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The genetics of adult-plant blackleg (*Leptosphaeria maculans*) resistance from *Brassica juncea* in *B. napus*

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Abstract The genetic control of adult-plant blackleg (*Leptosphaeria maculans*) resistance in a *Brassica napus* line (579NO48-109-DG-1589), designated "R13" possessing *Brassica juncea*-like resistance (JR), was elucidated by the analysis of segregation ratios in F_2 and F_3 populations from a cross between "R13" and the highly blackleg-susceptible *B. napus* cultivar "Tower". The F_2 segregration ratios were bimodal, demonstrating that blackleg resistance in "R13" was controlled by major genes. Analysis of the segregation ratios for 13 F_3 families indicated that blackleg resistance in these families was controlled by three nuclear genes, which exhibited a complex interaction. Randomly sampled plants of F_3 progeny all had the normal diploid somatic chromosome number for *B. napus*. The similarities between the action of the three genes found in this study with those controlling blackleg resistance in *B. juncea* is discussed.

Key words Genetics · Blacking resistance · *Brassica napus* · *Brassica juncea* · *Leptosphaeria maculans*

Introduction

All current rapeseed cultivars in Australia possess only adult-plant resistance to blackleg. As seedlings, plants of these cultivars develop cotyledon and leaf infections, but mostly remain canker-free at harvest. The lack of adequate seedling resistance in *B. napus* to blackleg has prompted efforts to transfer this resistance from related *Brassica* species (such as *B. juncea*) which are resistant at both the seedling and adult-plant stages. Roy (1978) produced hybrids between *B. juncea* ("BJ168", genomes AABB) and *B. napus* (cv "Cresus-o-Precose"; blackleg-susceptible; genomes AACC) by conventional crossing methods, and found that about 40% of the resulting F_2 plants showed *B. juncea*-type (JR) seedling resistance (no cotyledon infection). All F_1 plants had previously displayed cotyledon lesions when infected with an ascospore suspension but none was cankered at flowering, which implied that the expression of this character may be controlled by a recessive gene(s).

In addition to recovering F_2 plants with JR resistance, Roy (1978) also reported the incorporation of adult-plant resistance (from *B. juncea*) in the susceptible *B. napus* background. Since F_2 plants possessing this resistance were highly fertile and had *B. napus*-type morphology, Roy (1978) surmised that genes conferring adult-plant resistance to *L. maculans* were located in the A genome of *B. juncea*, which were thus likely to be readily transferred to the A genome of *B. napus* through homologous chromosome pairing.

Between 1978 and 1981, a large number of lines (F_4–F_7) were developed by Roy (1984) from a single F_3 plant, designated "Onap^{JR}", selected from the above F_2 populations on the basis of low erucic-acid content, JR resistance, and *B. napus*-type morphology. He was unable, however, to establish lines homozygous for JR resistance, as even the advanced generation lines (F_7) were found to give variable proportions of JR-type plants. Roy (1984) attributed this failure to his selection procedure, which may have favoured plants heterozygous for JR resistance. The segregation patterns in the F_5–F_7 for JR resistance indicated its simple inheritance but no attempts were made to determine the number of segregration genes. Cytological studies on "Onap^{JR}" revealed the presence of the normal chromosome number for *B. napus* ($2n = 38$) and regular pairing (19 bivalents) at diakinesis (Roy 1984). These studies, coupled with observations of normal seed fertility in "Onap^{JR}", indicated that JR resistance had been stably incorporated in *B. napus*. Recently, however, Rimmer and van den Berg (1992) reported than most (but not all)

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of such lines were aneuploids, with chromosome numbers typically in the range of 39–40.

In the present study, the inheritance of adult-plant blackleg resistance in populations obtained from crosses between resistant lines derived from "Onap^{JR}" ("ROY" accessions) and the susceptible cultivar "Tower" was investigated.

Materials and mathods

Fungal cultures

A single-ascospore isolate of *L. maculans*, designated MB2, was used in all inoculations. This highly virulent isolate, from Mt. Barker (Western Australia), had been used in studies detailed in a previous paper (Pang and Halloran 1995).

Production of experimental populations and cultural conditions

The accession used in this study (579N048-109-DG-1589), designated "R13", was provided by Dr. N. N. Roy via Dr. P. A. Salisbury, of the Victorian Institute for Dryland Agriculture (VIDA), Horsham, Victoria. The accession was derived from backcrosses between Onap^{JR} and Wesroona (recurrent parent, which posseses adult-plant resistance to blackleg). This accession was found to be characterized by high levels of blackleg resistance when tested at the VIDA blackleg nursery at Lake Bolac, Victoria (Salisbury, personal communication). Plants within this accession which displayed no, or only minor, leaf infection at the seedling stage (JR resistance), and which were subsequently canker-free at flowering, were selfed. Seeds of these single-plant selections (made at Lake Bolac, Victoria) were used in the present experiment.

