Stabilization of Resistance to *Leptosphaeria maculans* in *Brassica napus*–*B. juncea* Recombinant Lines and Its Introgression into Spring-Type *Brassica napus*

A.-M. Chèvre, INRA, UMR118, F-35000 Rennes, France; H. Brun, INRA, UMR1099, F-35000 Rennes, France; F. Eber, J.-C. Letanneur, and P. Vallee, INRA, UMR118, F-35000 Rennes, France; M. Ermel and I. Glais, INRA, UMR1099, F-35000 Rennes, France; Hua Li, School of Plant Biology, The University of Western Australia, Crawley, W.A. 6009, Australia; K. Sivasithamparam, School of Earth and Geographical Sciences, The University of Western Australia, Crawley, W.A. 6009, Australia; and M. J. Barbetti, School of Plant Biology, The University of Western Australia, Crawley, W.A. 6009, Australia and Department of Agriculture and Food Western Australia, Baron-Hay Court, South Perth, W.A. 6151, Australia

ABSTRACT

Chèvre, A.-M., Brun, H., Eber, F., Letanneur, J.-C., Vallee, P., Ermel, M., Glais, I., Hua Li, Sivasithamparam, K., and Barbetti, M. J. 2008. Stabilization of resistance to *Leptosphaeria maculans* in *Brassica napus*–*B. juncea* recombinant lines and its introgression into spring-type *Brassica napus*. Plant Dis. 92:1208–1214.

The value of Katanning Early Maturing (KEM) breeding lines from Western Australia, derived from *Brassica napus* × *B. juncea* crosses, was assessed as a source of germplasm for resistance to blackleg disease (caused by *Leptosphaeria maculans*) in spring-type oilseed rape cultivars. The stability of blackleg resistance in these KEM lines was related to key cytological characteristics to determine why there are poor levels of introgression of this resistance into progeny. Promising recombinant KEM lines were crossed with the spring-type *B. napus* cv. Dunkeld, which has useful polygenic resistance to blackleg, and screened for resistance. The lines were analyzed cytologically for pairing of bivalents in each generation to aid in the selection of stable recombinant lines. KEM recombinant lines showing regular meiotic behavior and a high level of blackleg resistance were obtained for the first time. We also showed that the stable introgression of the *B. juncea* resistance from the KEM lines into a 'Dunkeld' background was possible. Inoculation of selfing and backcross populations with isolates of *L. maculans* having different *AvrLm* genes indicated that the *B. juncea* resistance gene, *Rlm6*, had been introgressed into a *B. napus* spring-type cultivar carrying polygenic resistance. The combination of both resistances would enhance the overall effectiveness of resistance against *L. maculans*. This is clearly needed in Australia and France where cultivars relying upon single dominant gene-based resistance for their effectiveness have proved not durable.

Additional keywords: blackleg, breeding, canola, oilseed rape, phoma

Blackleg (causal fungus, *Leptosphaeria maculans* (Desm.) Ces. et de Not.) causes severe epidemics and major economic losses in the main oilseed rape (*Brassica napus* L.) growing regions of Canada, Europe, and Australia despite differences among these regions in growing seasons, cultivars, agricultural practices, and climates (18,44). The fungus causes cotyledon and leaf lesions during the vegetative phase of the host plant. Crown canker is the most damaging phase of the disease and can lead to dramatic yield losses of oilseed rape (44). In Australia, yield losses result predominantly from the crown canker phase of the disease but also from infections that occur at the early seedling

stage (5,6,7,41), especially from cotyledon infections that occur above (7) and below (22,23) ground.

The use of host resistance is the major method of managing the disease in Australia (6,41), Europe (12), and Canada (30,31). Resistance to *L. maculans* is usually categorized as seedling and/or adult-stage resistance. Because there is a high degree of correlation, particularly in Australia, between cotyledon infection and subsequent crown canker development (10,20,24,25), resistance at the cotyledon and adult phases is being sought by oilseed rape breeders (40,41,44). However, the resistance conferred at the seedling stage is considered to be under control of monogenic dominant genes, each having its corresponding avirulence gene in the pathogen population (2). For this reason, the efficacy of such resistance is highly dependant on the genotype structure of the pathogen populations and the frequency of races that are able to overcome that resistance (20,32). Adult-stage resistance is generally considered to be partial, nonspe-

cific, polygenic, and influenced by environmental conditions (17,28) compared with seedling resistance. The spring-type *B. napus* cv. Dunkeld has been known to carry polygenic resistance to blackleg (19; http://www.regional.org.au/au/cs/2004/poster/3/7/3/975_gororo.htm). A diversification of the resistance sources is needed for selection of durable resistant genotypes.

