

F₁ HYBRIDS — ADVANTAGES AND SEED PRODUCTION

RICHARD H. JONES

Yates Seeds

P.O. Box 124

Narromine, New South Wales

INTRODUCTION

Crop improvement through hybridisation of selected parent plants has been practised for many years. In its broadest sense hybridisation is simply a cross between two plants but in practical terms hybridisation is usually considered to be the crossing of individuals of unlike constitution such as species of the same genera or inbreds of species which have different genetical characteristics.

In the natural environment hybrid crosses between species of the same genus may occur indiscriminately but the number of natural hybrids which develop is limited. However in commercial species hybrid seed production utilises inbreds which are developed with certain desirable characteristics and hybridisation of these lines produces hybrids with specific advantages.

With certain vegetables, grains and flowers species different genetic systems are used to enforce controlled hybridisation in the production of commercial hybrid cultivars. In this paper I want to outline the advantages of hybrids, the basis of hybrid seed production, and some of its associated problems.

What are the advantages of hybrids? With vegetables, trial work and field evaluation has indicated that there are usually significant advantages in using hybrid cultivars. Since their introduction the number of hybrids in use has increased considerably (14); e.g. in 1976 hybrids constituted 65 percent of Brussels sprout cultivars, 69 percent of cucumber cultivars, 40 percent of cabbage cultivars, and 65 percent of tomato cultivars. Dorsman (2) summarises these advantages as including:

(i) Productivity. There may be yield advantages with onions and carrots and there are significant yield advantages with *Brassica* spp.

(ii) Earliness. With cabbages and kohlrabi the hybrids tend to be earlier in maturity.

(iii) Uniformity. Generally hybrid cultivars tend to mature uniformly which assists in reducing costs in those crops where "once-over" harvesting is not the usual practice, e.g. cabbage, cauliflower, and broccoli.

(iv) Disease resistance. Evidence is available for improved disease resistance in cabbage hybrids (13); Gabelman and other onion breeders have made significant gains in root disease resistance in onions, in particular Fusarium (*Fusarium oxysporum*) and pink root (*Pyrenochaeta terrestris*) (personal communication).

(v) Selection for quality in hybrids has shown results such as better skin quality in onions which is essential for the export trade.

With flowers, e.g. petunias and marigolds, the hybrids are more attractive with larger flowers and greater uniformity, and work is proceeding to improve disease resistance.

Basis of F₁ Hybrid Seed Production. It is possible to utilise several methods for producing hybrids based on the genetic characters of the species involved (See Table 1).

Table 1. Systems of F₁ Seed Production.

System	Crop
A. Genetic/cytoplasmic	Carrot
(i) Male sterility	Leek
	Onion
	Petunia
	Radish
	Tagetes
	Tomato
(ii) Self-incompatibility (sporophytic or gametophytic)	Ageratum
	Bellis
	Broccoli
	Brussels sprout
	Cabbage
(iii) Dioecy	Spinach
(iv) Monoecy	Sweetcorn
B. Mechanical or Manual	Snapdragon
	Sweetcorn
	Tomato
	Pansy
	Cucurbits
C. Chemical (male suppression or gametocidal)	Cucurbits

A. Genetic.

1. Cytoplasmic male sterility

Cytoplasmic male sterility is not a regular mechanism for controlling hybridity in natural populations (1). Male sterile plants have been detected, probably as the result of mutations, but they do not have any advantages. However, in domesticated plants cytoplasmic sterility can be utilised to produce hybrid cultivars.

Some species, such as onion (*Allium cepa*), carrot (*Daucus*

carota) and *Petunia* have a specific sterile cytoplasm (S cytoplasm) which renders the plants sterile, either because the anthers are nonfunctional, or no viable pollen is produced. These sterile plants have the genotype, S msms, and an identical line with normal cytoplasm, N msms, used as the pollen parent to maintain the sterile line.

For crops where only the vegetative or floral product is required, e.g. vegetables and flowers, the resultant hybrid from a cross of the sterile inbred and a pollen parent may be fertile or sterile. However, where seed is harvested, e.g. sorghum and corn, pollen restoring genes are essential in the pollen parent. The pollen parent must have genotype, N MsMs, and the presence of the Ms gene with the sterile cytoplasm, i.e. S Msms, in the hybrid will result in fertility.

