

TABLE 1. Characters of an elite Ms / Rf allele

Characters of an elite male sterile allele :	
*	normal plant phenotype with respect to vegetative and quality characters
*	tolerance to glufosinate-ammonium at commercial application rates with no delay in plant development and no delay in flowering date
*	normal 1:1 segregation for glufosinate-ammonium susceptibility/tolerance and in flower phenotype, sterile/fertile
*	perfect restoration in combination with designated elite Rf alleles to produce a 100 % restored hybrid
*	stable expression of sterility in different Brassica species under diverse environmental conditions and in different genotypic backgrounds
Characters of an elite restorer allele :	
*	normal plant phenotype with respect to vegetative and quality characters
*	tolerance to glufosinate-ammonium at commercial application rates with no delay in plant development and no delay in flowering date
*	perfect restoration in combination with designated elite Ms alleles to produce a 100 % restored hybrid
*	stable expression of restoration of fertility in hybrid combinations, in different Brassica species under diverse environmental conditions and in different genotypic backgrounds

The elite allele selection is performed in different steps (levels). Level 0 and 1 are executed in the greenhouse and level 2 and 3 are executed in the field.

Level 0 evaluation consists of selecting simple integration pattern transformants (single copy) and observing primary transformants for morphology, vigour, flower phenotype, herbicide resistance.

In level 1 testing the expression of the inserted traits is evaluated.

Level 2 testing consists of a first field evaluation (in 2 locations, 2 replicates), where germination, establishment, vigour, herbicide tolerance level, segregation, flowering date, restoration and seed set are evaluated.

In level 3 a multi-site evaluation (5 to 8 locations, 4 replicate RCB trial) comprises evaluation of herbicide tolerance level, stability of the male sterility and restoration, and agronomic performance. In these trials male sterile plants were individually bagged and checked for the presence/absence of seedset in order to evaluate the stability of the sterility.

Interspecific crosses

The original transformations were executed in spring *B. napus* lines. Elite alleles selected in *B. napus* were subsequently introduced by interspecific crosses in a wide germplasm base of both spring and winter *B. napus*, spring *B. rapa* and *B. juncea*.

Seed production

In the field seed of female and male parent is seeded in alternating strips. The roguing of the herbicide sensitive, male fertile female plants is done by application of glufosinate-ammonium on the entire field at the 2-4 leaves stage.

The male parent strips are mechanically removed after pollination.

The hybridity % of the final product was determined using PCR-based technology.

RESULTS

The major problems related to other pollination control systems in oilseed rape, have been instability of the male sterility in certain environments, biological penalty in virtue of the cytoplasm and incomplete restoration of fertility in the hybrid crosses. Therefore, we have put special emphasis on the evaluation of these characters in the elite allele selection.

Using the elite allele selection protocols, we have today selected 7 elite Ms alleles and 4 elite Rf alleles. Four of the Ms lines and two of the Rf lines have passed level 3 successfully. Three additional Ms elite alleles and two additional Rf elite alleles were selected after level 2 testing and are currently in level 3 testing.

Stability of the male sterility

Level 3 testing of spring *B. napus* was performed in 14 different locations, in 7 different countries. In all these trials, stability of the male sterility was demonstrated through determination of the level of seedset in properly isolated male sterile racemes, which turned out to be nil.

The male sterility, restoration and herbicide tolerance genes were transferred successfully in *B. napus* winter types, in *B. rapa* and *B. juncea* germplasm. Stability of the male sterility under various climatic conditions was demonstrated. (amongst others Varma et al., 1995)

Absence of biological penalty

Level 3 testing comprises an agronomical evaluation of the crosses between elite male sterile and restorer alleles. In these trials characters as emergence and establishment, vigour, days to flower, days to maturity, seed yield and quality parameters (oil content, protein content, fatty acid composition, glucosinolates content) have been determined in comparison to the non-transformed line.

Table 2 gives an overview of the results for some of these characters, as determined in level 3 testing in 1994.

TABLE 2. Absence of biological penalty in the hybrid combinations.