A set of reciprocal crosses was made between blackleg-resistant plants of the accession "R13" and plants of the Canadian blackleg-susceptible cultivar "Tower". A total of ten F_1 populations were produced. A single F_1 population (with "R13" as the female parent) was subsequently selected for the inheritance study of blackleg resistance, based on the wide separation between the resistance/susceptibility scores of its parents.

Thirty to forty seeds from the selected F_1 population were pre-germinated, as described above, and were subsequently transferred to 18 × 17-cm pots containing a peat-sand-soil mix. Seedling were thinned to three per pot 1 week after transfer. The glasshouse was maintained at 15 °C (night) and 25 °C (day) with a 13–11-photoperiod (natural daylength). Thirty plants of the F_1 were selfed to produce the F_2, and were also each backcrossed to both the resistant and susceptible parents to produce the BCP_1 (to "R13") and BCP_2 (to "Tower") populations.

Plants from the parental, F_1, F_2, and first-backcross populations were grown in a glasshouse under a regime of 15 °C(night) and 25 °C(day), and a 11–13-h photoperiod. Seeds from these populations were sown in a peat-sand-soil mix in 17-cm-diameter pots. Seedlings were thinned to four per pot 10 days after germination. The pots were randomly allocated among the benches in the glasshouse 1 week prior to inoculation.

After disease assessment, 13 F_2 plants, with varying levels of blackleg resistance (external symptoms only), were retained and selfed to produce F_3 families. Benlate (2g/l) was applied after assessment to stop further disease development. At ripening, each F_2 plant was scored for internal lesion development (percentage of internal infection, %I I). Seeds from each F_3 family were pre-germinated as described above and were subsequently transferred to 17-cm-diameter pots containing a peat-sand-soil mix. The trial was conducted under natural daylength; glasshouse temperatures were as described above. Seedlings were thinned to four per pot 10 days after germination. Each F_3 family was to be represented by a total of 40 plants but, due to accidental deaths, a few of them had fewer than 40 plants at inoculation time. Pots were randomly allocated among benches in the glasshouse 1 week prior to inoculation.

Infection procedure and disease-severity assessment

The preparation of inoculum, application of pycnidiospores and would-inoculation procedures were as described in a previous paper (Pang and Halloran 1995). Plants from the seven populations (including the F_3) were inoculated at growth stage 2.4–2.5 on the Harper and Berkenkamp (1975) scale. To encourage infection, transparent polythene hoods were placed over benches containing inoculated plants for 3 days to maintain high humidity.

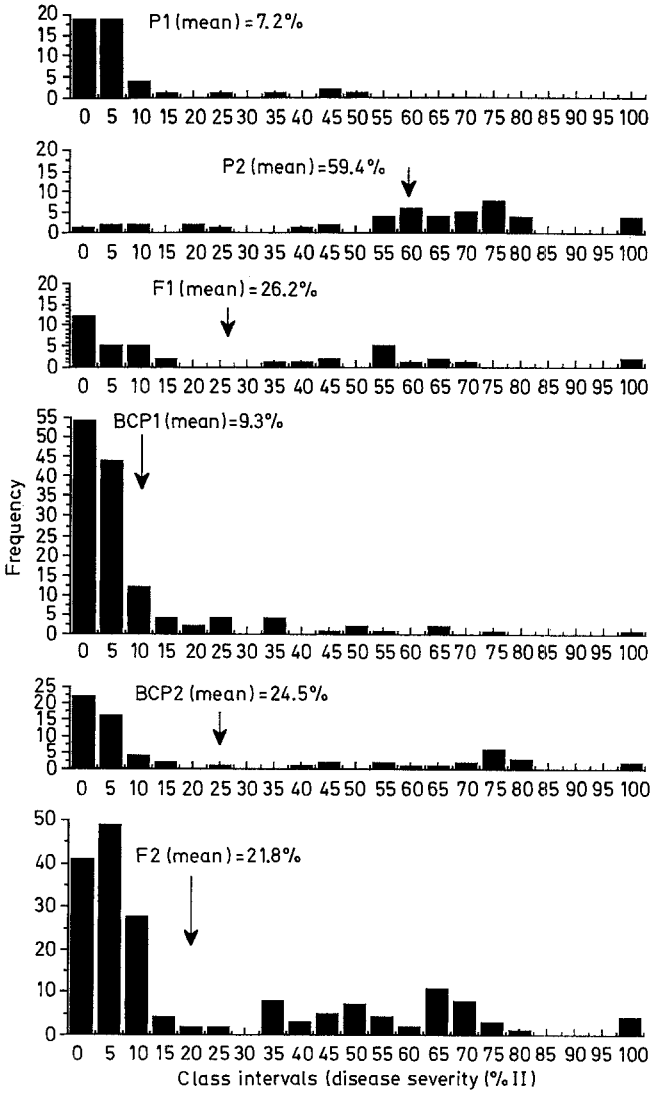
Assessment of stem-canker development was conducted 5 weeks after inoculation. The percentage of infection (%I I) was used to represent disease severity. The following formula was used to calculate %I I:

Percentage of internal infection

$$(\%\text{I I}) = \frac{\text{internal lesion area}}{\text{transverse area of the crown}} \times 100$$

No transformation was performed on the data, as the coefficients of variation and the standard errors (of the means) for the seven

Fig. 1 Frequency distributions of blackleg disease severity (%I I) for parental, F_1, F_2 and first backcross populations of "R13" (P_1) × "Tower" (P_2)



population indicated that the variances were uncorrelated with their respective means.

Chromosome counts

Chromosome counts were performed to ascertain the ploidy levels of the F_3 progeny. Root tips from randomly selected F_3 seedlings were and pre-treated at ambient temperature for 4–5 h in a solution of 0.29 g/l of 8-hydroxyquinoline (BDH England), fixed using a 3:1 solution of ethanol : glacial acetic acid for 30 h at 4 °C, hydrolysed at 60 °C for 13 min in N HCl, and finally stained with Feulgen reagent (Sharma and Sharma 1980) for 30 min at ambient temperature. The root tips were then squashed on slides in 40% glacial acetic acid. Chromosome counts were performed using a Leitz-Dialux (20 EB) bright-field microscope. Counts were made from at least ten cells per root tip.

Results

Inheritance of blackleg resistance: F_2 and first backcross populations

The P_1 and P_2 populations were clearly separated for levels of disease serving using %I I (Fig. 1) with most plants in P_1 population exhibiting %I I scores of less than 20%, while those of the P_2 were predominantly suspectible, with %I I scores of over 40%. The F_1 distribution was clearly bimodal, indicating that the resistant "R13" parent was heterozygous for blackleg resistance. The BCP_1 and BCP_2 distributions were heavily positively skewed, and the majority of plants in both populations possessed %I I scores of less than 20%. The form of these distributions indicated strong dominance at loci controlling blackleg resistance. The F_2 distribution was clearly bimodal, the modes of the two phenotypic classes coinciding with those observed in the P_1 and P_2 populations (Fig. 1). Due to the possible heterozygosity of the "R13" parent, no segregation ratios were fitted to the observed ratios in the F_2.

Inheritance of blackleg resistance: F_3 families

In the determination of segregation ratios, F_3 plants in the 0, 5, 10, and 15% classes (%I I) were considered resistant, while those in classes at or above 20% were considered susceptible. This demarcation was based on the observed discontinuities in distributions of the P_1, F_1, BCP_1, BCP_2 and F_2 populations (Fig. 1).

One-gene control of blackleg resistance

The segregation ratios in F_3 families derived from resistant F_2 plants indicated that blackleg resistance was likely to be determined by a single, dominant major gene (Table 1). Chi-square tests of a one-gene hypothesis (3 resistant:1 susceptible) for segregation in F_3 families 4,5,7,11, and 13 indicated a good fit of observed to expected ratios ($P > 0.025$ for most families; Table 1). However, while there appeared to be a single-gene inheritance of blackleg resistance in the above families, the one-gene hypothesis did not satisfactorily explain the large number of resistant outliers in F_3 families derived from susceptible F_2 parents, namely 1, 2, 3, 6, and 12.

Table 1 Chi-square test of a one-gene hypothesis for segregation for blackleg resistance (using % I I) in the F_3 of "R13" (P_1) × "Tower" (P_2)