The major problem with cytoplasmic sterility is the maintenance of the male sterile line. In certain species such as carrots (2) if the temperature rises above a certain level there can be restoration of fertility which can result in sibbing during seed production. This phenomena has been suspected in onion (Pryke pers. comm.), sorghum, corn, and sunflower. In petunia cytoplasmic male sterility is known but is closely linked to flower bud abortion and the problem has not yet been overcome (5).

Another problem often encountered in maintaining male sterile lines is the usual occurrence of a low frequency of fertility restorer plants (i.e. NMsms, NMsmMs) in the maintainer male, probably as a result of mutation. This causes little problems when producing stock seed but in the subsequent commercial seed production exercises, the presence of SMsms individuals necessitates extensive roguing to keep the level of inbreds in the hybrid seed to a minimum.

In recent years cytoplasmic sterility has been used experimentally for hybrid *Brassica* seed production. A sterile R cytoplasm was developed from *Raphanus* spp. (12) but it has some inherent problems. The seedlings tend to be chlorotic and the female flower, which lacks anthers, may also have poor stigma development and nectaries may be absent. The latter factors can affect hybrid seed production by reducing the attractiveness of the plants to pollen vectors such as bees, but Williams (pers. comm.) believes that these factors could be selected against.

(ii) *Self Incompatibility*

Self incompatibility is an important mechanism for maintaining cross pollination in natural communities and has been used for the production of commercial hybrids of *Ageratum* and members of the *Brassica* genus (see Table 1).

Self incompatibility reduces the ability of pollen from one plant to fertilise the ovum of the same or similar plant type and thus prevents selfing or sibbing. Prevention of selfing in natural population reduces the risk of inbreeding depression which is known to reduce the vigour and survival of species. The degree of incompatibility in species varies from 100 per cent to a slight preference for foreign pollen.

There are two main types of incompatibility — sporophytic or gametophytic (1,6,9). Sporophytic incompatibility is due to pollen-stigma interaction where pollen does not germinate because of lack of "recognition" or stimulation by the stigma. With gametophytic incompatibility the growth of pollen tubes is slow and deformed and proceeds very slowly if at all and blossoms may abort before fertilisation. In addition the pollen tubes may lose direction and never find the ovule.

Nieuwhof (9) designated self incompatibility as being controlled by a series of alleles, namely $S_1S_2S_3 \dots$. In natural populations there are a range of S alleles which will allow for crosspollination.

With sporophytic incompatibility, as in Brussels sprouts, the pollen will only germinate if the plants possess no corresponding S factors, e.g. $S_{12} \times S_3S_4$, but will not germinate if both the parents have 1 or more S factors in common, e.g. $S_1S_2 \times S_1S_3$.

The S alleles do not always act independently and at times one S factor may be dominant over another and varying responses to the S alleles between stigma and pollen may give rise to pollination in some cases (1,9).

Gametophytic incompatibility is not as complex and with $S_1S_2 \times S_3S_4$ crosses half the pollen will be compatible and pollen tube growth will be slow and ineffective (1). With commercial hybrid seed production, selections are made so that the inbreds are homozygous for their S factor and that crosses are made between inbreds that are compatible with each other. With the wide range of S alleles this should not be difficult.

Self incompatibility in *Brassica* is strongest in freshly opened flower when the flowers are most attractive to insect pollinators. However, a few days after the flower opens the incompatibility breaks down (9) and self pollination occurs. Factors which influence breakdown include excessively high temperatures (over about 30°C, Ascher pers. comm.) and the age of the flower.

Usually *Brassica* are maintained by bud pollination, i.e. prior to the flower opening the bud is opened manually, and pollen is transferred to the stigma. At this stage of develop-

ment the incompatibility of the flower is not complete and pollination can occur (9).

The major problem with the use of self incompatible reactions for hybrid production is the possible breakdown of the incompatibility reaction with the resultant sibbing due to poor maintenance in inbreds or due to environmental conditions. Occasionally there can be problems with insect vectors but this is not a serious problem.

B. Mechanical

To ensure cross pollination and therefore hybridisation it may be necessary to physically emasculate one inbred. With sweet corn the tassel of the female plant is removed by hand or machine and thus the pollen from the male plant is the only pollen source.