Brassica species with the B-genome, e.g., the diploid species *B. nigra* L. Koch (BB, 2n = 16) and the two derived amphidiploids, *B. juncea* L. Czern (AABB, 2n = 36) and *B. carinata* A. Br. (BBCC, 2n = 34), show complete resistance to blackleg that remains effective throughout the life of the plant (31,35). In *B. juncea*, several studies showed that the resistance is under control of few genes (26), and two *AvrLm* genes of the pathogen were described as revealing two dominant genes in *B. juncea* (3). One of them, *Rlm6*, was successfully introduced into winter-type *B. napus* (11). *B. napus* lines containing resistance introgressed from *B. juncea* (11,14,34,37,43) demonstrated a clear link between cotyledon susceptibility/resistance and responses of adult plants, whereas tests performed on different organs (cotyledon, leaf, petiole, and stem) indicated that several genes might be implicated in the resistance from *B. juncea* (29,36–39).

Roy (35) reported the creation of a recombinant line of *B. napus*, OnapJR, carrying a *B. juncea* (JR) resistance gene(s) effective at the adult stage. The use of recombinant lines in breeding programs remains difficult because of the loss of introgression (40) or production of unstable aneuploid lines (31). Subsequently, in 1986, a single dwarf, early maturing selection plant within a selection taken from the 82N105 material was used to produce further early maturing and/or dwarf selections at Katanning, Western Australia (S. Morgan, D. Bowran, and P. Fels, unpublished data). Two Katanning Early Maturity (KEM) lines, viz. KEM 165 and KEM 167, were selected for high levels of resistance to blackleg disease under field conditions in Western Australia. However, crosses made with these KEM populations did not consistently produce offspring

Corresponding author: M. J. Barbetti
E-mail: mbarbett@cyllene.uwa.edu.au

Accepted for publication 21 April 2008.

doi:10.1094/PDIS-92-8-1208
© 2008 The American Phytopathological Society

resistant to blackleg, suggesting that the resistance was not stably introgressed, and precluding its ready utilization by oilseed rape breeders.

The objective of this study was to reassess the value of KEM populations as a source of resistance for improving blackleg resistance in spring-type oilseed rape cultivars. For this purpose, blackleg resistance in selfing progeny of KEM populations was related to cytological evidence for introgression of this resistance. The performance of progenies from backcrosses of the KEM plants with the spring-type *B. napus* cv. Dunkeld was evaluated in cytological studies and with pathogenicity assays using different races of *L. maculans* to identify the gene(s) carried.

MATERIALS AND METHODS

Plant materials. An original recombinant line of spring-type *B. napus* containing resistance transferred from *B. juncea* (82N105) was produced by N. N. Roy (formerly of the Department of Agriculture and Food Western Australia, South Perth, Western Australia). Full details of the pedigree are provided in Figure 1. In 1986 in Western Australia, a single dwarf, early maturing plant selection taken from the original 82N105 material was used to produce further early maturing and/or dwarf selections (M. J. Barbetti, unpublished data). In the 1990s, two KEM populations,

viz., KEM 165 and KEM 167, were selected for resistance to blackleg under field conditions at Mt. Barker, Western Australia. Subsequent selections from these two KEM populations were made at Rennes, France. Cotyledon screening, undertaken utilizing different *L. maculans* isolates used to identify specific resistances, and cytogenetic analyses, based on numbers of bivalents, were performed on selfing progeny of both populations to fix the resistance gene(s). From the KEM165 population chosen, the genealogy we followed to produce the material is presented (Fig. 2). For introduction of the resistance gene into a spring-type oilseed rape genotype of interest, 'Dunkeld' was crossed to S1 plants selected (Fig. 2). After the first cross to 'Dunkeld' (1), one additional selfing generation was performed just for the plants selected with S7 *L. maculans* isolate because of the poor seed set of the initial F1 resistant plant chosen. Three complementary backcross progenies to 'Dunkeld' were analyzed. Each year the recurrent parent 'Dunkeld' was screened at the same time as the progeny and a susceptible control comparison and the least diseased plants against each *L. maculans* isolate were used for the backcrosses. 'Westar' and an introgressed line with the B genome, viz., Samourai MX carrying *Rlm6* (11), were used as control comparisons for each isolate.

