



Breeding F₁ hybrid varieties of vegetables

T. J. Riggs

To cite this article: T. J. Riggs (1988) Breeding F₁ hybrid varieties of vegetables, Journal of Horticultural Science, 63:3, 369-382, DOI: [10.1080/14620316.1988.11515871](https://doi.org/10.1080/14620316.1988.11515871)

To link to this article: <https://doi.org/10.1080/14620316.1988.11515871>



Published online: 27 Nov 2015.



Submit your article to this journal [↗](#)



Article views: 14



View related articles [↗](#)



Citing articles: 5 View citing articles [↗](#)

Review Article

Breeding F_1 hybrid varieties of vegetables*

By T. J. RIGGS

Institute of Horticultural Research, Wellesbourne, Warwick CV35 9EF, UK



SUMMARY

The last fifty years have seen the development of methods for breeding F_1 hybrids in vegetables, opening new possibilities for improvements in earliness, uniformity and quality and giving breeders the advantage of built-in exclusivity for seed production and sale. Total productivity in vegetables is often less important than marketable yield, which is largely determined by uniformity and quality. Many vegetable crops are harvested before full maturity for their greater tenderness or to meet specific size requirements. Phenotypic uniformity of an F_1 hybrid depends upon genetic homozygosity in the inbred parents but inbreeding depression may rapidly reduce the number of surviving lines. Ideally, large numbers of inbreds should be selected for phenotype and for general and specific combining abilities. Natural mechanisms to ensure cross-fertilisation between parent inbreds are used in large-scale seed production, although hand emasculation or chemical control of sex expression may be used in some crops. Chemical hybridizing agents must conform to stringent criteria to be really useful and are not yet widely used in vegetables. The success of hybrid seed production may be reduced by low vigour in the inbred female parent or by the preference of pollinating insects for particular parental lines, such that there is insufficient transfer of pollen from one to the other. The occurrence of 'sibs', resulting from weak self-incompatibility, can seriously contaminate a hybrid seed sample. Future prospects include the use of DNA clones to identify S-alleles and of somatic hybridization to transfer cytoplasmic male-sterility.

F_1 HYBRID breeding is prominent among the methods used in the improvement of vegetable crops, especially in out-breeding species. In a review of heterosis in vegetable crops, Pearson (1983) cites a report by Hayes and Jones (1916) on first-generation crosses in cucumber as probably the first suggestion that hybrid vigour be exploited in vegetable breeding. Pearson himself, in 1932, proposed field-scale production of hybrid seed in cabbage using the natural self-incompatibility system, and Jones and Clarke (1943) described practical methods for the commercial production of F_1 hybrid onion varieties using cytoplasmic male-sterility. Today hybrid varieties pre-

dominate in several vegetable crops and are available in many others (Table I), despite the higher cost of seed (Table II).

Modern European horticulture is, influenced by rising costs of labour and energy, increasing international competition and the changing demands of the consumer and the processing industries. New varieties must be superior in reliability of yield and quality, and for some crops, in their adaptability to mechanical harvesting. Hybrids offer opportunities for improvements in productivity, earliness, uniformity and quality and for the rapid deployment of dominant genes for resistance to diseases and pests. Breeders and seed producers charge higher prices for seed of hybrid varieties (due in part to the higher costs of production) and also benefit from the built-in exclusivity conferred by the ownership and maintenance of the parent lines of a successful hybrid. Cultivars

* This review is based on a paper commissioned by the Food and Agriculture Organisation of the United Nations (FAO) for a Regional Seminar on hybrid seed production organised by FAO/DANIDA at Surabaya, Indonesia in November 1986, and published by permission of that organisation.

of crops grown for a vegetative product are not required to produce seed, and an F_1 hybrid variety may be self-sterile; even if fertile however, seed produced from it will not be genetically uniform. The grower must therefore return to the breeder or his agent for each new seed supply.

TABLE I
Vegetable crops for which F_1 hybrids now exist and the approximate number of years since their introduction. (From Wuttwer, 1983)

Crop	Time since hybrid introduction
Sweet corn	40-50
Onion	40-50
Tomato	40-50
Summer squash	40-50
Ordinary cabbage	35-45
Cucumber	30-40
Watermelon	30-35
Muskmelon	30-35
Asparagus	30-35
Pepper	25-30
Spinach	20-30
Broccoli	20-25
Chinese cabbage	20-25
Eggplant	15-20
Brussels sprouts	15-20
Chinese radish	10-20
Carrots	10-20
Red beet	5-8
Celery	5-8

TABLE II
Proportions and prices of F_1 hybrids of vegetables in the United Kingdom

Crop	% F_1 varieties	Price range per kg seed (£)	
		OP ¹	F_1
Brussels sprouts	94	80-90	484-585
Cabbage	55	44-177	220-410
Carrot	39	30-47	136-180
Onion	49	35-90	66-225
Tomato	69	4-5 ²	19-23 ²

¹ Open-pollinated varieties
² per 1000 seeds

Data from 1986 catalogues.

TABLE III
Outlets available for carrots (from Fordham and Biggs, 1985)

Purpose	Shoulder diameter (mm)
Freezing	up to 26
Small roots for canning whole	19-32
Medium roots for canning whole	32-45
Prepacking	20-50
Loose packs for market	25-70
Large roots for slicing, dicing	Over 45

The advantages of F_1 hybrids in terms of crop production may be difficult to quantify. Yield *per se* is often of little significance in vegetable crops since many are harvested before full maturity and may be graded before marketing. Thus marketable yield is a variable proportion of the total yield at harvest, which frequently does not represent the biological potential of the crop.

Economic factors play an important part in determining when and how a crop is harvested (Fordham and Biggs, 1985). In the case of vegetables grown for their storage organs, delaying the harvest may result in higher yields but often at lower unit prices. Similarly, hand-harvesting and trimming of Brussels sprouts or cauliflower may achieve high premia for quality but at increased labour costs. The tenderness and flavour of vegetables is often best before full maturity and in addition there may be a market preference for small size, as in courgettes or pickling cucumbers. In carrots, size is particularly important, depending on the marketing outlet (Table III) and may be controlled partly by choice of variety and by population density but also by time of harvest.

