Progress in the utilisation of *Brassica nigra* in breeding for resistance to blackleg (*Leptosphaeria maculans*)

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ABSTRACT

The blackleg disease, caused by *Leptosphaeria maculans,* is one of the most important diseases of rapeseed worldwide. The use of resistant cultivars is frequently the most economical and effective means of controlling plant disease. Previous research identified *B*. *nigra* as highly resistant to the blackleg fungus and a potential source of resistance for *B*. *napus*. Interspecific crosses (*B*. *napus* and *B*. *nigra*) were made to produce a triploid hybrid, which was gamma-irradiated and treated with colchicine. The resultant hexaploid F_1 was backcrossed twice to *B*. *napus* and evaluated for resistance to blackleg under glasshouse conditions. Twenty eight percent of these BC_2F_1 plants displayed high levels of resistance to blackleg and indicated the likelihood of gene transfer from *B*. *nigra* to *B. napus.* Doubled haploids from crosses involving these *B*. *nigra* derived lines and *B. napus* elite cultivars were produced and evaluated for blackleg resistance by the Victorian canola breeding program. Three lines were identified with high levels of blackleg resistance confirming the likelihood of transfer of chromosome segments of *B. nigra* carrying blackleg resistance genes in *B*. *napus* background. These lines are now being used as parents in the crossing program, thereby contributing towards broadening the germplasm base in breeding blackleg resistant canola cultivars.

Key words: ionising, homoeologous, recombination, disease

INTRODUCTION

The production of canola in Australia has increased considerably in recent years and a substantial contribution to both the level and stability of production levels has been due to the release of cultivars moderately resistant to blackleg. However, high levels of effective resistance, as in *B. nigra,* have yet to be achieved. Among the cultivated *Brassica* species, different levels of chromosome pairing occur in hybrids between the three ancestral diploid species, *B. campestris* (AA), *B. oleracea* (CC), and *B. nigra* (BB). Amphihaploids involving *B. nigra,* AB (n = 18) and and BC (n = 17) showed relatively low frequency of chromosome pairing than those involving *B*. *campestris* and *B*. *oleracea* (AC, n=17) (Attia and Robbelen 1986). The major limitation to the exploitation of *B. nigra* as a source of useful genetic traits for *B. napus* improvement is the lack of chromosome pairing in interspecific hybrids between *B. napus* and *B. nigra*.

Evidence from other crop species suggests that significant advances have been made in plant breeding by the use of ionising radiation. This technique relies on in inducing 'recombination' between the chromosomes of a donor species and crop species in interspecific hybrids, in situations where there is little to no pairing between the chromosome sets of the different parental species. Thus, homoeologous pairing between *B. nigra* and *B. napus* chromosomes would need to be induced to transfer *B. nigra* genes *e.g* blackleg resistance to *B. napus* presumably onto homoeologous *B. napus* chromosomes. This is on the assumption that effective gene incorporation and subsequent expression must entail its incorporation in a homoeologously equivalent position in the related genome. Against this background, the aim of this study is to effect radiation-induced translocation between chromosomes of *B. nigra* to *B. napus* and to subsequently select for translocations that confer the blackleg resistance from *B*. *nigra* in fully viable and fertile genotypes of *B*. *napus*.

MATERIALS AND METHODS

Plant materials: 269 BC_2F_1 lines derived from gamma-irradiated triploid hybrid of the cross, (*B. napus* /*B. nigra*) x *B. napus* were used for this study. The experiment was carried out in a glasshouse

maintained at 25°C (day) and 15°C (night) with a natural daylength of 10-12 hours during the growing period.

Fungal cultures of six *L. maculans* isolates (G2, Ga2, Md2, N2, OJ4 and P2), were used for the infection of plants. The inoculum consisted of a mixture of equal volumes of pycnidiospores (10^6 spores/ml) from the six *L. maculans* isolates. Infection was achieved using the wound-inoculation, at growth stage 2.4-2.5. After inoculation, the plants were incubated at 100 % relative humidity for 5 days in the glasshouse.