***L. maculans* isolates.** Selection for resistance was performed in France with cotyledon inoculation of the KEM 165 and 167 populations and the original Dunkeld 1 population with three single ascospore isolates obtained from pseudothecia produced on plant debris infested with *L. maculans*. They were previously characterized for the avirulent gene (*AvrLm*) profiles on a differential set of plant hosts (12) following the nomenclature proposed by Balesdent et al. (4). Isolate S7 (*AvrLm*1-5-6-7-[8]), isolated from pseudothecia developed on a *B. napus*-*B. nigra* addition line carrying a resistance gene from *B. nigra*, overcomes this *B. nigra* resistance (9); isolate MX4.3 (*AvrLm*1-5-7-[8]), isolated from pseudothecia developed on a *B. napus*-*B. juncea* recombinant line carrying *Rlm6*, a *B. juncea* resistance gene, overcomes this *B. juncea* resistance (42); and isolate 290 (*AvrLm*5-6-7-8), isolated from *B. napus* pseudothecia, does not overcome either the *B. juncea* or the *B. nigra* resistances (42). Spore suspensions for inoculation of each of these isolates were prepared by a method modified from De March et al. (13). De March et al. (13) transferred single germinated spores of *L. maculans* to V8 juice agar containing Rose Bengal (40 mg/l) and streptomycin sulfate (100 mg/l). They then grew cultures for 10 days (21°C, 12-h photoperiod) on V8 for production of pycnidia, and then conidial suspensions

Fig. 1. Pedigree details for the *Brassica napus*-*B. juncea* recombinant line 82N105 based on details from N. N. Roy (35).

obtained by flooding the surface of the colonies with sterile water were filtered through cheesecloth under sterile conditions and the concentration adjusted to 10^7

spores ml^{-1} . We did not add Rose Bengal to the V8 juice and colonies were incubated under 300 to 400 nm black light (OSRAM L 40W/75) to enhance sporula-

tion. Isolates were maintained at 4°C on malt (2%) agar (2%) medium.

Experimental conditions and inoculations and disease assessments. All black-leg cotyledon assessments were undertaken in France. Inoculations were performed as described by Williams and Delwiche (45). For each cross and all generations, a minimum of 20 seeds were sown in a greenhouse in a randomized block design with two replications (a minimum of 10 plants per seedling tray [$30 \times 44 \times 10 \text{ cm}$]). Each seedling tray was filled with a sand/loam/compost mixture of 1:1:1. When plants were 12 to 13 days old, each lobe of each cotyledon of each seedling was wounded in the center with a needle and inoculated with a 10- μl drop of inoculum containing 10^7 pycnidiospores ml^{-1} (i.e., each experimental unit consisted of one point inoculation per lobe of each cotyledon and so four inoculation points per seedling). After incubation in darkness for 24 h at 20°C , each plant was placed under a plastic cover to maintain a saturated humidity in a growth chamber and then seedlings were maintained in a growth chamber (20°C , 16 h light/8 h night, around 80% relative humidity). Symptoms were scored 14 and 21 days after inoculation. The level of cotyledon infection was scored on a 0 to 11 scale in which 0 = no disease and 11 = cotyledons collapsed from blackleg. The scale was modified from the 0 to 9 scale described earlier by Williams and Delwiche (45) in which 0 = no darkening around wound – typical response of noninoculated controls or controls inoculated with water only; 1 = limited blackening around wound and lesion diameter of 0.5 to 1.5 mm; 3 = dark necrotic lesion with diameter of 1.5 to 3.0 mm; 5 = nonsporulating, 3 to 6 mm in diameter, lesion sharply delimited by darkened necrotic tissue; 7 = grayish green

Fig. 2. Genealogy of the original KEM 165 *Brassica napus*-*B. juncea* line and progenies following screening against three *Leptosphaeria maculans* isolates. S7 overcomes the *B. napus*-*B. nigra* addition line resistance, (*AvrLm*-5-6-7-[8]); 290 overcomes the *Rlm1* resistance gene, (*AvrLm*-5-6-7-8). The identity of the plants selected at each generation is given in between brackets. The different generations are represented as follows: original selfed = S0; first selfed = S1; first cross to 'Dunkeld' = F1; and first, second, and third backcross generations = B1, B2, and B3, respectively.

Table 1. Number of resistant, intermediate, and susceptible plants of the *Brassica napus*-*B. juncea* lines and their progenies on the basis of inoculation with isolates S7, 290, and MX4.3 of *Leptosphaeria maculans* in selfed and backcrossed generations

Isolates ^a	Generations ^b	Resistant	Intermediate	Susceptible	Ratio R:S	χ^2
S7 (<i>AvrLm</i> 1-5-6-7-[8])	S0 KEM165					
	KEM167	27	2	5		
	S1	10	0	0		
	F1	23	8	0	0:1	0* ^c
	B1	23	0	13	1:1	2.78*
	B2	21	0	0	1:1	21
	B3	26	0	0	1:1	26
290 (<i>AvrLm</i> 5-6-7-8)	S0 KEM165	29	1	0		
	KEM167	29	0	4		
	S1	13	0	3		
	F1	20	0	0	0:1	0*
	B1	8	1	11	1:1	0.22*
	B2	3	5	12	1:1	0.8*
	B3	10	1	10	1:1	0.05*
MX4.3 (<i>AvrLm</i> 1-5-7-[8])	S0 KEM165	0	0	33		
	KEM167	1	0	34		

^a S7 overcomes the *B. napus*-*B. nigra* addition line resistance; 290 overcomes the *Rlm1* resistance gene; and MX4.3 overcomes the *Rlm6* *B. juncea* resistance. For the *AvrLm* figures within brackets, isolates were not assessed on the corresponding resistant host.