The trend in modern varieties of open-pollinating (OP) vegetables has been towards greater genetic uniformity to meet the stringent requirements of the industry. Breeders are using fewer, more thoroughly selected parents, and in several crops F_1 hybrids are displacing OP varieties. These hybrids represent a fundamentally different type of crop population, with enhanced uniformity and a reduced genetic base (Simmonds, 1979). The trend towards F_1 hybrids may be neither necessary nor desirable in countries where vegetable production is less mechanized, where markets are local rather than remote, and where spreading the harvest may, in the absence of storage facilities, assist in maintaining an even supply. Again, dependence on a seed company for supplies of costly hybrid seed could be a problem to farmers in developing countries who might be better advised to grow their own seed of locally selected OP cultivars.

HETEROSIS FOR YIELD AND QUALITY

The genetical basis of heterosis

The phenomenon of hybrid vigour, expressed particularly in the first generation (F_1) following the crossing of cultivars or inbred lines, has been known for more than a hundred years. The term heterosis, coined by G. H. Shull, suggests a mechanism dependent on heterozygosity and therefore not fixable in the homozygous state. This would be the case if hybrid vigour were due to true overdominance or to tight linkage in the repulsion phase at some incompletely dominant loci (Jinks, 1983). Much evidence suggests that apparent overdominance is, in fact, due to non-allelic interaction and linkage disequilibrium and that heterosis is principally a result of the bringing together of unidirectionally dominant alleles dispersed between the parental lines. If this simple additive-dominance explanation holds, with little contribution due to tight linkages or to true overdominance, then heterozygosity is not an essential prerequisite for high performance, uniformity and stability of performance (Jinks, 1983). From a pragmatic point of view the mechanism of heterosis is of less concern to the breeder than the fact that F_1 hybrid breeding has been highly effective (Sprague, 1983), but the dispersion explanation implies that the properties of inbred lines produced from a cross should be predictable. The expected frequency of lines with performance equal to or better than the F_1 may be estimated and considered by the breeder in terms of the chances and advantages of selecting the best inbred, rather than seeking to produce the heterozygous equivalent as an F_1 hybrid (Jinks, 1983).

Plant breeders at the Institute of Horticultural Research (Wellesbourne) have produced large numbers of inbred lines of Brussels sprout and onion by single seed descent in an attempt to identify lines which are as vigorous and high-yielding as current F_1 hybrids (Smith *et al.*, 1985) or which represent a significant improvement over current inbreds used as parents of F_1 hybrids (Werner *et al.*, 1988). First results suggest that outstanding inbreds have not been

produced in the first cycle of selection (Smith *et al.*, 1986; Dowker *et al.*, 1987). Hybridization of the best lines of Brussels sprout, followed by a second cycle of inbred line production, using anther culture (Ockendon, 1984, 1985) may generate lines with improved performance. If sufficiently self-compatible these could be marketed as varieties in their own right; others may be used as vigorous parental lines for F_1 hybrids. In either case, the cost of seed production should be reduced.

The studies described above have revealed considerable inbreeding depression, particularly amongst the inbred onion lines, and it is this lack of vigour in the inbred parents of F_1 hybrids that limits the yields of hybrid seed. It is well known that cross-fertilizing species carry relatively large loads of deleterious recessives in a heterozygous condition. Sprague and Schuler (1961) described an experiment in which an OP variety of maize was self-pollinated and reconstituted from S1 seed from ears exhibiting no detectable segregation for seed and seedling characters. After only four generations of open pollination this reconstituted stock, which had been purged of deleterious recessives, showed a frequency of segregation of approximately half that of the original population. The authors concluded that such rapid accumulation of new mutations indicated a high mutation rate or strong heterozygote advantage. This suggests that vigorous inbreds, selected as parents for F_1 hybrids, could deteriorate during their maintenance unless periodically checked.

Yield

For reasons discussed above, the advantages of hybrid cultivars in many vegetable crops may have more to do with enhanced uniformity and quality, resulting in lower harvest costs and higher marketable yields, than with greater productivity *per se*. Moreover, Dowker and Gordon (1983) pointed out that heterosis for yield may be misleading if calculated simply with respect to the better parent line. F_1 hybrids have commercial value only if

they out-perform the best available open-pollinated varieties. After examining the literature for onion, a crop in which the yield advantages of F_1 hybrids have been extolled for 40 years, these authors concluded that little clear evidence had been published to show the commercial advantages of hybrids. Thus Sypien *et al.*, (1978) compared OP varieties and F_1 hybrids in Poland over three years and found little difference in total yield, although the F_1 gave slightly higher marketable yields because of better uniformity and shape with fewer rotten bulbs. On the other hand, data from a single trial conducted by Dowker at Wellesbourne and quoted by Dowker and Gordon (1983) demonstrated substantial yield advantages (from 14% to 67%) for 12 F_1 hybrids over four commercially grown OP varieties.

In carrot, French private breeders, using material supplied by INRA, produced hybrids which, although not higher yielding than the good standard selections, were more homogenous and earlier, with better colour and a much lower frequency of green shoulders, giving better grading on the market (Bonnet 1978). Baker (1978) estimated that hybrid carrots in Michigan, USA were conservatively 25% higher-yielding with 20% more "pack-out" than OP varieties. Results from Sweden (Axelsson, 1976) also indicated considerable heterosis for yield.

In Brussels sprout, F_1 hybrids have changed the whole character of the crop from one that was highly variable, wasteful and hand-picked, giving yields of 6 to 7 t ha⁻¹, to one that is now highly uniform, machine-harvested with no waste and yielding 17.5 to 25 t ha⁻¹ of high quality produce (Watts, 1980).

In tomato, a self-pollinating crop but one which can be emasculated and hybridized by hand relatively easily, hybrid varieties dominate. Tomato breeders thus have exclusive control over seed production, and this, apart from any improvements in performance resulting from heterosis, probably provides the principal incentive for concentrating on F_1 hybrid breeding. Reported expressions of heterosis include

earliness, increased total yield, higher adaptability to unfavourable environments, uniformity of produce and greater plant vigour (Yordanov, 1983). According to Yordanov, heterosis for yield and earliness is most pronounced at the beginning of the fruit-bearing period and gradually declines thereafter. However, the data presented were from one parental combination only and may not be typical. Heterosis for yield appears to be due to the greater number of fruit rather than greater mean fruit weight. This is the case also for cucumbers, though the number which are acceptable for use is the economic measure. In trials in New York State, hybrid varieties produced a higher proportion of arrested or aborted fruit – possibly due to overloading of the plant – than OP varieties (Pearson 1983).

