TRANSFRER OF SELF-INCOMPATIBILITY FACTORS FROM BRASSICA OLERACEA TO B. NAPUS

V.L. Ripley, W.D. Beversdorf

Department of Crop Science, University of Guelph, Guelph, Ont., Canada N1G 2W1

INTRODUCTION

Currently in Canada there is a major effort underway to produce hybrid in canola (Brassica napus). Among the requirements needed to facilitate large scale hybrid production is the need for an efficient pollination control mechanism (Grant and Beversdorf, 1985). Self-incompatibility may represent an efficient method of pollination control in B. napus.

The major disadvantage associated with self-incompatibility (SI), lies in the variability of expression. SI has already been transferred from B. campestris and B. napus var rapifera (rutabaga) into B. napus (Mackay, 1977; Banks, 1988). however the expression of the alleles once in the B. napus background has been extremely variable. We have chosen B. oleracea var italica as a source of new S-alleles for introgression into B. napus, mainly because the S-alleles in B. oleracea, have been very well characterized, based on phenotypic expression (Ockendon, 1974) as well as at the molecular level (Nishio and Hinata, 1977).

In this study we report on our initial attempts at interspecific hybridization and study of the glycoprotein profiles of the first hybrids obtained.

MATERIALS AND METHODS

Plant Materials

Lines and cultivars used in the present study were: B. napus, doubled haploids from each of: G231, Topas, Regent, Ariel, Lergo, Karat, Westar, Marnoo and Global (obtained from Allelix Crop Technologies, Georgetown, Ontario), and B. oleracea commercial F1 hybrid broccoli, Cape Queen, Dandy, Green Valiant, Improved Comet and Lazer (supplied by Paul Arnison, Paladin Hybrids Inc., Ottawa, Ontario) and F1 line crosses given my designation of: BRH1, BRH2, BRH3, BRH4 and BRH5, (supplied by Dr. G. Ruttencutter, Asgrow Inc., San Juan Bautista, California). Plants were grown under a 20°C day/ 15°C night, 16 hour photoperiod regime. Light was supplied by incandescent and fluorescent lamps at 170 $\mu Em^{-2}/s$. Plants were fertilized once weekly with a 20-20-20 nutrient solution.

Embryo Rescue

All crosses were performed using B. napus as the female parent, by application of pollen from single B. oleracea plants to the stigma of hand emasculated immature flower buds. Embryo rescue was attempted at eight days post pollination as previously reported (Ripley and Arnison, 1990). The following modifications were made: to the original NN medium, 300 mg/L casein hydrolysate and 200 mg/L of L-glutamine were added. The embryo regeneration procedure was modified to follow the procedure of Coventry et al., 1988.

Stigma Glycoprotein Analysis

Of the approximately 60 S-alleles in B. oleracea approximately two thirds can be distinguished from one another on the basis of stigma glycoprotein profile. The actual procedure used in this study was isoelectric focusing (I.E.F.).

Stigmas from twenty pistils were obtained from parental and F1 hybrid plants when the plants were in full flower. To avoid pollen contamination, stigmas were taken from buds which were just about to open (yellow bud stage). All samples were kept on ice and stored at -70°C until protein

extraction. Total protein was extracted from the stigmas in 120 ul of water through grinding. Total protein was determined using the BCA assay (Pierce) to allow for loading 5 ug of protein per sample onto the I.E.F. gel.

Isoelectric Focusing

The samples were run on a 1 mm thick polyacrylamide gel to which an ampholyte solution (Pharmalyte, pH 3-10) was added to allow for the establishment of a pH gradient in the gel. All runs were done on an FBE-3000 flatbed apparatus (Pharmacia) at a constant power. The gels were pre-focused at 15 watts for 20 minutes followed by a run at 30 watts for a total of 1.5-2.0 hours (i.e. until total volt*hours exceed 3000).

