Progress towards hybrid rapeseed using recessive self-incompatibility and possibily atrazine resistance

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In England winter rape often flowers in cold, damp weather, when cross-pollination between plants by insects or wind is minimal. Movement of flower heads by wind probably self-fertilises flowers by knocking pollen from dehisced anthers onto the stigma of the same flower. Thus it is essential that any hybrids, to be grown commercially by farmers, are self-fertile to ensure a high proportion of flowers developing into pods with a good seed set.

Method for three-way cross hybrids

A possible commercial method of producing three-way cross hybrid seed, using two recessive self-incompatible (RSI) lines and one self-compatible (SC) line to give a self-fertile farm crop was described by Thompson (1979). A simplified outline of the method is given in Fig. 1. The two RSI lines (A and B) must be reciprocally cross-compatible and active for different incompatibility factors or

Fig. 1. Three-way cross method to produce hybrids using recessive self-incompatibility.

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Lines        A      x      B
           (RSI)    |    (RSI)
                    |
                   AB      x      C
                 (RSI)     |    (SC)
                           |
                          ABC  -  Seed for farmers
                          (SC)    from AB blocks
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RSI = Recessive self-incompatible
SC = Self-compatible or self-fertile

S alleles. The RSI F_1 hybrid is crossed by an SC pollinator (C) to produce the SC three-way cross seed (ABC). This seed is marketed to farmers by bulking the blocks of the RSI F_1 hybrids in the crossing nursery (Fig. 2). Seed from the alternating blocks of the SC pollinator is bulked separately and crushed for oil and meal production.

Nakanishi and Hinata (1975) found that high concentrations of carbon dioxide overcame self-incompatibility in cabbage. Fairly large quantities of selfed seed of the two RSI lines, A and B, can be produced in polythene tunnels, in which the carbon dioxide content of the air can be increased to 3 to 4 per cent by the introduction of dry ice (solid carbon dioxide) pellets twice a week during flowering (Thompson, 1979; Taylor, 1982).

Producing different RSI lines from synthetic Brassica napus

It has not been possible to use the three-way cross method because only one RSI type has been found in oilseed rape. In order to obtain other RSI types, cross-compatible with the original RSI rape line, seven interspecific hybrids between marrow-stem kale (Brassica oleracea) and spring turnip rape (B. campestris) were produced by embryo culture. These hybrids each contained the same S allele from turnip rape and one of seven different kale S alleles. Synthetic B. napus plants were produced by doubling the chromosome numbers of these hybrids, but with one exception these true breeding synthetic B. napus plants had poor pollen and poor seed fertility.

The kale S alleles, used to synthesise B. napus lines, were allele 16, high in the dominance series, alleles 8, 13 and 25, which are intermediate and alleles 2, 5 and 15, which are low in the dominance series (Thompson and Taylor, 1966). The turnip rape S allele, α (originally η) was the lowest in the dominance series of five alleles tested by Badrul Alam (1980). Alleles mainly intermediate or low in the dominance series were chosen because previous work had shown that an independent self-compatibility gene in a kale inbred line was expressed only in the absence of an S allele, high in the dominance series (Thompson and Taylor, 1971). Also as self-incompatibility is often dominant to self-compatibility in forage rape (Mackay, 1976; 1977), S alleles, intermediate or low in the dominance series, are more likely to be recessive to self-compatibility in oilseed rape.

To determine whether the particular S alleles in the different synthetic B. napus lines were recessive to self-compatibility (RSI), either the synthetic B. napus plant or better the F_1 hybrids, from crossing with the RSI oilseed rape type, were crossed with a SC homozygous diploid line and the progeny tested for self-compatibility. The kale S alleles, 13, 15 and 25, in combination with the turnip rape S allele, α, were recessive to self-compatibility and gave RSI hybrids when crossed to the RSI rape line (Table 1). However, the synthetic B. napus plant, containing S allele 2 and α, gave only SC plants on crossing to the RSI rape line. Such competitive interaction between S alleles to give self-compatibilty also occurred between alleles 2 and 15 in kale (Thompson, 1972).