Quality and associated characters

Marketable yields in vegetables is a function of total productivity and of quality of product. Size of usable product may be a quality character as well as a component of yield and indeed, as discussed above, quality considerations, in terms of size and shape, may necessitate some sacrifice of gross yield. In most Brassica crops, large size is not desirable, although in Australia extra large cabbage heads are desired and are sold to the consumer in segments (Pearson, 1983).

Gabelman (1974) stressed the importance of synchronised maturity of the onion crop in establishing uniformity of quality for storage. OP varieties grown in the northern United States are subject to infection by neck rot and soft-rot organisms when stored. This occurs in immature bulbs or those which matured too early and remained too long in the field. These problems are eliminated when a hybrid of appropriate maturity is grown. In a report on the leading varieties of onion in eastern Europe, Doruchowski (1984) referred frequently to the better keeping quality of the new F_1 hybrid varieties. In Israel short-day yellow onions have been intensively bred from a narrow base. Hybrids have been produced (Peters, 1984) which surpass the two main OP varieties for yield and are

also better for firmness, uniformity of shape and skin adhesiveness. Several very early hybrids showed improved storage quality over the OP varieties.

It is well known that in spaced vegetables such as sprouts and cabbage, F_1 hybrids can give remarkably good plant-to-plant uniformity. The situation is different in row crops such as carrot and onion where there is irregular spacing of plants within the row and high density, causing competition between plants. Work at Wellesbourne (Dowker 1978) showed no consistent differences between the variability of root size between hybrid and OP varieties of carrot. Dowker (1978) and others have referred to the importance of emergence time of individual plants in determining final root size of carrot. Uniformity of emergence is associated with uniformity of embryo length (Gray *et al.*, 1986) but studies by Umlauf (1978) and by Dowker and Gray (1985) could not detect any reduction in the variability of embryo size, the spread of emergence or seedling weight associated with increased genetic uniformity of the seed population. Since the seed sources used in these experiments were not grown under the same conditions or harvested at the same stage of maturity, Gray and Steckel (1986) compared the variation in embryo length of an F_1 hybrid and an OP cultivar of carrot when the seed of both was produced and harvested under identical conditions, with the OP cultivar acting as pollinator for an adjacent male sterile. Again, there was no evidence of lower variability in embryo length or seedling weight in F_1 hybrid compared with OP seed. The use of an OP pollinator would have reduced the genetic uniformity normally to be expected from a commercial F_1 hybrid produced from two inbred lines and the question therefore remains open. Bonnet (1978) reported trial results demonstrating that F_1 hybrid carrots were more homogenous and earlier than standard varieties.

Many vegetables for the fresh market must meet certain cosmetic requirements such as freedom from surface blemish, good colour and shape. Uniformity for such

characters may be extremely important and may be greater in hybrids than in OP varieties. Heterosis for early maturity is common in hybrid varieties of vegetables and may greatly increase economic yield when price differentials for early marketing are high.

BREEDING F_1 HYBRIDS

Improvement of cross-pollinating crops such as onion, carrot, cabbage, Brussels sprout, broccoli, cauliflower and sweetcorn has traditionally involved mass selection or recurrent selection to increase gene frequencies for desired traits. Improved populations can be used as sources of new open-pollinated cultivars or for the extraction of superior inbreds for use in synthetic populations or as parents of F_1 hybrids (Hosfield *et al.*, 1977; Coyne, 1980).

Phenotypic uniformity of an F_1 hybrid depends upon genetic homozygosity in the inbred parents. Out-breeding crops are more or less intolerant of inbreeding, which exposes and fixes unfavourable recessive alleles and leads to inbreeding depression. Cole crops permit inbreeding to I_5 , albeit with low seed production, but onion and carrot can scarcely be taken to I_3 and in leek inbreeding depression may be as high as 35% at I_1 (Dorsman, 1976; Currah, 1986). This loss of vigour results in low yields of hybrid seed and difficulties in maintaining the inbreds. Seed production is expensive and the cost to the grower may be three to six times that of seed of OP varieties (Table II). [Single crosses give the greatest opportunity for hybrid vigour with maximum uniformity]. However, where lack of parental vigour seriously affects seed yield, it may be necessary to use an F_1 hybrid as the female parent in a three-way cross or to make double crosses, using F_1 single-cross hybrids for both parents. The consequent loss of uniformity in the hybrid variety may be minimised by using a "modified" single-cross system (Wright 1980) in which the initial single cross is made between two progeny, A and A', from the same population and therefore closely related. $A \times A'$ may give a better seed yield than either inbred alone, and when crossed

with an unrelated inbred, B, can give heterosis with good uniformity.

Inbreeding depression may rapidly reduce the number of surviving lines unless compromises are made in the degree to which inbreeding is enforced. Phenotypic selection among second and third generation inbreds may be followed by mass-pollination between the best of them to give an improved population for a further cycle of selfing. At some point the general and specific combining abilities of selected inbreds will be determined. General combining ability (GCA) can be estimated in top-crosses. This involves crossing each of the inbreds to a widely-based tester stock, usually an OP variety. Identification of outstanding combinations of inbreds, those depending on specific combining ability (SCA) on top of GCA, is the next important step (Simmonds, 1979). In biennial vegetable crops such as onion, carrot and Brussels sprout the problems of producing and assessing large numbers of inbred lines for phenotypic characters and of carrying out controlled crosses to a tester for combining ability assessment are formidable and perhaps impractical, yet outstanding inbreds will be rare and without this effort the potential of F_1 hybrids will not be fully realised. Ideally, improved inbred lines should be sought in separate populations so that there is some diversity; unrelatedness of inbreds is a significant component of heterosis in their hybrids (Simmonds, 1979).