The parents and the derived BC_2F_1 plants were assessed for their level of infection to *L. maculans* eight weeks after inoculation using two disease measures, namely external lesion length (E) and percentage of stem girdled by the lesion (% G). A modified method of Newman's (1984) was used for disease assessment in which plants with scores of:

(i) 0 for E and %G were considered to be resistant (class 1),

(ii) 1-10 mm, E and less than 25% G, were considered as moderately resistant (class 2 which includes those with hypersensitive reaction)

(iii) > 10mm but less than 19mm and > 25% G, they were considered susceptible (class 3),

(iv) those with E greater than 30mm and 75 - 100% G were designated as class 4

(v) plants which collapsed following severe incidence of blackleg infection were placed in class 5. However, only plants in classes 1 and 2, were considered as resistant.

Field evaluation of DH lines: In 2001, 130 DH lines involving BC2F1 lines and *cv.* Mystic (Figure 1). were screened in a blackleg field nursery. Individual rows of each DH were selected based on disease resistance and plant type (highly heritable traits). A survival percentage method was used to evaluate DH line for resistance to blackleg. Briefly, lines were scored visually for resistance (0-4 scale with 0 =susceptible and 4 =highly resistant) to blackleg and plants were counted at establishment and maturity to allow survival rates to be calculated.

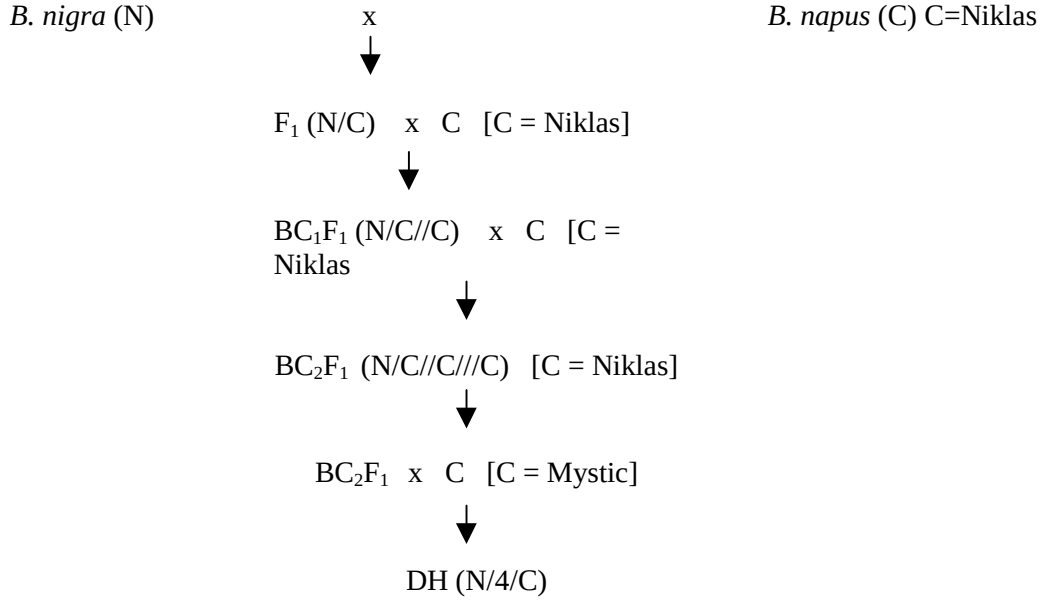

Fig. 1. Scheme showing the production of doubled haploid lines derived from crossing *B. nigra* with *B.napus.*

RESULTS AND DISCUSSION

Approximately 8% of the 197 BC_2F_1 plants were highly resistant to *L. maculans* infection using a combined scale of external lesion size and the proportion of stem girdled by the pathogen. 20% displayed a hypersensitive-type reaction while 71% were susceptible. In comparison, plants of the susceptible parent *B. napus* 'Niklas' displayed a high level of susceptibility with 50% of such plants displaying 75 - 100%G in which the entire stem were girdled.

