TRANSFER OF DISEASE RESISTANCE TO OILSEED RAPE FROM WILD BRASSICA SPECIES

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INTRODUCTION

The wild taxa of the Brassica oleracea L. 2n=18 complex form a polymorphic aggregate of perennial species distributed on limestone maritime cliffs of southern and western Europe and north Africa (Snogerup et al., 1990). All of these species are at least partially interfertile with each other and with cultivated forms of B.oleracea, and are considered to possess the Brassica C genome (Harberd, 1972; Snogerup, 1980). The C genome also occurs within the allotetraploid species B.napus L. (genome AACC, n=19) which arose through the spontaneous hybridisation of B.oleracea and B.rapa L. (AA, n=10) followed by chromosome doubling to restore fertility.

A number of characters occur within the wild C genome taxa which are not observed within the cultivated Brassica species. Several of these characters may have agronomic value. For example, the intense hairiness of the B.incana-rupestris complex may confer pest resistance and the thickened pods of B.macrocarpa (Guss.) Caruel and B.hillarionis Post appear to be partially resistant to pod shattering. Resistance to the fungal pathogens Peronospora parasitica and Leptosphaeria maculans have been shown to occur within these wild forms (Greenhalgh and Mitchell, 1976; Mithen et al., 1987). It is likely that other characters of agronomic importance such as improved water use efficiency and pest resistance may be present within these taxa.

Novel forms of B.napus can be routinely made by interspecific hybridisation of the diploid progenitors and subsequent in vitro culture of embryos (Inomata, 1978; Chen and Heenan, 1989). The majority of these synthetic forms have been derived from a limited range of B.oleracea and B.rapa genotypes, and none have been developed from wild forms, although the production of embryos from a cross between B.rapa and B.cretica Lam. has been reported (Inomata, 1985). The potential for using cultivated and wild forms of the diploid progenitors of B.napus in oilseed rape breeding programmes is likely to increase considerably with the development of molecular markers in Brassica. This technology will significantly reduce the time required for introgressing the desired genes into an agronomically superior oilseed rape background.

This paper examines the development of synthetic B.napus lines from wild species of the B.oleracea complex and the expression of resistance to foliar infection by Leptosphaeria maculans.

MATERIALS AND METHODS

Plant Material

The origin of the Brassica lines used in this study are shown in Table 1. Inbred lines had been developed from the cultivars by bud pollination. The B.atlantica and B.incana accessions were received from Professor Snogerup, University of Lund and the other wild accessions from Professor Gomez-Campo, Universidad Politecnica, Madrid. Plants were cultivated in John Innes no.1 compost in 8" pots in an insect proof glasshouse with a minimum temperature of 12°C.

Table 1. The Brassica lines used in the present study.

GENOME	TAXON	ORIGIN
A	B.rapa subsp. oleifera	cultivar Maleksberger
C	B.oleracea var. alboglabra 8	cultivar, Japan
C	B.oleracea var. alboglabra 11	cultivar, Japan
C	B.insularis Moris	Cape Caccia, Sardinia
C	B.atlantica (Coss.) Schultz	Mt Bou Kourein, Tunisia
C	B.incana Ten.	Mt Alburni, Italy
C	B.montana Pourr.	Cape Norfeo, Spain
C	B.macrocarpa (Guss.) Caruel	Egadi Islands, Sicily

Development of synthetic B.napus lines.

Flowers of B.rapa were emasculated and pollinated immediately with fresh pollen from each of the C genome taxa. After six days, the pistils were removed, surfaced sterilised in 10% sodium hypochlorite solution and cultured on Nitsch and Nitsch media (Sigma Ltd) containing 3% sucrose and 0.6% agar. After 25-30 days, embryos were dissected from the ovaries and cultured on Gamborgs B5 medium with 3% sucrose and 0.6% agar. Secondary embryos and adventitious meristems which developed from the enlarged hypocotyl of the primary embryos were subcultured onto fresh B5 medium. If organised meristems had not developed from the primary embryos after three weeks, the tissue was placed on Murashige and Skoog medium B (Sigma Ltd) to induce shoot development, and then transferred back onto B5 for root development. After a good root system had developed in culture, the plants were transferred to JI no.1 compost in a heated glasshouse.

When flower buds could be seen developing within the apex, the apical meristem was cut off and the epidermis on the petiole and stem around each axillary meristem was removed by scraping with a scalpel. A 0.1% aqueous colchicine solution was then applied to the meristems and allowed to dry. When inflorescences developed which appeared to be fertile by virtue of fully developed

stamens and viable pollen (determined by staining in 0.4% acetocarmine solution), open flowers were self pollinated and the inflorescence enclosed within a polythene bag into which CO_2 was injected to raise the internal concentration to above 5%. The bag was removed after 72 hours. Seeds obtained from the colchicine treated plants were germinated and plants raised in conditions similar to above. Upon flowering, they were selfed by either bud pollination or with CO_2 and crossed to a homozygous spring rape line derived by microspore culture of the cultivar Westar. Hybrids with the winter rape cultivars Cobra and Tapidor were also obtained.

Inoculation with L.maculans

A highly aggressive isolate of L.maculans designated RFMLM11 was cultured on V8 agar at 15°C. Upon sporulation, the culture was flooded with sterile distilled water and left for 5 minutes for discharge of conidia into the water. The spore suspension was then pipetted from the plate and the concentration of spores adjusted to 1 x 10^6 spores ml^{-1}. Plants of the synthetic B.napus lines were grown under the conditions described above and inoculated by placing a 10μl drop of spore suspension over a pin-prick wound in the cotyledons. A further batch of plants were inoculated at six sites on leaves two and three. Plants were assessed after three weeks by measuring the diameter of the lesions.