MECHANISMS FOR HYBRID SEED PRODUCTION IN VEGETABLES

Large-scale production of hybrid seed requires that there be some mechanism for ensuring that cross-pollination occurs between the parental lines with a minimum of self-fertilization. Hand emasculatation is considerably more difficult in species with hermaphrodite flowers than in maize, which can be emasculated by detasseling. The economics of hand emasculatation will depend upon the ease with which it can be carried out, the return of seeds per pollination, the price of hybrid seed in relation to the cost of production, and the

seed rate (Frankel, 1973a). In tomato, emasculatation is generally performed by hand in mass hybrid seed production (Yordanov, 1983).

Two naturally occurring mechanisms for ensuring cross-fertilisation in monoecious, or hermaphrodite, species are male-sterility and self-incompatibility. The use of both these mechanisms in hybrid seed production is reviewed by Frankel (1973a 1973b). Apart from the need to prevent selfing of the female line, it is necessary that pollen of the male line is effectively transferred to the female line. This requires either artificial pollination or an efficient pollen dispersal mechanism.

Male-sterility

Male-sterility may be genic or cytoplasmic. In the former case the male-sterile (A) line is maintained by crossing to a maintainer (B) line, heterozygous for the recessive male-sterility allele. Thus half the progeny will also be heterozygous and fertile and must therefore be removed. The pollinator (C) line must be homozygous fertile to ensure that all F_1 hybrid plants are fertile, though in most vegetable crops this would not be necessary. Genic male-sterility is far less useful for hybrid seed production than cytoplasmic male-sterility (CMS) interacting with nuclear genes. The potential of CMS for F_1 hybrid production was first realised following the discovery of a male-sterile plant in a Californian crop of the onion cv. Italian Red (Jones and Emsweller 1936) and the subsequent determination by Jones and Clarke (1943) of the inheritance of the cytoplasmic factor 'S' and its interaction with the recessive nuclear gene, 'ms'. The genotype N msms with normal cytoplasm, 'N', is male-fertile and can be used as a 'maintainer' for the male-sterile, S msms, since all the progeny will inherit the maternal 'S' cytoplasm and thus be sterile. After repeated back-crossing the male-sterile 'A' line and the maintainer 'B' lines become virtually isogenic. To produce hybrid seed, the A line is interplanted with the pollinator or C line, selected to flower synchronously.

Onion is an outbreeder with protandrous

flowers (Currah and Ockendon, 1978; Ockenden and Currah, 1979), a factor that should minimise selfing. However, the flowers develop in successive zones of a dense umbel so that there is opportunity for pollen transfer between adjacent flowers. Except for experimental purposes it is quite impractical to produce hybrid seed by hand emasculation and pollination. The CMS system can easily be used to produce seed of single, double or three-way hybrids (Figure 1). The fact that half the plants of a double-cross will be male-sterile is not important for vegetable crops that are not grown for fruit or seed. Thus the pollinating C line need not carry a restorer gene. A different cytoplasmic system of male-sterility in onion is that based on 'T' cytoplasm (Schweisguth, 1973). This system involves three recessive genes which are common in European varieties although the 'T' cytoplasm is not. Many fertility restoring lines for the 'S' cytoplasm are maintainers for 'T' cytoplasm and can thus be easily converted to male-sterile equivalents. This would be prudent to avoid the dangers of genetic vulnerability associated with the use of a single cytoplasm.

The expression of CMS in onion may be affected by environmental factors, particularly high temperatures; unstable lines can be predominantly male-sterile at 14°C and predominantly male-fertile at 23°C (Van der Meer, 1978). Reversion to male-fertility in an A line would have disastrous

consequences for commercial hybrid seed production.

F₁ hybrid production in carrot became a possibility when CMS was found in the variety Tendersweet in the 1940s (Welch and Grimball, 1947); this was the brown-anther form in which the pollen does not develop beyond the microspore stage. Another, quite distinct, type was discovered in wild carrot in 1953 by H.M. Munger (Thompson, 1961). This is the petaloid form in which the five anthers are transformed during development into petal-like structures and do not produce pollen. According to Thomson (1961) there are at least two and probably three duplicate dominant maintainer genes and an epistatic restorer operating in the inheritance of male-sterility in both 'Tendersweet' and the wild petaloid type. However, Pearson (1983) states that the cytoplasm responds to different systems of restorers: sterility in the brown anther type is controlled by two complementary recessive genes, interacting with the cytoplasm, whereas the petaloid type is controlled by two complementary dominant genes, interacting with a different cytoplasm. The brown-anther recessives occur in many stocks, but the petaloid dominants occur much more rarely. Most commercial F₁ hybrids are from the petaloid CMS which generally produces lower seed yields than the brown anther type but has a more stable expression (Erickson and Peterson, 1979). The shapes of the petaloid

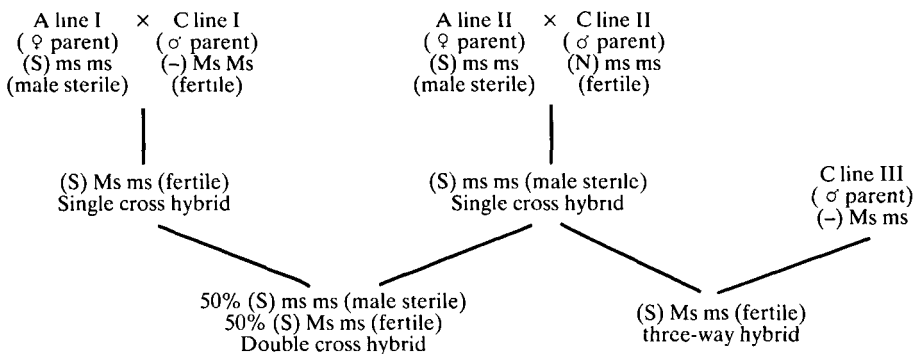


FIG 1
Examples of schemes for single- and double-cross and three-way hybrid seed production using cytoplasmic male sterility and fertility restoration.

anthers vary from strongly petal-like to filamentous (Eisa and Wallace, 1969) but the open flowers are generally easily distinguishable from normal flowers.