^b Generations were original selfed = S0; first selfed = S1; first cross to 'Dunkeld' = F1; and first, second, and third backcross generations = B1, B2, and B3, respectively.

^c * Indicates not significant (NS) at $P = 0.05$

tissue collapse, lesion of 3 to 5 mm, with sharply delimited, nondarkening margin; and 9 = rapid tissue collapse at approximately 10 days, accompanied by profuse sporulation in large lesion (greater than 5 mm), and lesions with diffuse, nondarkened margins. There are no 2, 4, 6, 8, or 10 points on this assessment scale. To this scale established by Williams and Delwiche (45), we added an additional category of "11 = cotyledon totally collapsed, very often dry" because this phenotype did not exist in the scale described above by Williams and Delwiche but is required to categorize the most susceptible plants. Plants of classes 0 to 5 were considered as resistant as well as plants in classes 5 to 7 showing typical resistant plant reaction with blackening and limited symptoms that did not develop further between the two assessment dates at 14 and 21 days after inoculation. Plants of classes 9 to 11 were classified as susceptible, with symptoms appearing quickly, no blackening observed in lesions and with rapid disease progression between the two dates of assessment (14 and 21 days after inoculation). The highest score of the four inoculation points was considered as representative of plant behavior for every seedling. During the selection procedure, more than 20 plants per genotype were tested in each generation. Because mother plants were either selfed or backcrossed, each plant of progeny is unique and the replication restricted to the four cotyledon lobes of each seedling. Segregation analyses using tests of chi-square were used to make comparisons of frequency distribution of resistant versus susceptible seedlings.

Cytogenetic studies. Because of a putative chromosome instability due to *B. juncea* introgression in KEM material, we checked the chromosome number and the meiotic behavior of the plants at each generation (viz., the original selfed and the four backcross generations). The method employed for meiotic analysis was previously described by Eber et al. (16). Floral buds were fixed in a Carnoy solution containing 6 vol. of ethanol per 3 vol. of chloroform per 1 vol. of acetic acid at room temperature. After 24 h, buds were stored in a 50% alcohol solution at 4°C. Anthers containing pollen mother cells (PMCs) at the M-I stage of meiosis were squashed in a drop of aceto-carmin solution; 10 to 30 PMCs were observed per plant using a compound microscope at $\times 1600$.

DNA amplification. To confirm the presence of the *B. juncea* resistance gene, *Rlm6*, the specific marker I1-*Hae*III was analyzed in the BC3 plants by contrasting resistant and susceptible and the recurrent parent 'Dunkeld'. DNA was extracted from young leaves according to the method of Doyle and Doyle (15). The specific marker of the introgression carrying *Rlm6* was used as described by Chèvre et al. (11). This I1 random 10-mer primer used was

purchased from Operon Technologies (Alemada, CA). After amplification, the *Hae*III restriction enzyme (1 unit per 12.5 μ l) was added for 1 h at 37°C to the amplified products that were visualized after running in 1.8 agarose gel stained by ethidium bromide (0.5 μ g/ml).

RESULTS

Selection among KEM populations for blackleg resistance. Specific host resistance genes were detected on the basis of host response to inoculation with the three specific *L. maculans* isolates (Table 1). The susceptibility of plants to the MX4.3 isolate (67 of 68 plants) indicated that *Rlm1*, 5, 7, and the *R* gene carried by *B. napus*-*B. nigra* addition lines were absent, whereas the resistance detected against isolates S7 and 290 (51 or 59 resistant plants of 66 or 63 plants tested, respectively) demonstrated that *Rlm6* was

most likely responsible for the resistance observed in those particular assays. On the basis of these data, resistant plants were selected only among the KEM165 population after testing with isolates S7 and 290 (Table 1).