The gels were produced by mixing 22 ml of I.E.F. monomer, (consisting of 12.125 g acrylamide, 0.375 g bis), 5.9 ml of glycerol and 2.2 ml of Pharmalyte ampholytes pH 3-10. The mixture was degassed under vaccuum for 45 minutes, after which 51 ul TEMED and 66 ul of 10% ammonium persulfate were added. The mixture was then loaded between two glass plates lined with Gelbond on one side and a plastic film on the other side. After polymerization, the glass plates were removed and the gel was placed onto the FBE-3000. All pre-focusing and running was done at 4°C. After running, the gel was fixed and stained with 0.1% silver nitrate stain. PI markers were run on every gel to provide a basis for determination of the PI values of unique bands in our samples.

RESULTS

Embryo Rescue

An experiment was performed to explore whether or not the response to interspecific hybridization was parent genotype dependent. When averaged over all crosses with B. oleracea, some genotypes such as G231 and Global exhibited the highest response, in terms of number of hybrids produced, Westar and Lergo exhibited an intermediate response, and Topas, Karat, Marnoo and Regent exhibited a poor response (Table 1). The effects of the B. oleracea parent were less clear. While the overall effects of the Asrow material vs. the commercial cultivars are very similar, the range of response is wider with the Asgrow material (13-46.5% vs. 16.6-38.9% respectively).

The response of the various B. napus genotypes to B. oleracea pollen indicates a wide range of response (i.e. from 0 to73.8% of the siliques developing). This initial response is not always indicative of the ultimate number of hybrids which can be produced.

Isoelectric Focusing

Protein was extracted from 20 stigmas of each F1 hybrid and run on an I.E.F. gel along with the B. napus parents used in the cross. From this analysis, three stigma protein profiles are evident (Fig. 1). B. napus parental material, lanes 3,8 and 10 have the same protein profile, while the F1 hybrids between B. napus and B. oleracea cv. Lazer show two distinct patterns. Hybrids in lane 5 and 6 exhibit a different unique band at pI 10.3; lanes 1, 3, 7, 9, 11 and 12 (see arrow on lane 8).

In order to confirm that these patterns are truly correlated to the S-phenotype, a diallel cross was performed with these F1 hybrid plants. The S reaction was observed through staining with analine blue and observing the stigmas under fluorescence microscopy (Table 2) (Fig. 2). A problem arises though, in that the plants have varying degrees of pollen viability i.e. ranging from 5-35% (data not shown). Therefore the hybrids cannot be compared on an equal level. To circumvent this problem, a procedure using male and female coefficients of crossability (Anderson et al., 1989), was employed. The MCC and FCC values are presented (Table 3), and are based on counts of pollen tubes (Table 2). Values of MCC and FCC approaching 1 for a specific cross, indicates that the two plants being crossed represent different S-phenotypes, while values approaching 0 indicate that the two plants are carrying the same S-allele. Pairs of MCC-FCC values which are

significantly different from each other indicate that some mechanism other than SI is acting (Anderson et al., 1989). Regressing FCC on MCC, an r value of 0.89 was calculated. Deviations of the FCC-MCC values from this regression line are evidence for other mechanisms, i.e. incongruity.

DISCUSSION

Embryo Rescue

The range of response of the B. napus parent is probably due in some part to the fact that individual plants were used in the crossing scheme (Quazi, 1988). Individual plants had to be used in the case of B. oleracea since our objective was to transfer S-alleles in a precise, defined way. By working with individual B. oleracea plants we could be assured that we would find only two S-alleles in the resulting progeny.

Individual plants were used for the B. napus parents in the cross for two reasons; firstly, to control any variability in stigma protein profiles which may complicate interpretation of results, and secondly to identify any highly responsive lines. While it is clear that there is a single line effect operating i.e. G231; 5004-2 vs. 5006-2, there also appears to be a genotype effect, as shown by the fact that all lines from some genotypes responded poorly i.e. Regent and Ariel. From this study, the best line from each genotype will be used in subsequent experiments to examine some of the factors contributing to success of interspecific hybridization through embryp rescue.