Self-incompatibility

Self-incompatibility (SI) systems are found in many species of flowering plants, among them important crop plants (Frankel, 1973b). Where SI is due to pistil-pollen interaction, the control may be gametophytic or sporophytic. The gametophytic form is controlled by the pollen grain whereas in the sporophytic type the reaction is between the exine of the pollen grain, which is sporophytic in origin, and the papillae of the stigma (Roggen, 1972). All types of *Brassica oleracea* possess a sporophytic SI system which varies in effectiveness in the different crop types, being strongest in kale and weakest in summer cauliflower (Watts, 1980), but there are many factors, genetic and environmental, which affect its expression (Ockenden, 1971). The S-allele system is complex, with about 50 alleles at a single locus, forming a dominance series in which, generally speaking, the dominant alleles are strong and the recessive alleles weak.

The first objective in breeding F_1 hybrids is to produce inbred lines as parents. Therefore the incompatibility reaction has to be overcome to allow self-pollination. This can be done in some species by pollinating the flowers in the bud stage before the SI mechanism is operative. It is known that glycoproteins have an important role in sporophytic SI and that the increased rate of synthesis of these S-locus specific glycoproteins in the developing stigma correlates with the onset of the incompatibility reaction (Nasrallah *et al.*, 1985).

Commercial production of F_1 hybrid seed in vegetable brassicas depends on crossing together two inbred lines, each homozygous for a different S-allele. Each line is required to be both sib- and self-incompatible but the two lines must be cross-compatible.

Manipulation of sex expression

Hybrid breeding in cucurbitous crops is

made possible by manipulation of sex expression. The use of genetic control of sex in cucumber has been described by Galun (1973). Cucumber plants are typically monoecious, producing more male flowers than female on the same plant. Genetically gynoeceious plants produce only female flowers and can be used to produce F_1 seed on a field scale. Multiplication of such lines is made possible by artificially inducing male flower production. This is done by applying gibberellic acid (GA4/7) or silver nitrate to seedling leaves (Pike and Peterson, 1969; Tolla and Peterson, 1979). Conversely, monoecious lines of squash can be rendered female only by applying ethephon to suppress male flower production (Shannon and Robinson, 1979).

Chemical hybridising agents (CHA's)

This term is used to describe chemicals which cause pollen abortion and render the treated plants male-sterile while not affecting ovule fertility. Such gametocides, if absolutely effective could be used to produce male-sterile versions of any breeding line, thus obviating the need for laborious back-crossing as required for the transfer of genic or cytoplasmic male-sterility. To be really useful in F_1 hybrid seed production, CHAs should cause only pollen abortion and not affect ovule fertility, have no mutagenic effects, be easy and economical to apply, with a wide window of application and should have no harmful side-effects. At present, large-scale use of CHAs in hybrid seed production is confined to the major cereal crops and even here it is a new development. The commercial value of vegetable varieties may be too low to attract research investment into the development of CHAs but positive responses have been achieved with GA3 and GA4/7 in lettuce and onion, with maleic hydrazide in tomato and onion, and with sodium 2,3-dichloroisobutyrate in tomato (Sneep *et al.*, 1979). Effective CHAs would remove the need to incorporate male-sterility into A-lines and, for seed crops, to introduce a restorer gene into a C-line. Many vegetables have an extended period of flowering such that a single spray

application of CHA would not be sufficient to affect all flowers. Flowering would either have to be more determinate or the CHA would need to be systemic and of long effect. Preliminary studies are being carried out at Wellesbourne into the effects on onion of two putative CHAs supplied by a chemical company with plant breeding interests.

FACTORS AFFECTING CROSS-FERTILIZATION IN HYBRID SEED PRODUCTION

Problems have occurred in crops where commercial hybrid seed production is dependent upon effective insect pollination of male-sterile inbreds. Pollen-collecting bees may tend to work the male-fertile inbreds and spend rather little time on male-sterile plants. Even where both parents are male-fertile, as in SI plants, the bees may prefer one parent to the other, perhaps because of petal colour or quantity and concentration of nectar. There is also sometimes a tendency for insects to stay on the inbred on which they first alighted rather than move randomly from one to another. Faulkner (1974) found that bees could discriminate between Brussels sprout inbreds, with a selfing to crossing movement of 30:1. Flower colour was probably a major factor since bees were found to distinguish between different shades of yellow, related to age of the flower. Plant height also affected bee behaviour.

The proportion of sib seed in commercial F_1 hybrid seed productions in Brassica crops can vary from nil to 80% (Wills *et al.*, 1979). This proportion can be estimated by growing-on tests but these may take weeks or months and may delay seed distribution. A biochemical test, based upon the separation by electrophoresis of the isoenzymes of acid phosphatase in seed extracts, can be used to distinguish inbred from hybrid seed, provided that the inbreds differ for the isoenzyme bands (Wills *et al.*, 1979).

In a study of flowering and pollination in onion, Ali *et al.* (1984) found that the number of flowers per umbel was lower, and the percentage defective pistils higher, in male-sterile inbreds than in inbred

pollinators. Number of flowers per umbel and period of stigma receptivity were greater in two male-sterile hybrids than in an inbred male-sterile line, suggesting that seed yields would be higher in the former. Campbell *et al.* (1968) in a study of hybrid seed production of onion in Idaho showed that yield losses in male-sterile inbreds were due to high proportions of abnormal flowers or aborted ovules.

Commercial production of seed from CMS carrots has frequently been disastrous, probably because of non-random foraging patterns of the pollinating insects (Erickson *et al.*, 1979). Comparing male-sterile lines (S) and their isogenic maintainers (M), Erickson and Peterson (1979) found that the S lines nearly always flowered later and had a shorter period of attractiveness to bees. This was true also of male-sterile F_1 s and so was not due entirely to inbreeding. In general nectar quantity was lower in S than in M lines and nectar secretion and release of volatiles was much later in the S lines. The anthers of male-fertile plants fluoresce in uv light and therefore provide a visual cue that is not present in male-sterile plants. Bonnet (1978) compared the brown anther CMS with the petaloid type against the same genetic background. Insects visited each equally and seed production was the same.