Genotype	Days to flower ¹	Yield ²	Oil % ¹
Ms1 x Rf1Rf1	66	105	46.5
Ms4 x Rf1Rf1	66.5	96	47.4
Ms5 x Rf1Rf1	66.5	101	47.3
Ms6 x Rf1Rf	66	104	45.5
Ms1 x Rf2Rf2	66.5	102	48.0
Ms4 x Rf2Rf2	65.5	102	47.1
Ms5 x Rf2Rf2	65.5	92	47.9
Ms6 x Rf2Rf2	65.5	98	46.1
Non-transformed control	66	100	45.8

¹ : observations in 1 specific location

² : relative to non-transformed line, average over 5 locations

Restoration of fertility

In level 3 testing the restoration of the fertility in the 'hybrid' combinations (cross between an elite Ms allele and an elite Rf allele) is evaluated by scoring at least 100 plants per plot for flower phenotype (sterile versus fertile).

Table 3 gives an overview of the results for this character, as determined in level 3 testing.

TABLE 3. Restoration of fertility in elite allele combinations.

Genotype	% fertile plants ¹
Ms1 x Rf1Rf1	100
Ms4 x Rf1Rf1	100
Ms5 x Rf1Rf1	100
Ms6 x Rf1Rf	100
Ms1 x Rf2Rf2	100
Ms4 x Rf2Rf2	100
Ms5 x Rf2Rf2	100
Ms6 x Rf2Rf2	100

¹ : average over 5 locations

All candidate elite alleles are being crossed into *B. napus* winter and spring, *B. rapa* and *B. juncea* germplasm. The restoration level in the hybrid combinations tested so far, was as expected i.e. similar as in the elite allele selection trials.

First candidate commercial hybrids

Several *B. napus* spring hybrids have been tested in private yield trials, mainly executed in Canada and northern Europe (Scandinavia, U.K.). Two of these hybrids have passed the first year of official registration trials in Canada (1994), two other hybrids have been entered in National List trials in the UK and another two in Sweden (1995).

Part of the results of the two Seedlink™ hybrids in canadian transgenic Co-operative trials are presented in table 4.

TABLE 4. Two Seedlink™ hybrids in CO-OP tests 1994.

<i>B. napus</i>	Seedyield as % checks ¹ Mid Season	Seedyield as % checks ¹ Long Season + Irrigated	Oil Content % dry weight Mid Season	Oil Content % dry weight Long Season + Irrigated
PGS1	115.6	119.4	47.3	44.9
PGS2	114.3	112.8	47.4	45.9
Excel	96.9	98.3	47.0	45.7
Profit	97.7	93.1	47.5	45.8
Legend	99.4	96.2	45.7	44.3
Delta	103.7	105.6	45.3	43.5

¹ : Checks were Excel, Legend, Delta (average seedyield 2350 kg/ha)

² : Checks were Excel, Legend, Delta (average seedyield 2880 kg/ha)

In winter *Brassica napus* restored hybrids have been obtained using some of the selected candidate elite alleles. The first yield data are expected in July 1995.

Two restorer and three male sterile elite alleles have been introduced into *B. rapa* germplasm. Several hybrids based on these parental lines are currently being tested in private trials in Canada.

Three male sterile and two restorer candidate elite alleles were backcrossed in *Brassica juncea* recurrent parents. Several hybrids are being produced based on these elite alleles, the agronomic performance will be evaluated during the next season.

Seed production based on Seedlink™

Although there is a valid scientific basis present to call Seedlink™ a successful pollination control system, it remains to be demonstrated that seed production using Seedlink™ on a commercial scale is technically and economically feasible.

Therefore, seed production trials have been set up in 1994 with spring *B. napus* parents in 5 locations across Canada and Europe.

The average yield of hybrid seed varied between 850 and 2000 lbs/acre. Hybridity level in the produced hybrid seed lots varied between 85 and 99 %.

In 1995 the first commercial seed production of two Seedlink™ hybrids takes place in Canada.

REFERENCES

Mariani, C. et al. (1990). Induction of male sterility in plants by a chimaeric ribonuclease gene. In *Nature* 347, pp. 737-741.

Mariani, C. et al. (1992). A chimaeric ribonuclease-inhibitor gene restores fertility to male sterile plants. In *Nature* 357, pp. 384-387.

Varma, N.S.; Ghosh, N.; Singh J. (1995). Development of hybrids of Indian mustard using PGS hybridization technology. *Proceedings of 9th International Rapeseed Congress* A26.