F_2 parent phenotype	Suspected F_2 genotype	F_3 line no.	F_3 genotype	Observed F_3 ratios	Expected F_3 ratios	χ^2	P
Susceptible (69.0%)[a]	aa	1	AA, Aa	12	0	N/A	N/A
			aa	26	38		
Susceptible (56.0%)[a]	aa	2	AA, Aa	18	0	N/A	N/A
			aa	19	37		
Susceptible (31.0%)[a]	aa	3	AA, Aa	9	0	N/A	N/A
			aa	31	40		
Resistant (6.0%)[a]	Aa	4	AA, Aa	32	30.75	0.20	0.50–0.75
			aa	9	10.25		
Resistant (3.0%)[a]	Aa	5	AA, Aa	26	29.25	1.44	0.10–0.25
			aa	13	9.75		
Susceptible (40.0%)[a]	aa	6	AA, Aa	14	0	N/A	N/A
			aa	26	40		
Resistant (2.0%)[a]	Aa	7	AA, Aa	27	30	1.20	0.25–0.50
			aa	13	10		
Resistant (4.0%)[a]	AA	8	AA, Aa	34	38	N/A	N/A
			aa	4	0		
Resistant (3.0%)[a]	AA	9	AA, Aa	35	40	N/A	N/A
			aa	5	0		
Resistant (8.0%)[a]	Aa	10	AA, Aa	32	30	0.53	0.25–0.50
			aa	8	10		
Resistant (2.0%)[a]	Aa	11	AA, Aa	33	30	1.20	0.25–0.50
			aa	7	10		
Susceptible (56.0%)[a]	aa	12	AA, Aa	16	0	N/A	N/A
			aa	24	40		
Resistant (1.0%)[a]	Aa	13	AA, Aa	28	29.25	0.21	0.50–0.75
			aa	11	9.75		

[a] Internal lesion scores (%I I) at harvest time. Figures were rounded to nearest integers. N/A = not applicable

Moreover, families 8 and 9, which were presumed to be derived from F_2 parents homozygous for the resistant allele, possessed a number of susceptible outliers (Table 1).

Two gene control of blackleg resistance in F_3 families derived from susceptible F_2 parents

Segregation ratios for resistance/susceptibility of families 1, 2, 3, 6 and 12 fitted either a 1R:3S or a 7R:9S ratio. These ratios would be obtained when blackleg resistance was determined by two independently-segregating, di-allelic genes acting in a complementary manner; resistance is then expressed only when one or both loci are homozygous for the recessive alleles. Chi-square tests of this hypothesis (Table 2) indicated a good fit of observed ratios to either a 1R:3S or a 7R:9S ratio for all families.

Overall: three-gene control of adult-plant blackleg resistance

When combined the results of chi-square tests of one- and two gene control (Tables 1 and 2), indicated a trigenic control of blackleg resistance. The proposed mode of control of resistance is as follows:
(1) The first gene locus determining resistance (henceforth known as Bl_1) in the presence of the dominant allele (i) confers blackleg resistance and (ii) masks the expression of the other two genes (epistasis).
(2) The other two genes (Bl_2 and Bl_3) act in a complementary manner as stated previously but due to epistatic gene effects of Bl_1, they are expressed only in plants homozygous for the recessive allele at the Bl_1 locus. This model of control is represented diagrammatically in Fig. 2.

Based on this hypothesis, the observed segregation ratios of each F_3 family were tested for goodness-of-fit to various expected ratios. Results of F_3 families derived from the same suspected F_2 genotype were pooled. Chi-square test of the three-gene hypothesis performed on these pooled ratios, indicated a good fit to expected ratios in each case (Table 3). A chi-square test performed on the segregration ratios in the P_1 distribution (Fig. 1) indicated that the original "R13" parent used was likely to be the genotype $Bl_1bl_1Bl_2bl_2Bl_3bl_3$ (55R:9S; $\chi^2 = 0.40$; $P = 0.50$–0.75).

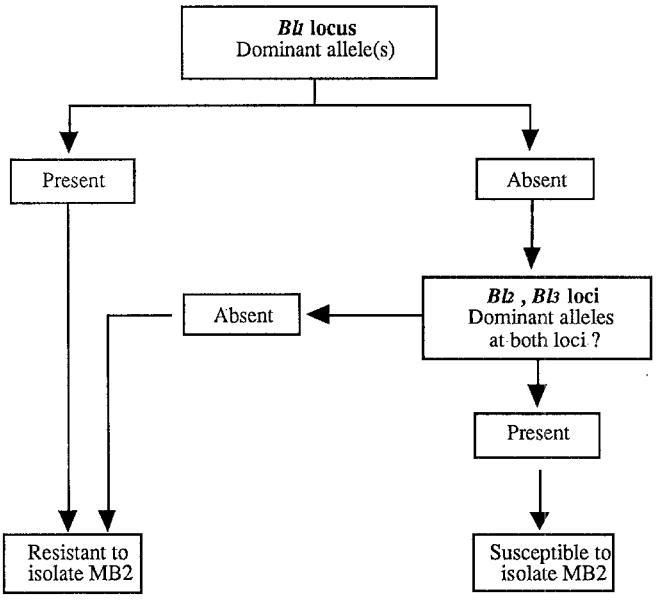


Fig. 2 Flow chart depicting the proposed genetic control of blackleg resistance in F_3 progeny of "R13" (P_1) × "Tower" (P_2)