FUTURE PROSPECTS

Recent advances have seen the cloning of DNA from the S_6 allele of *Brassica oleracea* (Nasrallah *et al.*, 1985). The use of such clones as probes may be a more convenient way to characterize plants for their S-locus genotype than using genetic and electrophoretic methods (Charlesworth, 1985).

Somatic hybridization by protoplast fusion could provide a means of directly combining a male-sterile cytoplasm with the nuclear genome of an improved inbred, thus avoiding the need for lengthy back-crossing. This technique could also be used to create unique and possibly heterotic material by combining different cytoplasmic to form cybrids. Conventional sexual hybrids contain only maternally derived cytoplasmic factors but somatic fusion combines both parental cytoplasmic. Subsequent cyto-

plasmic segregation results in the elimination of one or the other cytoplasm (Aviv *et al.*, 1980) but evidence from restriction analysis of mitochondrial DNA suggests that recombination occurs between parental mitochondrial DNAs prior to cytoplasmic segregation (Belliard *et al.*, 1979). Thus protoplast fusion can give rise to unique nuclear-cytoplasmic combinations, not available using conventional methods (Sharp *et al.*, 1984). Breeding lines so produced, if free from deleterious somaclonal variation, might be useful in conventional hybrid breeding.

Development of a CMS system in *Brassica* would offer a mechanism for hybrid seed production which is well worked out (Pearson, 1972). Two types have been found in *B. nigra* but in one, a petaloid form, the flowers have no nectaries and are not visited by insects, and in neither is there a satisfactory maintainer system (Pearson, 1972). Nevertheless recent progress at the New York State Agricultural Experiment Station, Geneva in the exploitation of CMS from *B. nigra* for F_1 hybrid calabrese looks promising (M.H. Dickson, pers. comm.). CMS from *Raphanus sativus* is a vestigial anther type with normal flowers and full seed fertility. Nectary development may not be normal but there seems to be scope for selection. The *B. oleracea* genome has been transferred to *R. sativus* cytoplasm (Bannerot *et al.*, 1975) but when grown at low temperatures the plants exhibit chlorosis. Recent researches in somatic hybridization (Pelletier *et al.*, 1983) have produced 'cybrids' in which the male-sterile cytoplasm from *R. sativus* has been combined with the chloroplasts of *B. napus*. Recombination between mitochondria is useful in separating CMS from "defective nectary", as both characters are mitochondrially determined. A lack of suitable restorers prevents the use of this source of CMS in rape (*B. napus*) but not in vegetable brassicas where restoration is unnecessary.

Clonal propagation techniques offer the potential for large-scale proliferation of genetically identical plants for commercial

use. Such techniques could be used to propagate the parental lines of an F_1 hybrid or even, if economically feasible, the F_1 hybrid plants themselves. Mass propagation of thousands of tiny somatic embryos in a batch culture system as can be done for carrot (Redebaugh *et al.*, 1986), followed by encapsulation or suspension in a gel carrier offers the prospect of somatic "seeds" of an F_1 hybrid in crops where this technique can be used (Sharp, *et al.*, 1984). These latter possibilities have disturbing implications for plant variety protection, since the biological exclusivity inherent in the ownership of the parental lines of an F_1 hybrid could be violated if the hybrid plants were vegetatively propagated in large numbers by unlicensed agents. Breeders are seeking legislation to prevent this. Clonal propagation could also be used to maintain parental lines at a constant level of heterozygosity, perhaps to ensure sufficient vigour for adequate seed production. Maintenance of a male-sterile parent by propagation could be advantageous in some situations, particularly when a suitable maintainer line is not available.

Molecular techniques for identifying polymorphic markers at the DNA level hold considerable promise for varietal identification, genetical analysis of economic traits and new breeding methodologies (Beckman and Soller, 1986). Restriction fragment length polymorphisms (RFLPs) might allow marker analysis of differences between potential parent lines in terms of quantitative trait loci and thus enable the planned construction of superior recombinants. This could prove more effective than conventional methods of field testing large numbers of recombinant inbred lines. However, Ellis (1986) considered it unlikely that RFLP's could be useful as markers for quantitative characters. Fragment length polymorphisms do, nevertheless, appear to offer a potentially powerful means of estimating the frequency of sibs in a sample of F_1 hybrid brassica seed compared with that provided by isozyme analysis.