Stigma Protein Profiles

When hybridizing a B. napus line with a B. oleracea line, many hybrids can be produced from a single cross. Crosses involving only a single B. oleracea parent which contains two S-alleles should result in two different S specificities in the F1 hybrids. Unfortunately, the F1 hybrids between B. napus and B. oleracea typically have low pollen viability. Therefore when performing a diallel cross of the F1 hybrids it is difficult to definitively assign an S-phenotype due to the variation in pollen viability. If we carry the analysis one step further and count the number of pollen tubes we can get an idea of the potential crossability of the hybrids.

As another approach to circumvent the problem of sterility in the F1 hybrids, a study of the stigma protein profile of the B. napus parents and of the hybrids proved informative (Fig. 1). B. napus tends to lack or have very weak expression of protein bands in the basic portion of the gel which has been identified as the site of the S-locus specific glycoproteins in B. oleracea (Nishio and Hinata, 1977). The F1 hybrids exhibit a different protein pattern from their B. napus parents, as well they exclusively exhibit only two patterns which may represent the two S-alleles from a common B. oleracea parent (Fig. 1).

Based on the analysis of the stigma protein profiles, the hybrids can be divided into two groups; group 1 (E249-1, E209-1, E200-2 and E205-1), and group 2 (E205-2 and E53-1). Verification of these two groupings is supported by the diallel cross data (Table 3). Further evidence of the correlation between the protein patterns seen and the S-phenotype will be provided in subsequent analysis involving SI/SC segregating backcross generations.

CONCLUSIONS

Initial results indicate that B. oleracea represents a potential source of S-alleles for B. napus. Data has been presented that shows that hybrids between a range of genotypes of the two species can be obtained. The S-phenotype of the B. oleracea parent remains in force in the F1 hybrids and through isoelectric focusing of stigma proteins and diallel analysis, the S-phenotype can be selected at the F1 hybrid generation.

REFERENCES

ANDERSON, N.O., LIEDL. B.E., ASCHER, P.D. and DESBOROUGH, S.L. 1989, Distinguishing between self-incompatibility and other reproductive barriers in plants using male (MCC) and female (FCC) coefficients of crossability. Sex. Plant Rep. 2: 116-126.

BANKS, P. 1988. Self-incompatability and rapeseed breeding. Ph.D. Dissertation, Crop Science Dept., University of Guelph, Guelph, Ont.

COVENTRY, J., KOTT, L. and BEVERSDORF, W.D. 1988. Manual for microspore culture technique for Brassica napus. Dept. of Crop Sci. Tech. Bull. OAC Publ. 0489. University of Guelph, Guelph, Ont. 12-13.

GRANT, I. and BEVERSDORF, W.D. 1985. Heterosis and combining ability estimates in spring-planted oilseed rape (Brassica napus L.). Can. J. Genet. Cytol. 27: 472-478.

MACKAY, G.R. 1977. The introgression of S-alleles onto forage rape, Brassica napus L., from turnip, Brassica campestris L. ssp. rapifera. Euphytica. 26: 511-519.

NISHIO, T. and HINATA, K. 1977. Analysis of S-specific proteins in stigma of Brassica oleracea L. by isoelectric focusing. Hered. 38(3): 391-396.

OCKENDON, D.J. 1974. Distribution of self-incompatability alleles and breeding structure of open-pollinated cultivars of Brussels sprouts. Hered. 33(2): 159-171.

QUAZI, M.H. 1988. Interspecific hybrids between Brassica napus L. and B. oleracea L. developed by embryo culture. Theor. Appl. Genet. 75: 309-318.

RIPLEY, V.L. and ARNISON, P.G. 1990. Hybridization of Sinapis alba L. and Brassica napus L. via embryo rescue. Plant Breeding. 104: 26-33.