After selfing of the selected S0 plants, 10 of 10 S1 plants inoculated with S7 were resistant and 13 of 16 S1 plants inoculated with 290 were resistant, indicating that the resistant gene(s) were either at the homozygous stage, with 100% of S1 resistant plants with S7 isolate, or at the heterozygous stage, with 3R:1S ($\chi^2 = 0.03$, $P < 0.05$) segregation for 290 isolate, in S0 mother plants selected (Table 1). None of the F1 seedlings of crosses with 'Dunkeld' were susceptible to S7 or 290 indicating that S1 plants contained the resistance gene at the homozygous stage (Table 1). During the following generations, the screenings of BC1, BC2, and BC3 back-

Fig. 3. Examples of meiotic behavior ($2n = 39$, $2n = 38$) in the progeny of the *Brassica napus*-*B. juncea* KEM 165 line. **A**, $2n = 39$ and 19 bivalents + 1 univalent indicated by arrow. **B**, $2n = 38$ with 19 bivalents.

cross generations with the isolate 290 confirmed the presence of one major gene, as in each case the segregation fitted a 1:1 ratio whereas, from BC2 and BC3 generations the plants inoculated to S7 showed an excess of resistant compared with the expected 1:1 ratio for one major gene.

Characterization of the plants selected. Among the 22 plants analyzed, we

found five aneuploid plants with $2n = 37$, 39, or 40 chromosomes at different generations (S0, S1, and B2); an example of this is presented in Figure 3. Fifty-six to ninety percent of cells were found to have 19 bivalents (Fig. 4).

Molecular characterization of the *Rlm6* introgression. The specific marker 11-*Hae*III was analyzed only in the F1 and

BC1 from the KEM population that showed the highest level of resistance at the cotyledon stage for each *L. maculans* isolate. For both isolates, S7 and 290, resistant and intermediate plants showed the markers (Fig. 5) indicating that *Rlm6* was in segregation in the populations. Only one intermediate plant tested with the S7 isolate did not carry the markers and this result might be due to some additional resistance gene(s) that could also be present in the progeny selected with this isolate.

DISCUSSION

Cytogenetic analyses combined with blackleg cotyledon assays at each generation allowed us to obtain KEM recombinant lines showing regular meiotic behavior and a high level of blackleg resistance at the cotyledon stage. In both initial KEM populations, dominant gene resistance was detected. Our findings in these studies supported the findings of Chèvre et al. (11) who showed that it was possible to select recombinant oilseed rape lines containing the *B. juncea* blackleg resistance from 82N105 sent to France by N. N. Roy in 1985. Roy (35) produced homozygous resistant lines with a regular meiotic behavior similar to that of oilseed rape (i.e., 19 bivalents) after several backcrosses of 82N105 to oilseed rape of the F1 *B. napus* × *B. juncea* hybrids followed by selfing. He named these lines OnapJR (35). Others working with this material have reported that most of the lines were aneuploid (31), and Salisbury et al. (40) indicated that their attempts to transfer this resistance to different oilseed rape lines were unsuccessful, most likely because of the failure of introgression. Our study is the first to exploit the KEM material.

Pang and Halloran (27) suggested that three genes were implicated in the *B. juncea*-like resistance in the Roy material, but were unable to identify the genomic origins of these genes, perhaps because of the selection of a disomic substitution line of a *B. juncea* chromosome. In contrast, in the backcross generation of KEM 165 selected with isolate 290, only one gene appeared to be present in our study. This gene likely corresponds to *Rlm6* because *L. maculans* isolates 290 and S7 carried *AvrLm6* and because the resistant plants always carried the marker identified with the introgression of the *B. juncea* resistance (11). However, in backcross generations obtained from selection with isolate S7, one or more additional specific resistance genes seem to be present. 'Dunkeld', which was used as the recurrent parent, has known polygenic resistance (21) and may carry some other specific resistance gene(s). This hypothesis was supported by additional cotyledon tests we performed on the selfing progeny of the 'Dunkeld' plants. Four isolates allowing the detection of resistance genes *Rlm1*, 5, and 7 were

Fig. 4. Stability of the meiotic behavior in the *Brassica napus*–*B. juncea* line and progenies as assessed by the percentage of cells with 19 bivalents at each generation. The generations are represented as follows: original selfed = S0; first selfed = S1; first cross to *B. napus* 'Dunkeld' = F1; and first and second backcross generations = B1 and B2, respectively.

Fig. 5. Analyses of the amplification products by I1 digested by *Hae*III revealed molecular markers (indicated by arrows) linked to the *Brassica juncea* blackleg resistance gene in BC1 resistant plants obtained from KEM 165 progeny (K3 and K20) and in the resistant control MXS carrying *Rlm6*. The markers were absent in the controls, e.g., in all the plants of the recurrent parent 'Dunkeld' (01 Du), in winter oilseed rape cvs. Samourai and Darmor, and in the line carrying *B. napus*–*B. nigra* addition line resistance (74.11).

used, one on each lobe of cotyledon of 'Dunkeld' progeny; for each isolate, variability was detected between plants of the same selfing progeny indicating that additional resistance genes might be present and in segregation in 'Dunkeld' plants used as the recurrent parent (data not shown). Additionally, 'Dunkeld' is known to carry *Rlm4* (33). Since the Roy material (35) used has in its genealogy, a selection from 79N47, (viz., 79N47-1) and as another selection from 79N47 (viz., 79N47-58) is in the 'Dunkeld' genealogy (1), it is highly likely that the *Rlm5* previously described in *B. juncea* by Balesdent et al. (3) could also be present in some of the 'Dunkeld' plants. Production of doubled haploid plants from 'Dunkeld' might allow the identification of the different specific resistance genes present in this variety.