REFERENCES

- ALI, M., DOWKER, B. D., CURRAH, L. and MUMFORD, P. M. (1984). Floral biology and pollen viability of parental lines of onion hybrids. *Annals of Applied Biology*, **104**, 167-74.
- AVIV, D., FLUHR, R., EDELMAN, M. and GALUN, E. (1980). Progeny analysis of the interspecific somatic hybrids: *Nicotiana tabacum* (cms) + *Nicotiana sylvestris* with respect to nuclear and chloroplast markers. *Theoretical and Applied Genetics*, **56**, 145-50.
- AXELSSON, F. (1976). Investigation on breeding F₁ hybrid varieties of carrots. *Agricultural and Horticultural Genetics*, **34**, 42-55.
- BAKER, L. R. (1978). Breeding of Spartan hybrid carrot varieties. *Biuletyn Warzywniczy*, **22**, 127-36.
- BANNEROT, H., BOULIDARD, L., CAUDERON, Y. and TEMPE, J. (1975). Transfer of cytoplasmic male sterility from *Raphanus sativus* to *Brassica oleracea*. *Proceedings of Eucarpia Meeting, Cruciferae*, September 25-27, 1974, 52-4.
- BECKMANN, J. S. and SOLLER, M. (1986). Restriction fragment length polymorphisms and genetic improvement of agricultural species. *Euphytica*, **5**, 111-24.
- BELLIARD, G., VEDEL, F. and PELLETIER, G. (1979). Mitochondrial recombination in cytoplasmic hybrids of *Nicotiana tabacum* by protoplast fusion. *Nature, UK*, **281**, 401-3.
- BONNET, A. (1978). Breeding of carrot F₁ hybrids in France. *Biuletyn Warzywniczy*, **22**, 147-50.
- CAMPBELL, W. F., COTNER, S. D. and POLLOCK, B. M. (1968). Preliminary analysis of the onion seed (*Allium cepa* L.) production problem, 1966 growing season. *HortScience*, **3**, 40-1.
- CHARLESWORTH, D. (1985). Molecular view of pollen rejection. *Nature, UK*, **318**, 231-2.
- COYNE, D. P. (1980). The role of genetics in vegetable improvement. *Scientific Horticulture*, **31**, 74-88.
- CURRAH, L. (1986). Leek breeding: a review. *Journal of Horticultural Science*, **61**, 407-15.
- CURRAH, L. and OCKENDON, D. J. (1978). Protandry and the sequence of flower opening in the onion (*Allium cepa* L.). *New Phytologist*, **81**, 419-28.
- DORSMAN, C. (1976). F₁ hybrids in some out-pollinating crops. In: *Heterosis in Plant Breeding. Proceedings of the 7th Congress of Eucarpia, Budapest 1974*, 197-208.
- DORUCHOWSKI, R. W. (1984). The leading cultivars of onions in Eastern Europe. *Allium Newsletter*, **1**, 40-1.
- DOWKER, B. D. (1978). A new approach to breeding for uniformity in competitive row crops. *Biuletyn Warzywniczy*, **22**, 161-70.
- DOWKER, B. D. and GORDON, G. H. (1983). Heterosis and hybrid cultivars in onions. In: *Monographs on Theoretical and Applied Genetics, Vol. 6 Heterosis*, (Frankel, R., Ed.), Springer-Verlag, Berlin, 220-33.
- DOWKER, B. D. and GRAY, D. (1985). Variation in embryo size in inbred lines of carrot. *Annals of Applied Biology*, **107**, 597-9.
- DOWKER, B. D., CROWTHER, T. C., JUSTICE, J. M., WERNER, C. P. and LAVERY, K. (1987). Breeding methods in onions. *Report of the National Vegetable Research Station for 1986/87*, 45.
- EISA, H. M. and WALLACE, D. H. (1969). Morphological and anatomical aspects of petaloidy in carrot. *Journal of the American Society of Horticultural Science*, **94**, 545-8.
- ELLIS, T. H. N. (1986). Restriction fragment length polymorphism markers in relation to quantitative characters. *Theoretical and Applied Genetics*, **72**, 1-2.
- ERICKSON, E. H. and PETERSON, C. E. T. (1979). Asynchrony of floral events and other differences in pollinator foraging stimuli between fertile and male-sterile carrot inbreds. *Journal of the American Society of Horticultural Science*, **104**, 639-43.

- ERICKSON, E. H., PETERSON, C. E. and WERNER, P. (1979). Honey bee foraging and resultant seed set among male fertile and cytoplasmic male sterile carrot inbreds and hybrid seed parents. *Journal of the American Society of Horticultural Science*, **104**, 635-8.
- FAULKNER, G. J. (1974). Factors affecting field-scale production of seed of F₁ hybrid Brussels sprouts. *Annals of Applied Biology*, **77**, 181-90.
- FORDHAM, R. and BIGGS, A. G. (1985). *Principles of Vegetable Crop Production*. Collins, London.
- FRANKEL, R. (1973a). The use of male-sterility in hybrid seed production. In: *Agricultural Genetics: Selected Topics*, (Moav, R., Ed.) John Wiley and Sons, New York, 85-94.
- FRANKEL, R. (1973b). Utilisation of self-incompatibility as an out-breeding mechanism in hybrid seed production. In: *Agricultural Genetics: Selected Topics*, (Moav, R., Ed.), John Wiley and Sons, New York, 95-107.
- GABELMAN, W. H. (1974). F₁ hybrids in vegetable production. *Proceedings of the 19th International horticultural Congress, Vol. III, Warsaw*, 419-28.
- GALUN, E. (1973). The use of genetic sex types for hybrid seed production in *Cucumis*. In: *Agricultural Genetics: Selected Topics*, (Moav, R., Ed.), John Wiley and Sons, New York, 23-56.
- GRAY, D. and STECKEL, J. R. A. (1986). Comparison of plant-to-plant variability from F₁ hybrid and open-pollinated carrot cultivars. *Journal of Agricultural Science, UK*, **106**, 199-200.
- GRAY, D., STECKEL, J. R. A., JONES, S. R. and SENIOR, D. (1986). Correlations between variability in carrot (*Daucus carota* L.) plant weight and variability in embryo length. *Journal of Horticultural Science*, **61**, 71-80.
- HAYES, H. K. and JONES, D. F. (1916). First generation crosses in cucumber. *Report of the Connecticut Agricultural Experimental Station for 1916*, 319-22.
- HOSFIELD, G. L., VEST, G. and PETERSON, C. E. (1977). Heterosis and combining ability in a diallel cross of onions. *Journal of the American Society of Horticultural Science*, **102**, 355-60.
- JINKS, J. L. (1983). Biometrical genetics of heterosis. In: *Monographs on Theoretical and Applied Genetics Vol. 6 Heterosis*, (Frankel, Ed.), Springer-Verlag, Berlin, 1-46.
- JONES, H. A. and CLARKE, A. E. (1943). Inheritance of male-sterility in the onion and the production of hybrid seed. *Proceedings of the American Society of Horticultural Science*, **43**, 189-94.
- JONES H. A. and EMSWELLER, S. L. (1936). A male-sterile onion. *Proceedings of the American Society of Horticultural Science*, **34**, 582-5.
- NASRALLAH, J. B., KAO, T.-H., GOLDBERG, M. L. and NASRALLAH, M. E. (1985). A cDNA clone encoding an S-locus specific glycoprotein from *Brassica oleracea*. *Nature, UK*, **318**, 263-7.
- OCKENDON, D. J. (1971). Incompatibility studies. *Report of the National Vegetable Research Station for 1971*, 28-29.
- OCKENDON, D. J. (1984). Anther culture in Brussels sprouts (*Brassica oleracea* var. *gemmifera*). I. Embryo yields and plant regeneration. *Annals of Applied Biology*, **105**, 285-91.
- OCKENDON, D. J. (1985). Another culture in Brussels sprouts (*Brassica oleracea* var. *gemmifera*). II. Effect of genotype on embryo yields. *Annals of Applied Biology*, **107**, 101-4.
- OCKENDON, D. J. and CURRAH, L. (1979). The effect of protandry on the amount of out-crossing in the onion (*Allium cepa* L.). *Horticultural Research*, **19**, 55-61.
- PEARSON, O. H. (1972). Cytoplasmically inherited male sterility characters and flavour components from the species cross *Brassica nigra* x *B. oleracea*. *Journal of the American Society of Horticultural Science*, **97**, 397-402.

