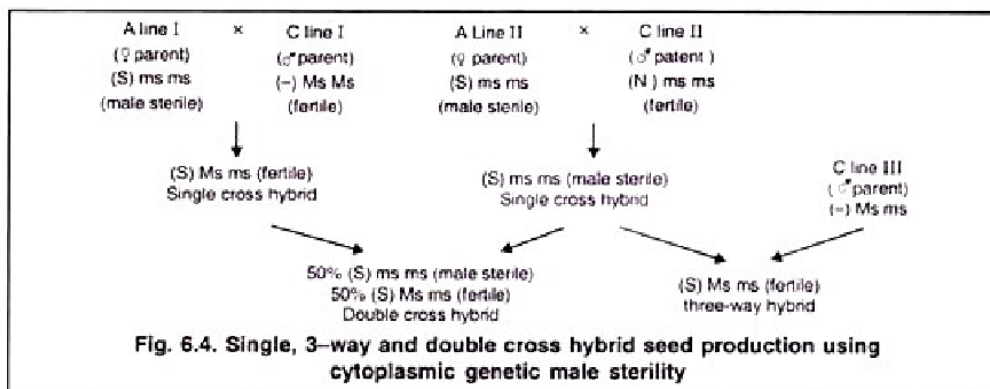
Cytoplasmic Genetic Male Sterility:

The cytoplasmic factor S interacts with recessive nuclear gene *ms* and produces a male sterile line of the genotype *Smsms* which is male sterile and is known as ‘A’ line in the hybrid seed production programme. The genotype with normal cytoplasm is known as *Nmsms* and is male fertile. This is referred to as ‘B’ line and is used as a maintainer for the male sterile line, *Smsms* (A line). After repeated backcrossing, the male sterile ‘A’ line and the maintainer ‘B’ lines become almost isogenic. To produce hybrid seed the ‘A’ line is inter-planted with the pollinator or ‘C’ line of the genotype *NMsMs*.

These steps are demonstrated as follows:



The CMS system can easily be used to produce seed of single, double or 3-way hybrids as given in Fig. proposed by Riggs (1988)



The discovery of cytoplasmic genetic male sterility in onion by Jones and Clarke has been instrumental in application of this system in hybrid seed production on large scale in several crops, particularly the 4 crops, namely, onions, pear millet, sunflower and grain sorghum, and now rice.

Self-Incompatibility:

The term ‘self-incompatibility’ is defined as inability of a plant to set seed when self-pollinated, even though it can form normal zygotes when cross-pollinated and its pollen can fertilize other plants. The genetic control of SI may be gametophytic or sporophytic.

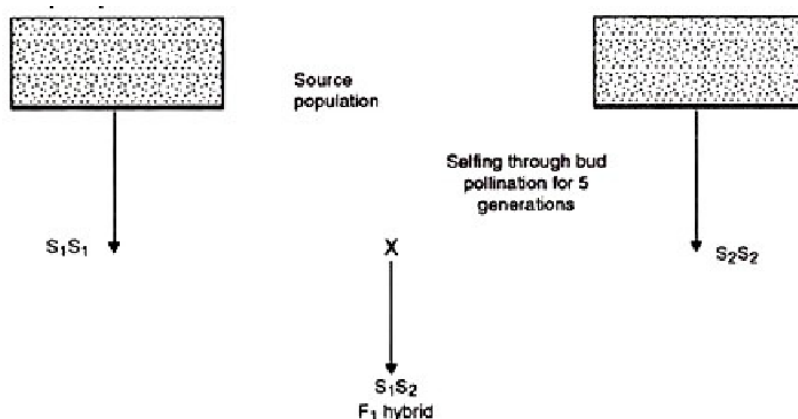
The gametophytic incompatibility is controlled by pollen grain, whereas in the sporophytic system the reaction is between the exine of the pollen grain, which is sporophytic in origin and the papillae of the stigma. This incompatibility is determined by the diploid nucleus of the sporophyte.

In other words, the behaviour of each pollen grain is determined by the diploid genotype. As a result, patterns of inter-allelic interaction ranging from independence to complete dominance may occur in both pollen and style. All kinds of brassicas have a sporophytic SI system being strongest in kale and weakest in summer cauliflower. The S-allele system is complex with about 50 alleles at a single locus.

It is known that glycoproteins have an important role in causing self-incompatibility. Increased rate of synthesis of these S- locus specific glycoproteins coincides with the onset of the incompatibility reaction.

Therefore, while developing parental inbred lines this reaction is overcome by pollinating the flowers in the bud stage during selfing. The commercial F₁ hybrids are produced by inter-planting 2 self-incompatible but cross-compatible inbred lines. The inbred lines are maintained through bud-pollination.

A simple system is shown below:



Thompson (1964) recommended production of ‘triple-cross’ hybrid kale, a hybrid type which can be produced only with the sporophytic SI. The triple-cross is a hybrid between 2 three- way crosses, that is (ABxC)x(DEXF).