In a blackleg field nursery during 2001, 3 of 130 DH lines had blackleg resistance greater than *cv*. Mystic.

Table 1. Percentage survival and mean disease score of *B. nigra* accession, canola cultivars, backcross and doubled haploid lines used in study

Parent	Plant Survival (%)
B. nigra	97
B. napus – cv. Niklas	0
B. napus – cv. Mystic	12
BC_2F_1 lines	0

DH line	Blackleg disease score (0 – 4)
DH 002	3
DH 152	3
DH 153	3
B. napus – cv. Mystic	1.9
B. napus – cv. Niklas	0
BC_2F_1 lines	0

Based on the degree of chomosome pairing in crosses between *B. napus* and *B. nigra*, the expectation was that there would be minimal recombination between the B-genome and those of A and C-genomes (Attia and Robbelen 1986). However, about 28% of the second backcross progenies to *B. napus* of the gamma-irradiated triploid hybrid displayed high levels of resistance to *L. maculans* in the glass-house. 8% of these showed disease response pattern typical of the *B. nigra* type resistance, suggesting a limited gene transfer from *B. nigra* to *B. napus* amongst the BC_2F_1 plants, assuming that this resistance is under a single gene control. Sjödin and Glimelius (1989) similarly reported the transfer of genes for resistance to *L. maculans* into *B. napus* in an asymmetric hybridization *via* protoplast fusion of *B. nigra* and *B. napus* but this was limited to only the triploid F_1 hybrid generation. Similar results were also reported by Gerdemann-Knock *et al*. (1995).

Further evaluation of these lines using three *B. nigra* specific primers showed that 30% of the 94 BC_2F_1 plants contained *B. nigra*-specific bands (data not shown). This supports the possibility for a substantial retention of genetic variation from *B. nigra* in this population though still segregating. These BC_2F_1 lines could constitute translocation, chromosome addition or substitution of *B. nigra* chromosomes to the *B. napus* complement with little likelihood of normal recombination. If this were recombination as a consequence of radiation-induced translocation, it would have to be validated at later backcross generations given that this is only the BC_2F_1 generation.

However, DH lines involving BC_2F_1 (*B. nigra* derived) lines and *cv.* Mystic with acceptable agronomy and blackleg resistance were identified under field conditions. This supports the likelihood of homoeologues gene recombination from B-genome of *B. nigra* into AC-genome of *B. napus* with the use of radiation-induced translocation. Homoeologous recombination between the B genome of *B. nigra* and A or C genomes were also suggested in the identification of two independent loci in near isogenic lines of *B. napus* derived from asymmetric somatic hybrids of *B. napus and B. nigra* (Dixelius 1999).

REFERENCES

Attia, T. and Robbelen, G. (1986). Cytogenetic relationship within cultivated *Brassica* analysed in amphidiploids from the three diploid ancestors. Can. J. Genet. Cytol. 28, 323-329.

Dixelius, C (1999). Inheritance of the resistance to Leptosphaeria maculans of *Brassica nigra* and *B. juncea* in near-isogenic lines of *B. napus*. Plant Breeding 118, 151-156.

Gerdemann-Knorck, M., Nielen, S., Tzscheetzsch, C., Iglisch, J., Schieder, O., Cassells, A. C., and Jones, P. W. (1995). Transfer of disease resistance within the genus Brassica through asymmetric somatic hybridization. Euphytica 85, 247-553.

Newman, P. L. (1984). Screening for disease resistance in winter oilseed rape. In Aspects of Applied Biology 6, Agronomy, physiology, plant breeding and crop protection of oilseed rape. 371-380.

Sjödin, C., and Glimelius, K. (1989). Transfer of resistance against *Phoma lingam* to *B. napus* by asymmetric somatic hybridization with toxin selection. Theor. Appl. Genet. 77, 651-656.