- PEARSON, O. H. (1983). Heterosis in vegetable crops. In: *Monographs of Theoretical and Applied Genetics, Vol. 6 Heterosis*, (Frankel, R., Ed.), Springer-Verlag, Berlin, 138-88.
- PELLETIER, G., PRIMARD, C., VEDEL, F., CHETRIT, P., REMY, R., ROUSSELLE, P. and RENARD, M. (1983). Intergeneric cytoplasmic hybridisation in cruciferae by protoplast fusion. *Molecular and General Genetics*, **191**, 244-50.
- PETERS, R. (1984). Hybrids versus open pollinated cultivars in short day onion (*Allium cepa* L.). *Eucarpia 3rd Allium Symposium, September 4-6 1984*, 60-4.
- PIKE, L. M. and PETERSON, C. E. (1969). Gibberellin A₄/A₇ for induction of staminate flowers on the gynoeceous cucumber. *Euphytica*, **18**, 106-9.
- REDENBAUGHM, K., PAASCH, B. D., NICHOL, J. W., KOSSTER, M. E., VISS, P.R. and WALKER, K. A. (1986). Somatic seeds: encapsulation of asexual plant embryos. *Biotechnology*, **4**, 797-801.
- ROGGEN, H. P. J. R. (1972). Scanning electron microscopical observation on compatible and incompatible pollen stigma interactions in Brassica. *Euphytica*, **21**, 1-10.
- SCHWEISGUTH, B. (1973). Study of a new type of male-sterility in onion, *Allium cepa* L. *Annales de l'Amélioration des Plantes*, **23**, 221-53.
- SHANNON, S. and ROBINSON, R. W. (1979). The use of ethephon to regulate sex expression of summer squash for hybrid seed production. *Journal of the American Society of Horticultural Science*, **104**, 674-7.
- SHARP, W. R., EVANS, D. A. and AMMIRATO, P. V. (1984). Plant genetic engineering: designing crops to meet food industry specifications. *Food Technology, February 1984*, 112-9.
- SIMMONDS, N. W. (1979). *Principles of Crop Improvement*. Longman, London.
- SMITH, B. M., HARVEY, E., SETTER, A. P., WERNER, C. P., KUBBA, J. and JOHNSTON, E. C. (1986). Breeding Brussels sprouts: self-compatible inbred cultivars. *Report of the National Vegetable Research Station for 1985*, 36.
- SMITH, B. M., ROGERS, W. J. and KEARSEY, M. J. (1985). The feasibility of producing inbred rather than F₁ hybrid cultivars of Brussels sprouts: a preliminary genetical analysis and choice of material for inbreeding. *Annals of Applied Biology*, **107**, 87-99.
- SNEEP, J. MURTY, B. R. and UTZ, H. F. (1979). In: *Plant Breeding Perspectives, D. J. van der Have 1879-1979*, (Sneep, J., Hendriksen, A. J. T. and Holbeck, O., Eds.). PUDOC, Wageningen, 131-4.
- SPRAGUE, G. F. (1983). Heterosis in maize: theory and practice. In: *Monographs on Theoretical and Applied Genetics Vol. 6 Heterosis*, (Frankel, R., Ed.), Springer-Verlag, Berlin, 47-70.
- SRAGUE, G. F. and SCHULLER, J. F. (1961). The frequencies of seed and seedling abnormalities in maize. *Genetics*, **46**, 1713-20.
- THOMPSON, D. J. (1961). Studies on the inheritance of male-sterility in the carrot *Daucus carota* L. var. *sativa*. *Proceedings of the American Society of Horticultural Science*, **78**, 332-8.
- TOLLA, G. E. and PETERSON, C. E. (1979). Comparison in gibberellin A₄/A₇ and silver nitrate for induction of staminate flowers in gynoeceous cucumber lines. *HortScience*, **14**, 542-4.
- UMLAUF, C. F. (1978). Investigations into the variation of germination properties and emergence characters in size-graded seeds lots of carrots (*Daucus carota* L.) using hybrid seed multiplications and open-pollinated cultivars for comparison. MSc thesis, University of Birmingham, UK.
- VAN DER MEER, Q. P. (1978). Results of recent investigations of male-sterility in onion (*Allium cepa* L.). *Biuletyn Warzywniczy*, **22**, 73-9.
- WATTS, L. (1980). *Flower and Vegetable Plant Breeding*. Grower Books, London.
- WELCH, J. D. and GRIMBALL, E. L. (1947). Male sterility in carrot. *Science, USA*, **106**, 594.

- WERNER, C. P., DOWKER, B. D., DeSOUZA, D. C., SETTER, A. P., CROWTHER, T. C. and HOROBIN, J. F. (1988). Triple test cross predictions of the performance of recombinant inbred lines from a wide cross in onions (*Allium cepa* L.). In preparation.
- WILLS, A. B., FYFE, S. K. and WISEMAN, E. M. (1979). Testing F_1 hybrids of *Brassica oleracea* for sibs by seed isozyme analysis. *Annals of Applied Biology*, **91**, 263-70.
- WITTWER, S. H. (1983). Frontiers for science and technology in vegetable production. *10th Anniversary Monograph Series, Asian Vegetable Research and Development Center Publication*, 83-190.
- WRIGHT, H. (1980). Commercial hybrid seed production. In: *Hybridisation of Crop Plants*, Fehr, W. R. and Hadley, H. H. American Society of Agronomy, Crop Science Society of America, 161-76.
- YORDANOV, M. (1983). Heterosis in tomato. In: *Monographs on Theoretical and Applied Genetics, Vol. 6 Heterosis*, (Frankel, R., Ed.), Springer-Verlag, Berlin, 187-219.

(Accepted 1 December 1987).