Synthetic B. napus plants, with poor seed fertility, were crossed to the original RSI rape line to recover plants, homozygous for the S alleles from synthetic B. napus, but with improved seed fertility. This was done by selfing F_1 hybrids from this cross, selecting in the F_2 generation for seed-fertile plants, cross-compatible with the RSI rape line and testing for homozygosity of S alleles in the F_3 generation. Anthers were also cultured from the F_1 hybrids and spontaneous diploids and doubled haploids from embryoids were tested for S allele constitution. Information on the kale S alleles used, their relationship to self-compatibility and the methods used to recover seed-fertile plants with S allele combinations from the synthetic B. napus plants are summarised in Table 1.

Table 1. Incompatibility activities in seed-fertile lines from synthetic B. napus.

S allele constitution	Dominance level of kale S allele	Relationship to SC	Activity in F_1 hybrid with RSI rape line	Method used for SI production
2,2 α, α	Low	-	SC	-
5,5 α, α	"	-	-	SI, seed-fertile syn. B. napus
15,15 α, α	"	RSI	SI	-
8,8 α, α	Intermediate	-	SI	-
13,13 α, α	"	RSI	SI	F_2 generation from syn. B. napus x RSI rape line
25,25 α, α	"	RSI	SI	Anther culture from F_1 hybrid
16,16 α, α	High	-	SI	-

RSI = recessive self-incompatibility
SI = self-incompatible
SC = self-compatible

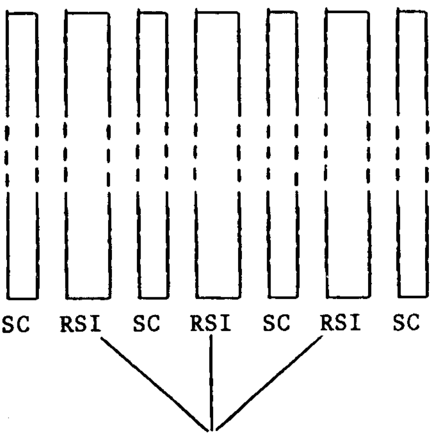
Different seed fertile RSI lines, cross-compatible with the original RSI rapeseed line, have been obtained from synthetic B. napus. Of these, the S allele combination, 25, 25, α, α, may be the best as allele 25 is the highest, so far, in the dominance series to give RSI lines and is likely to give the most self-incompatible lines. These S alleles must be transferred into agronomically-acceptable, low glucosinolate rape lines.

Application of atrazine resistance to seed multiplication of hybrids

The proposed production of the final three-way cross hybrid seed is from blocks of RSI F_1 hybrids, five to ten metres wide, alternating with narrower blocks of the SC pollinator line (Fig. 2). This arrangement might give poor cross-fertilisation in cool, wet weather, when few insect pollinators work on the crop. Only seed from the RSI F_1 hybrid blocks would be bulked and marketed to the farmer as hybrid seed, but with lodging of the crop in the field it might be difficult to avoid mixture with seed from the SC blocks, unless the latter were cut down after flowering.

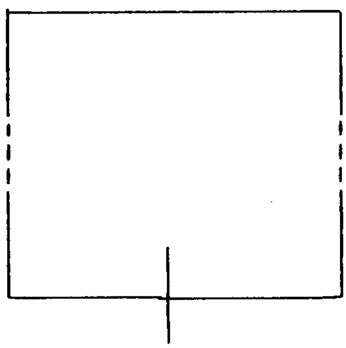
Fig. 2. Production of three-way cross hybrid seed in the field.

(a) without atrazine resistance



Bulked as hybrid seed for farmer.

(b) with atrazine resistant RSI lines



Single block of atrazine resistant, RSI F_1 seed mixed with atrazine-susceptible SC seed.