With tomato and petunia and some hybrid cotton the flowers are hand emasculated with tweezers and pollen is transferred by hand from the pollen parent and placed on the receptive stigma. Thus the F_1 hybrid seed is expensive.

C. Chemical

Hybrid cucurbit seed can be produced if one line is sprayed with ethephon (Ethrel) to suppress male flower production. Timing is critical to ensure that the female plants do not produce pollen.

Pollination as a Factor in Hybridisation. To ensure maximum seed set in both natural populations and controlled hybrid seed production units, it is essential that (i) anthesis in the donor parent coincides with the period when the stigma on the female parent is receptive and, (ii) the agent effecting pollination is available.

There are two major types of pollinating agents — abiotic such as wind and water, and biotic such as insects, birds, and bats (6). Wind pollination, which is probably the most significant abiotic agent is important in the Graminae, Cyperaceae, and Juncaceae, but it is very inefficient because vast masses of pollen must be released and the efficiency of pollination depends on the wind velocity and direction. Plants adapted to this pollination process have light, smooth dry pollen and in some cases have evolved air sacs to increase buoyancy (6). Pollen can only be released under warm, dry conditions and is rapidly lost from the atmosphere if rain occurs. Pollen viability in the atmosphere is short lived due to external factors, including ultra violet radiation.

Biotic agents often have a fixed relationship with the blossom to be pollinated. The pollinating agent usually is attracted to the flower because of some specific attractant such as pol-

len, nectar, or odour (6). Pollen, a major attractant, is a primary source of protein as well as fat and sugars. It is eaten directly by many insects and in cases such as bees is an intrinsic part of the diet of the larvae. Insects will actively forage for the pollen primarily as its food source but during foraging become covered with pollen (which adheres to body hairs) and pollination is achieved when the foraging insect brushes against a receptive stigma. Pollen from many insect-pollinated crops tends to be sticky and the grains adhere in lumps to the vector. In some cases the pollen may be dry but the flower structure is such that the insect's body becomes sticky from contact with the stigma and the pollen can adhere to body hairs (6).

Nectar is also recognised as a primary attractant for many insects and is found in most angiosperms (6). Nectar, which is a primary source of soluble sugars, is secreted from nectaries located within the blossoms at the base of the corolla and their secretions tend to coincide with the periodicity of the pollination process. Thus secretions are high when pollen release occurs to act as an insect attractant. Nectar gathering insects become coated with pollen in much the same way as pollen collecting insects do.

Thus pollination may be classified as an accidental process, which occurs as a result of active foraging for a food source by insects. However, plants have evolved so that they can attract specific pollen vectors so as to achieve pollination. Insects other than bees usually only benefit specific crops at specific times. Moths, butterflies, and wasps only consume nectar for their bodily requirements and not to provide for their nest (8) and thus pollen adhering to their bodies as they forage for nectar can facilitate pollination.

With commercial seed production dependant on biotic pollination it is essential to maximise all conditions to ensure that adequate pollination is achieved. Natural insect population such as ants, aphids, bees (honeybees and other colonial, gregarious, and solitary bees) beetles, butterflies, midges, moths, thrips, and wasps are involved, but the most important vector is bees (8), and their population can be controlled.

Flies have a rôle in pollination of open-pollinated crops such as carrot (*Daucus carota*) and onion (*Allium cepa*) and have been used by plant breeders as pollinating agents in small scale seed production cages. However flies are not of much benefit in hybrid seed production in field situations.

To achieve hybridisation in commercial fields it is essential that the insect population, especially bees, is maintained at a high level but the level of bees in a seed production unit is

not necessarily indicative of the pollination potential. With cytoplasmic male sterility the level of bees in both male-sterile rows and pollen rows should be evaluated. Foraging bees rarely collect both nectar and pollen at the same time. Since individual bees have been programmed to collect either pollen or nectar, pollen-collecting bees are unlikely to visit the male sterile rows, except by chance, because of lack of pollen. Once bees have found a food source they will show remarkable fidelity to that row. Erickson, *et al.* (3) has studied this phenomena in carrot (*Daucus carota*) and has demonstrated that significant numbers of bees will stay on the same flower type (male sterile or normal) with only a low percentage of the population drifting onto a different flower type within a few days. To ensure that pollen transfer will occur the bee population must include a high proportion of nectar collecting bees which will forage throughout the seed crop. Where possible the visual differences between inbreds should be minimised to reduce the ability of bees to differentiate between the two inbreds.