In our study, cytogenetic analysis combined with resistance assays allowed us to keep only the resistant plants with 38 chromosomes for the following generations. The presence of aneuploid plants even in the advanced BC2 generation and the percentage of cells with 19 bivalents in the $2n = 38$ recombination indicated that monitoring of the chromosome number and meiotic stability will need to be maintained through the advanced generations. The instability we observed may be related to a disturbed meiotic behavior that can give rise to unbalanced gametes.

We showed that the stable introgression of the *B. napus*-*B. juncea* recombinant resistance from the KEM lines into a 'Dunkeld' background is possible. However, the *Rlm6* gene has been shown to be potentially not durable when used solely on its own under experimental conditions in France (8) and isolates with the ability to overcome the *B. juncea* resistance have been reported in Australia (21). Combining this gene with polygenic resistance might not only improve the durability of the *Rlm6* gene, but also prevent collapse of the crop if this gene is overcome. The optimal use of the *Rlm6* gene for durable resistance in any commercial deployment requires checks to be made of the efficacy of the *Rlm6* gene and that assessments be made of the structure of the local pathogen populations to monitor the evolution of *AvrLm6* frequency in the pathogen populations (4,21). Otherwise, such resistance could be overcome rapidly by resistance-adapted isolates of *L. maculans*, such as those occurring in France against *Rlm1* resistance (32) and in Australia against the *B. rapa* subsp. *sylvestris* and other single dominant gene-based resistances (21).

ACKNOWLEDGMENTS

We acknowledge the team of the Experimental Unit (Le Rheu, France) for their assistance and G. Walton from the Department of Agriculture and Food Western Australia for seed of the KEM165 and KEM167 breeding lines.