Strong resistance to the herbicide, atrazine, was found in bird's rape (B. campestris) and transferred to B. napus by Beversdorf et al (1980). Resistance is determined entirely by the cytoplasm or more accurately in the chloroplast (Weiss and Beversdorf, 1981). All seeds from an atrazine-resistant plant, whether from selfing or by crossing with a susceptible plant, are atrazine resistant, while all seeds from an atrazine-susceptible plant are susceptible even if crossed by an atrazine-resistant plant. Thus, use of atrazine-resistant RSI lines and an atrazine-susceptible, SC line would enable RSI F_1 hybrid and SC seed to be mixed, grown and harvested as one large unit (Fig. 2) for three-way cross hybrid seed. A ratio of 2 or 3:1 of RSI:SC seed for seed multiplication would probably give a satisfactory seed set on RSI plants. The harvested bulk would contain 30 to 50 per cent of atrazine-susceptible SC seed. The farmer would drill the commercial crop at a higher seed rate than normally, spray with atrazine and only atrazine-resistant hybrids with a few resistant selfs or sibs should survive.

Apart from easier production of atrazine-resistant hybrid seed, atrazine would give farmers a cheaper pre-emergence herbicide to control volunteer cereals, which compete most strongly with seedling rape in the six weeks after sowing. Also in fields that had grown oilseed rape in the last 20 years, high glucosinolate volunteers would be killed.

However there may be difficulties and penalties from using atrazine resistance. Atrazine resistant B. napus plants may be inherently lower yielding; this could reduce the yield advantage of hybrids. Hume et al. (1982) said that in most atrazine-tolerant weeds, photosynthesis and growth rates are depressed, compared to normal counterparts; although the fifth backcross to Candle (B. campestris) had yielded 20-30 per cent less seed than Candle, the fifth backcross to Tower (B. napus) had given only 5 to 10 per cent less than Tower, while selections from the fifth backcross bulk to Tower had appeared to yield as well as Tower. Reciprocal crosses between two winter rape lines, one resistant to atrazine, are in trial at two sites near Cambridge to determine the effect on yield of the atrazine-resistant cytoplasm in winter rape. Another problem for farmers could be damage to the following crop from atrazine residues, although in England after a year's rainfall it should only occur if there was overlapping of spraying. Finally in a dry autumn, atrazine might not kill the majority of atrazine-susceptible rape seedlings, sown with the resistant hybrid.

In conclusion, the recovery of seed-fertile RSI lines from synthetic B. napus, cross-compatible with the original RSI rapeseed line, makes large scale production of three-way cross hybrid seed possible. Routine backcrossing of these S alleles into canker-resistant, low glucosinolate lines will be necessary to generate breeding lines useful for hybrid production. Use of atrazine-resistant RSI lines would increase the percentage of hybrid seed and simplify seed multiplication in the field, but more information is required on the effect of atrazine-resistant cytoplasm on yield.

References

Badrul Alam, A.F.M. (1980) M. Phil. Thesis. University of Cambridge

Beversdorf, W.D., Weiss-Lerman, J., Erickson, L.R. and Souza Mahado, V. (1980) Canadian Journal Genetics and Cytology 22, 167-172

Hume, D.J., Donnelly, M.J. and Wade Montminy (1982) Annual Report, Department of Crop Science, Ontario Agricultural College, University of Guelph, Canada. pp180-186

Mackay, G.R. (1976) Incompatibility Newsletter 7, 4-8

Mackay, G.R. (1977) Euphytica 26, 511-519

Nakanishi, T. and Hinata, K. (1975) Euphytica 24, 117-120

Taylor, J.P. (1982) Euphytica 31, 957-964

Thompson, K.F. (1972) Heredity, Lond. 28, 1-7

Thompson, K.F. (1979) Proceedings 5th International Rapeseed Conference, Malmo, Sweden. Vol. 1. pp56-59

Thompson, K.F. and Taylor, J.P. (1966) Heredity, Lond. 21, 345-362

Thompson, K.F. and Taylor, J.P. (1971) Heredity, Lond. 27, 459-471

Weiss, J. and Beversdorf, W.D. (1981) Canadian Journal of Plant Science 61, 723-726