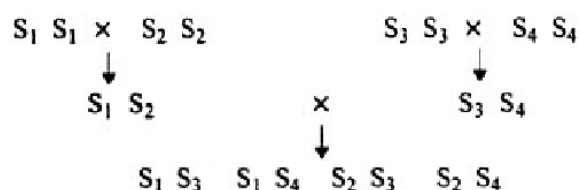
With this type of cross there is a minimum of bud- pollination (needed to maintain inbred lines), and the simple cross, three-way cross and triple-cross can also be made without emasculation if proper self-incompatibility alleles are present in the 6 inbred lines. If kale has gametophytic system of incompatibility, the triple-cross could have been impossible because some plants in each three-way cross could be cross compatible.

In cole crops (*Brassica oleracea* L), hybrid seed production makes use of the sporophytic incompatibility mechanism. Due to the great number of alleles, cabbage populations, generally, consist of plants that are heterozygous at the incompatibility locus. With bud pollinations, inbred lines may be developed from such a population.

If inbred lines with the constitution $S_1 S_1$ and $S_2 S_2$ which have been tested for specific combining ability, are produced by bud-selfing and grown next to one another, **seeds only with the allelic combination $S_1 S_2$ will be formed.**

This is due to the fact that the S_1 pollen cannot fertilize any egg cells of the $S_1 S_1$ plants, nor can S_2 pollen fertilize any egg cells of the $S_2 S_2$ plants. To prevent occurrence of pseudo-fertility and pollination of parent lines by selfing during hybrid seed production, it is necessary to develop inbred lines with **strong dominant alleles.**

If the seed production after a single cross is too low and therefore, uneconomical, double crosses can also be produced according to the following scheme:



As a result of the pseudo-fertility, e.g. due to high temperatures or late flowers, pollination within parental lines leading to the development of selfed or sibbed seeds, must always be taken into account. Depending upon the cole crop species, the plant material, environmental conditions, and modifying genes, their proportion may amount to as high as 40%.

Manipulation of Sex Expression:

Production of hybrids in certain crops like cucurbits is possible through manipulation of sex expression. Cucumber plants are typically monoecious producing male and female flowers on the same plant. Now gynoecious lines are available. These lines produce only female flowers and can be easily used as female parent to produce F_1 seed of cucumber on large scale.

Multiplication of such lines is made possible by induction of male flowers through spraying of gibberellic acid i.e., GA 4/7 (2000 ppm) or Ag NO₃ on seeding leaves. On the other hand, monoecious lines of squash can be rendered female by applying ethephon to suppress male flower production. Two applications of ethephon @ 600 ppm at the 2-and 4-leaf stage result in complete male flower suppression during the fruiting stage. Ethephon is being used on commercial scale for production of hybrid seeds in squash.

Chemical Hybridizing Agents (CHAs):

The CHAs are defined as the chemicals which cause pollen abortion and render the treated plants male sterile without affecting ovule fertility. These chemicals are also called as gametocides. To be really useful in F₁ hybrid seed production, CHAs should have no mutagenic effect, be easy and economical to apply, have wider applications and have no harmful side effects.

Although CHAs have relatively larger application in major cereal crops, their use in vegetables is yet to be commercialized. Positive responses have been achieved with GA₃ and GA 4/7 in lettuce and onion, with maleic hydrazide in tomato and onion, and with sodium 2, 3-dichloroisobutyrate in tomato.

Biotechnological Approaches in Hybrid Seed Production:

The conventional F₁ hybrid seed production systems have depended on hand emasculating, genetic, cytoplasmic genetic male sterility and self-incompatibility, etc. In most cases these systems have some disadvantages, involving cost, efficacy, ease of use, reliability and effect of environmental factors leading to breakdown of the systems.

The production of cell-toxic **ribonuclease enzyme in the tapetal cells (which nourish cells that develop into male gametes), thereby causing male sterility**. For technical reasons, mother gene, bar conferring resistance to herbicide glufosinate was hooked to the barnase cytotoxic gene.

This gene cassette helps in retaining only male sterile plants from mixed progenies in commercial hybrid seed production plots. For commercial crop production, the F₁ hybrids have to bear normal bisexual flowers. To restore male fertility in the F₁ hybrids, they used *barstar* gene from the same bacterium, *B. amyloliquefaciens*. The product of *barstar* forms a complex with *barnase* enzyme and nullifies the toxic effect of *Rnase*.

Incorporating *barstar* in the male parent which can be used to pollinate *barnase* carrying male sterile, but female fertile plants provided a perfect system for commercial hybrid seed production. This system has been patented by M/s Plant Genetic Systems (PGS) Ltd., Belgium.

In 1994, Proagro Seed Company Ltd. (PSCL) made a joint venture company with Plant Genetic Systems, Belgium to develop transgenic plants in mustard (*Brassica juncea*) and in vegetables. The technology of Plant Genetic Systems known as Seed-link technology was already tested in *B. napus* in Canada. Proagro Seed Company has converted *B. juncea* lines with the help of transgenes, *barnase*, *barstar* and *bar*, and stabilized the hybridisation system in mustard to produce hybrid seed on commercial scale. The limited field study trials for environmental and food safety as per the requirements of Department of Biotechnology (DBT) were conducted in India but commercial release did not materialize.