RESULTS

Development of synthetic lines

Interspecific embryos were derived from each of the crosses at similar frequencies (Table 2). Within each cross, some pods contained up to three or four embryos while the majority were empty. Pods which contained embryos were frequently, but not exclusively, those that had developed roots from the basal end of the pedicel. Of the 75 primary embryos cultured, only two grew directly into plants. However, following shoot and root induction, plantlets were derived from embryos of each cross. After colchicine treatment, fertile plants and seed were obtained.

Table 2. The number of interspecific embryos obtained from crosses between B.rapa and of C genome taxa.

MALE PARENT	OVARIES CULTURED	EMBRYOS	EMBRYOS / OVARY
var. alboglabra 8	20	3	0.15
var. alboglabra 11	34	5	0.15
B.insularis	107	16	0.15
B.atlantica	113	17	0.15
B.incana	60	11	0.18
B.montana	50	8	0.16
B.macrocarpa	132	15	0.11

Growth and fertility of synthetic lines and F1 hybrids.

The synthetic lines grew vigorously. Flowering time of the synthetic hybrids was intermediate between the two diploid parents but it was not possible to predict precisely. Pollen from each of the lines exhibited a high degree of fertility (Table 3), although in each case the fertility was lower than that of the B.napus Westar line, or that observed with other cultivated forms of B.napus. All the lines were self-incompatible, but could be selfed through bud pollination or by CO_2 treatment.

Each of the F1 hybrids were vigourous, and flowered within two weeks of each other. Pollen from each of the F1 lines exhibited levels of fertility similar to the synthetic lines (Table 3). All the F1 hybrids were self-compatible, with seeds being produced through self pollinating open flowers, except for the lines derived from B.oleracea var alboglabra 8, which were self-incompatible. Self seed were obtained from this F1 hybrid by bud pollination.

Table 3. Fertility of synthetic allopolyploids and F1 hybrids with B.napus cv. Westar.

	POLLEN VIABILITY MEAN ± SE	
C GENOME	SYNTHETIC AACC	F1 WITH B.NAPUS
var. alboglabra 8	85.9 ± 2.75	80.7 ± 3.17
var. alboglabra 11	87.1 ± 1.92	96.3 ± 1.11
B.insularis	92.7 ± 1.20	90.0 ± 4.00
B.atlantica	95.0 ± 3.50	91.6 ± 2.49
B.incana	89.2 ± 2.31	98.0 ± 1.05
B.montana	83.3 ± 4.41	92.0 ± 2.31
B.macrocarpa	83.6 ± 2.86	90.0 ± 7.29

Several morphological characters were expressed in the synthetic lines which were derived from the C genome parent, including the short, non-shattering pods of B.macrocarpa and the trichomes of B.incana.

Resistance to L.maculans.

Cotyledons of all the lines were very susceptible to infection by L.maculans, and no significant difference between the lines were apparent. In contrast, true leaves of lines derived from B.insularis, B.macrocarpa and B.atlantica were less susceptible to infection by L.maculans than lines derived from the other taxa (Table 4). Resistance was expressed by a reduction in lesion size and a greater amount of cell necrosis around the site of inoculation. Following surface sterilisation, the fungus could be re-isolated from leaves of all the lines.

Table 4. Susceptibility of leaves of synthetic lines to L.maculans.

C genome of synthetic allopolyploid	Lesion diameter mean ± SE
var. alboglabra 8	10.42 ± 3.01
var. alboglabra 11	9.00 ± 0.71
B.atlantica	0.56 ± 0.30
B.insularis	1.85 ± 0.43
B.montana	7.26 ± 0.83
B.macrocarpa	0.70 ± 0.31
B.incana	not tested

DISCUSSION

The development of synthetic B.napus lines

It was possible to develop synthetic allopolyploids between B.rapa and all of the C genome taxa used in this study. Each of these lines were sexually compatible with B.napus and there were no major reductions in fertility. Hence, there appears to be no significant barriers to the introduction of traits from the wild taxa into B.napus breeding lines. The reduction in fertility of the synthetic lines and the F1 hybrids suggests that there are some meiotic irregularities. It is likely that these are due to attempted pairing of homoeologous chromosomes within the A and C genome, as has been reported by Attia and Robbelen (1986).

Several characters were expressed within the synthetic lines which have not been observed within naturally occurring B.napus lines or synthetic lines derived from cultivated diploid taxa. The inheritance and potential agronomic value of these characters are currently being evaluated.

Resistance to Leptosphaeria maculans.

The reduction in lesion size following infection by L.maculans on synthetic B.napus lines derived from B.atlantica, B.macrocarpa and B.insularis is similar to that reported to occur within the wild diploid taxa themselves (Mithen et al., 1987). This form of resistance is not expressed in the cotyledons. It has previously been suggested that the reduction in fungal growth on leaves of wild Brassica species is a result of the high levels of alkenyl glucosinolates found in the leaves of these taxa (Mithen et al., 1987). These glucosinolates undergo hydrolysis to give antifungal products. The expression and inheritance of leaf glucosinolates in the synthetic lines reported in this study and their relationship to disease resistance will be considered in a separate publication.

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