LITERATURE CITED

- Anonymous. Canola (rapeseed), *Brassica napus*, 'Dunkeld' Registration Application No: 1994/050 by Daratech Pty Ltd., accepted 16 February 1994.
- Ansari-Melayah, D., Balesdent, M. H., Delourme, R., Pilet, M. L., Tanguy, X., Renard, M., and Rouxel, T. 1998. Genes for race-specific resistance against blackleg disease in *Brassica napus* L. Plant Breed. 117:373-378.
- Balesdent, M. H., Attard, A., Kuhn, M. L., and Rouxel, T. 2002. New avirulence genes in the phytopathogenic fungus *Leptosphaeria maculans*. Phytopathology 92:1122-1133.
- Balesdent, M. H., Barbetti, M. J., Hua Li, Sivasithamparam, K., Gout, L., and Rouxel, T. 2005. Analysis of *Leptosphaeria maculans* race structure in a worldwide collection of isolates. Phytopathology 95:1061-1071.
- Barbetti, M. J. 1975. Effects of temperature on development and progression in rape crown canker caused by *Leptosphaeria maculans*. Aust. J. Exp. Agric. Anim. Husb. 15:705-708.
- Barbetti, M. J., and Khangura, R. K. 1999. Management of blackleg in disease-prone environments of Western Australia. (CD-ROM proceedings). Page 100 in: Proc. GCIRC 10th Int. Rapeseed Cong. Canberra, Australia.
- Bokor, A., Barbetti, M. J., Brown, A. G. P., MacNish, G. C., Poole, M. L., and Wood, P. McR. 1975. Blackleg- A major hazard to the rapeseed industry. J. Agric. West. Aust. 16:7-10.
- Brun, H., Levivier, S., Somda, I., Ruer, D., Renard, M., and Chèvre, A. M., 2000. A field method for evaluating the potential durability of new resistance sources: Application to the *Leptosphaeria maculans*-*Brassica napus* pathosystem. Phytopathology 90:961-966.
- Brun, H., Ruer, D., Levivier, S., Somda, I., Chèvre, A. M., and Renard, M. 2001. Presence in *Leptosphaeria maculans* populations of isolates virulent on resistance introgressed into *Brassica napus* from the *B. nigra* B genome. Plant Pathol. 50:69-74.
- Cargeeg, L. A., and Thurling, N. 1979. Seedling and adult plant resistance to blackleg (*Leptosphaeria maculans* (Desm.) Ces. Et de Not.) in spring rape (*Brassica napus* L.). Aust. J. Agric. Res. 30:37-46.
- Chèvre, A.-M., Barret, P., Eber, F., Dupuy, P., Brun, H., Tanguy, X., and Renard, M. 1997. Selection of stable *Brassica napus*-*B. juncea* recombinant lines resistant to blackleg (*Leptosphaeria maculans*) 1. Identification of molecular markers, chromosomal and genomic origin of the introgression. Theor. Appl. Genet. 95:1104-1111.
- Delourme, R., Chèvre, A.-M., Brun, H., Rouxel, T., Balesdent, M.-H., Dias, J. S., Salisbury, P., Renard, M., and Rimmer, S., R. 2006. Major genes and polygenic resistance to *Leptosphaeria maculans* in oilseed rape (*Brassica napus*). Eur. J. Plant Pathol. 114: 41-52.
- De March, G., Seguin-Swartz, G., and Petri, G. A. 1986. Virulence and culture filtrate phytotoxicity in *Leptosphaeria maculans*: Perspectives for *in vitro* selection. Can. J. Plant Pathol. 8:422-428.
- Dixelius, C. 1999. Inheritance of the resistance to *Leptosphaeria maculans* of *Brassica nigra* and *B. juncea* in near isogenic lines of *B. napus*. Plant Breed. 118:151-156.
- Doyle, J. J., and Doyle, J. L. 1990. Isolation of plant DNA from fresh tissue. Focus 12:13-15.
- Eber, F., Chèvre, A. M., Baranger, A., Vallée, P., Tanguy, X., and Renard, M. 1994. Spontaneous hybridization between a male-sterile oilseed rape and two weeds. Theor. Appl. Genet. 88:362-368.
- Ferreira, M. E., Rimmer, S. R., Williams, P. H., and Osborn, T. C. 1995. Mapping loci controlling *Brassica napus* resistance to *Leptosphaeria maculans* under different screening conditions. Phytopathology 85:213-217.
- Fitt, B. D. L., Brun, H., Barbetti, M. J., and Rimmer, S. R. 2006. World-wide importance of phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*) on oilseed rape (*Brassica napus*). Eur. J. Plant Pathol. 114:3-15.
- Gororo, N., Salisbury, P., and Marcoft, S. 2004. Dunkeld and Rainbow: A tale of two Victorian canola cultivars. In: Proc. 4th Int. Crop Sci. Cong. Brisbane, Queensland, Australia.
- Hua Li, Barbetti, M. J., and Sivasithamparam, K. 2003. Responses of *Brassica napus* cultivars to *Leptosphaeria maculans* field isolates from Western Australia. Brassica 5:25-34.
- Hua Li, Barbetti, M. J., and Sivasithamparam, K. 2005. Hazard from reliance on cruciferous hosts as sources of major gene based resistance for managing blackleg (*Leptosphaeria maculans*) disease. Field Crops Res. 91:185-198.
- Hua Li, Sivasithamparam, K., and Barbetti, M. J. 2006. Evidence supporting the polycyclic nature of blackleg disease (*Leptosphaeria maculans*) of oilseed rape in Australia and implications for disease management. Brassica 8:65-69.
- Hua Li, Sivasithamparam, K., and Barbetti, M. J. 2007. Blackleg disease (*Leptosphaeria maculans*) on oilseed rape - evidence for it being a polycyclic disease in Australia and implications for disease management. Vol. I. Pages 416-419 in: Proc. 10th Int. Rapeseed Cong. Wuhan, China. Science Press USA Inc., Monmouth Junction, NJ.
- Hua Li, Damour, L., Sivasithamparam, K., and Barbetti, M. J. 2004. Increased virulence and physiological specialisation among Western Australian isolates of *Leptosphaeria maculans* breaking down existing single dominant gene-based resistance in six cultivars of *Brassica napus*. Brassica 6:9-16.
- Hua Li, Smyth, F., Barbetti, M. J., and Sivasithamparam, K. 2006. Relationship in *Brassica napus* seedling and adult plant responses to *Leptosphaeria maculans* is determined by plant growth stage at infestation and temperature regime. Field Crops Res. 96:428-437.
- Keri, M., van den Berg, C. G. J., McVetty, P. B. E., and Rimmer, S. R. 1997. Inheritance of resistance to *Leptosphaeria maculans* in *Brassica juncea*. Phytopathology 87:594-598.
- Pang, E. C. K., and Halloran, G. M. 1996. The genetics of adult-plant blackleg (*Leptosphaeria maculans*) resistance from *Brassica juncea* in *B. napus*. Theor. Appl. Genet. 92:382-387.
- Pilet, M. L., Delourme, R., Foisset, N., and Renard, M. 1998. Identification of loci contributing to quantitative field resistance to blackleg disease, causal agent *Leptosphaeria maculans* (Desm.) Ces. et de Not., in winter rapeseed (*Brassica napus* L.). Theor. Appl. Genet. 96:23-30.
- Plieske, J., Struss, D., and Röbbelen, G. 1998. Inheritance of resistance derived from the B-genome of *Brassica* against *Phoma lingam* in rapeseed and the development of molecular markers. Theor. Appl. Genet. 97:929-936.
- Rimmer, S. R. 2006. Resistance genes to *Leptosphaeria maculans* in *Brassica napus*. Can. J. Plant Pathol. 28:288-297.
- Rimmer, S. R., and van den Berg, C. G. J. 1992. Resistance of oilseed *Brassica* spp. to blackleg caused by *Leptosphaeria maculans*. Can. J. Plant Pathol. 14:56-66.
- Rouxel, T., Penaud, A., Pinochet, X., Brun, H., Gout, L., Delourme, R., Schmit, J., and Balesdent, M. H. 2003. A 10-year survey of populations of *Leptosphaeria maculans* in France indicates a rapid adaptation towards the *Rlm1* resistance gene of oilseed rape. Eur. J. Plant Pathol. 109:871-881.
- Rouxel, T., Willner, E., Coudard, L., and