To maximise the transfer of pollen it is essential that the female:male ratio is not too large. Erickson and Gabelman (3) found that with onion, seed set in female rows 7 feet from a pollen source had decreased by 50 percent when compared to female plants adjacent to the pollen. In *Brassica* the ratio is usually 4:2 (F:M) whilst in carrot and onion it is 3:1 (F:M).

Even with an adequate insect population in the area, hybrid seed production may still fail. Bees show marked preference for specific crops and invariably prefer to be working on clovers and lucerne (alfalfa) or native vegetation than onions (11). Thus to ensure maximum seed set it is essential to maintain a high level of native bees, i.e. bees which are new to the field, during the period of maximum seed set. Factors which may adversely affect attractiveness of onions include high nectar sugar levels which may be affected by the exposed nature of the flower, high potassium levels of the nectar, odour, and lack of visual attractiveness of the flower (Peterson pers. comm.).

If the level of nectar produced is inadequate, as can occur with the new R cytoplasmic sterile *Brassica*, then the bee population in the sterile row will be insufficient to ensure pollen transfer and seed set.

Hybrid seed, using self-incompatible inbreds, with both lines male-fertile, is not without problems. Bees are able to detect variability within colour of different inbreds of brassicas and thus may retain their fidelity for one row and not ensure hybridisation (7). The ultraviolet light reflection of the

petals differs and bees can detect this difference. A similar observation was made in carrots (Erickson pers. comm.).

SUMMARY

Utilisation of hybridisation to produce new hybrids in natural environments or under controlled conditions such as commercial seed production units requires systems to enforce pollen transfer for seed set and a mechanism for pollen transfer. In this paper I have outlined the genetic basis for hybridisation and the mechanisms for pollen transfer available to ensure effective seed set.

LITERATURE CITED

1. Allard, R.W., 1960. Principles of Plant Breeding. John Wiley & Sons, Inc., New York.
2. Dorsman, 1976. in Heterosis in Plant Breeding, A. Jonassy and F.G.H. Luptan eds., Elsevier Scientific Publishing Co. Amsterdam, pp 197-208.
3. Erickson, H.T. and Gabelman, W.H., 1956. The effect of distance and direction on cross pollination in onions. *Proc. Amer. Soc. Hort. Sci.* 68:351-7.
4. Erickson, D.F., Peterson, C.E. and Wemer, P., 1979. Honey bee foraging and resultant seed set among Male-fertile and cytoplasmically male-sterile carrot inbreds and hybrid seed parents. *Jour. Amer. Soc. Hort. Sci.* 104:635-638.
5. Ewart, L.C. 1981. Utilization of flower germplasm. *HortScience*, 16(2):-135-139.
6. Faegri, K. and van der Pijl L., 1979. The Principles of Pollination Ecology 3rd ed. Pergamon Press Oxford.
7. Faulkner, G.J., 1974. Factors affecting field scale production of F₁ hybrid Brussels sprouts. *Ann. Appl. Biol.* 77:181-190.
8. McGregor S.E., 1976. Insect Pollination of Cultivated Crop Plants. Agriculture Handbook No. 496 A.R.S. USDA.
9. Nieuwhof, M., 1969. Cole Crops. World Crops Books, Leonard Hill, London.
10. Poehlman J.M., 1959. Breeding Field Crops, Holt, Rinehart, and Winston, Inc. N.Y.
11. Waller, G.D., Waters, N.D., Erickson, E.H., and Martin, J.H., 1976. The use of potassium to identify onion nectar collecting bees. *Envir. Ent.* 5(4) 780-782.
12. Williams P.H. and Heyn, 1981. in Chinese Cabbage — Proceedings of the First International Symposium, AVRDC. Taiwan, pp 293-300.
13. Williams P.H., Walker, J.L. and Pound G.S., 1968, *Phytopathology* 58:79-796
14. Wills A.B. and North C., 1978, in: Symposium on seed problems in horticulture. *Acta Hort.* No. 83 pp 31-37.