Table 1. Results of interspecific crosses of B. napus doubled haploids eith B. oleracea var italica

Genotype	Line I.D.	# Crosses	# Pods Developed	% Developed	# Hybrids
G231	5004-1	54	6	11.1	5
	5004-2	45	18	40.0	18
	5006-1	42	21	50.0	1
	5006-2	36	9	25.0	0
Topas	5059-1	22	1	4.5	0
	5059-2	34	5	14.7	1
	5062-1	82	21	25.6	1
Regent	5098-1	70	3	4.3	0
	5098-2	11	3	27.2	0
	5103-1	65	11	16.9	0
	5103-2	10	0	0	0
Ariel	5309-1	32	5	15.6	0
	5309-2	25	9	36.0	0
	5315-1	6	1	16.7	1
	5315-2	24	3	12.5	1
Lergo	5317-1	47	26	55.3	0
	5317-2	24	0	0	0
	5322-1	48	18	37.5	1
	5322-2	66	25	37.9	5
Karat	5323-1	42	31	73.8	3
	5327-1	36	3	8.3	0
	5327-2	36	1	2.7	0
Westar	5521-1	21	2	9.5	7
	5521-2	51	21	41.5	5
	5524-1	27	3	11.1	3
	5524-2	6	0	0	0
Global	5635-1	97	22	22.6	2
	5635-2	58	25	43.1	20
	5637-2	34	10	29.4	0
Marnoo	5638-2	47	0	0	0

Table 2. Number of pollen tubes observed from crosses of interspecific hybrids having the common B. oleracea parent Lazer

	E249-1	E205-2	E209-1	E53-1	E200-2
E249-1	3.7	15.2	0.3	1.0	6.7
E205-2	0.8	2.7	2.7	0	5.3
E209-1	0	2.3	0	0.5	0.7
E53-1	13.3	15.4	34.3	10.0	31.2
E200-2	0.2	13.3	33.8	4.0	0.8

Table 3. MCC and Fcc coefficients for diallel of F1 interspecific hybrids

	E249-1		E205-2		E209-1		E53-1		E200-1	
	MCC	FCC	MCC	FCC	MCC	FCC	MCC	FCC	MCC	FCC
E249-1	0.286	0.250	0.451	1.000	0.009	0.019	0.059	0.066	0.215	0.441
E205-2	0.060	0.028	0.080	0.093	0.087	0.103	1.000	1.000	0.169	0.183
E209-1	0	0	0.068	1.000	0	0	0.029	0.217	0.022	0.304
E53-1	1.000	0.39	0.379	0.373	1.000	1.000	0.588	0.292	1.000	0.909
E200-2	0.015	0.006	0.395	0.393	0.985	1.000	0.235	0.118	0.026	0.024.

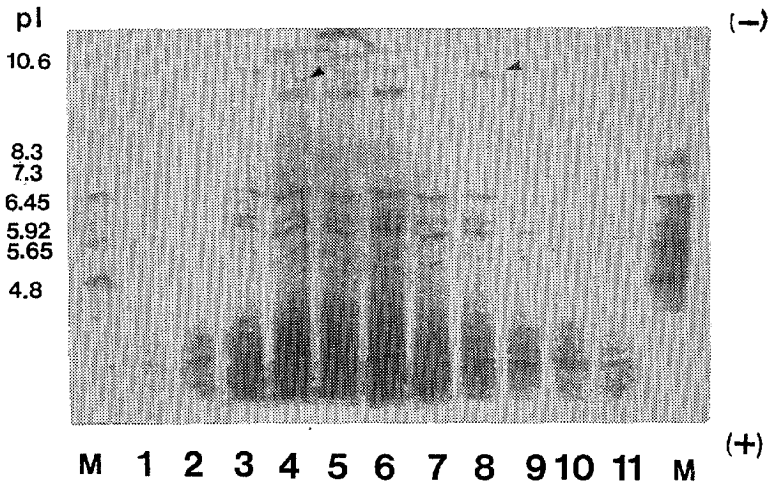

Fig. 1 Isoelectric focusing of stigma proteins of B. napus and F1 interspecific hybrids; 1= E53-1, 2= G231, 3= E205, 4= E205-2, 5= E205-4, 6= E200-1, 7= Global, 8= E200-2, 9= Karat, 10= E209-1, 11= E249-1 (M- denotes marker lanes)

(100 ×)

Fig. 2 Fluorescence micrograph of an incompatible stigma-pollen interaction exhibiting stunted pollen tubes (arrow)