- Balesdent, M. H. 2003. Screening and identification of resistance to *Leptosphaeria maculans* (stem canker) in *Brassica napus* accessions. *Euphytica* 133:219-231.
34. Roy, N. N. 1978. A study on disease variation in the population of an interspecific cross of *Brassica juncea* L. $\times$ *B. napus* L. *Euphytica* 27:145-149.
 35. Roy, N. N. 1984. Interspecific transfer of *Brassica juncea*-type high blackleg resistance to *Brassica napus*. *Euphytica* 33:295-303.
 36. Saal, B., Brun, H., Glais, I., and Struss, A. 2004. Identification of a *Brassica juncea*-derived recessive gene conferring resistance to *Leptosphaeria maculans* in oilseed rape. *Plant Breed.* 123:505-511.
 37. Saal, B., and Struss, D. 2005. RGA- and RADP-derived SCAR for a *Brassica* B-genome introgression conferring resistance to blackleg in oilseed rape. *Theor. Appl. Genet.* 101:281-290.
 38. Sacristán, M. D., and Gerdemann, M. 1985. Resistenzerstellung gegen *Phoma lingam* mit Hilfe von *in vitro*-systemen; Vererbung und Übertragung von Resistenzmerkmalen in *Brassica napus*. *Vortr. Pflanzenzüchtg.* 9:83-90.
 39. Sacristán, M. D., and Gerdemann, M. 1986. Different behavior of *Brassica juncea* and *B. carinata* as sources of *Phoma lingam* resistance in experiments of interspecific transfer to *B. napus*. *Plant Breed.* 97:304-314.
 40. Salisbury, P. A., Ballinger, D. J., Wratten, N., Plummer, K. M., and Howlett, B. J. 1995. Blackleg disease on oilseed Brassica in Australia: A review. *Aust. J. Exp. Agric.* 35:665-672.
 41. Sivasithamparan, K., Barbetti, M. J., and Hua Li. 2005. Recurring challenges from a necrotrophic fungal plant pathogen: A case study with the phytopathogenic fungus *Leptosphaeria maculans* (causal agent of blackleg disease in Brassicas) in Western Australia. *Ann. Bot.* 96:363-377.
 42. Somda, I., Delourme, R., Renard, M., and Brun, H. 1999. Pathogenicity of *Leptosphaeria maculans* isolates on a *Brassica napus*-*B. juncea* recombinant line. *Phytopathology* 89:169-175.
 43. Somda, I., Renard, M., and Brun, H. 1998. Seedling and adult plant reactions of *Brassica napus*-*B. juncea* recombinant lines towards A- and B-group isolates of *Leptosphaeria maculans*. *Ann. Appl. Biol.* 132:187-196.
 44. West, J. S., Kharbanda, P. D., Barbetti, M. J., and Fitt, B. D. L. 2001. Epidemiology and management of *Leptosphaeria maculans* (phoma stem canker) on oilseed rape in Australia, Canada and Europe. *Plant Pathol.* 50:10-27.
 45. Williams, P. H., and Delwiche, P. A. 1979. Screening for resistance to blackleg of crucifers in the seedling stage. Pages 164-170 in: *Proc. Eucarpia Conf. on the Breeding of Cruciferous Crops*. Wageningen, The Netherlands.