Chromosome counts on F_3 plants

All F_3 plants examined possessed 38 chromosomes, the normal somatic number for *B. napus*. As no observa-

Table 2 Chi-square test of a two-gene hypothesis for segregation for blackleg resistance (using %I I) in the F_3 families derived from susceptible F_2 parents of "R13" (P_1) × "Tower" (P_2)

F_2 parent phenotype	F_3 line no.	F_3 genotype	F_3 phenotype	Observed F_3 ratios	Suspected F_2 Genotype					
					XxYY,XXYy			XxYy		
					Expected F_3 ratios (1:3)	χ^2	P	Expected F_3 ratios (7:9)	χ^2	P
Susceptible (69.0%)[a]	1	Xy, xY	Resistant	12	9.5	0.88	0.25–0.50	16.63	2.29	0.10–0.25
		XY	Susceptible	26	28.5			21.37		
Susceptible (56.0%)[a]	2	Xy, xY	Resistant	18	9.25	11.08	< 0.005	16.19	0.36	0.50–0.75
		XY	Susceptible	19	27.75			20.81		
Susceptible (31.0%)[a]	3	Xy, xY	Resistant	9	10	0.13	0.50–0.75	17.5	5.71	0.01–0.03
		XY	Susceptible	31	30			22.5		
Susceptible (40.0%)[a]	6	Xy, xY	Resistant	14	10	2.13	0.10–0.25	17.5	1.24	0.10–0.25
		XY	Susceptible	26	30			22.5		
Susceptible (56.0%)[a]	12	Xy, xY	Resistant	16	10	4.8	0.03–0.05	17.5	0.23	0.50–0.75
		XY	Susceptible	24	30			22.5		

[a] Internal lesion scores (%I I) at harvest time. Figures were rounded to nearest integers

Table 3 Chi-square test of a three-gene hypothesis for segregation for blackleg resistance (using %I I) in the F_3 of "R13" (P_1) × "Tower" (P_2). R = resistant; S = susceptible

F_2 parent phenotype	Suspected F_2 genotype	F_3 families	Observed F_3 ratios (pooled)	Expected F_3 ratios (pooled)	χ^2	P
Resistant	$Bl_1bl_1Bl_2bl_2Bl_3bl_3$ (55R:9S)[a]	8, 9	69R 9S	67R 11S	0.42	0.50–0.75
Resistant	$Bl_1bl_1Bl_2bl_2Bl_3bl_3 + Bl_1bl_1Bl_2Bl_2Bl_3bl_3$ (13R:3S)[a]	4, 10, 11	97R 24S	98.3R 22.7S	0.09	0.75–0.90
Resistant	$Bl_1bl_1Bl_2Bl_2Bl_3Bl_3$ (3R:1S)[a]	5, 7, 13	81R 37S	88.5R 29.5S	2.54	0.10–0.25
Susceptible	$bl_1bl_1Bl_2bl_2Bl_3bl_3$ (7R:9S)[a]	2, 6, 12	48R 69S	51.19R 65.81S	0.35	0.50–0.75
Susceptible	$bl_1bl_1Bl_2Bl_2Bl_3bl_3 + bl_1bl_1Bl_2bl_2Bl_3Bl_3$ (1R:3S)[a]	1, 3	21R 57S	19.50R 58.50S	0.15	0.50–0.75

[a] Expected segregation ratios in the F_3

tions of meiotic chromosome pairing were made, it is unknown whether regular bivalent formation occurred at meiosis in these plants.

Discussion

The discrete segregation for JR resistance in F_2–F_3 populations of $Onap^{JR}$ crosses (to *B. napus)* indicated its simple inheritance (Roy 1984). In the present study, the action of the genes of designated Bl_1 and Bl_2 (or alternatively, Bl_3) and their interaction (Fig. 2) is identical to that described by Keri (1991) for two genes controlling blackleg resistance in *B. juncea*. It is likely, therefore, that Bl_1 and Bl_2 (alternatively, Bl_3) which control blackleg resistance in the F_3 from "R13" × "Tower" crosses, are the same as those found in *B. juncea, sensu* Keri (1991). Together with the observations of normal chromosome numbers (2n = 38), this finding implies that stable introgression of blackleg resistance genes from *B. juncea* into *B. napus* had been achieved, supporting Roy's (1984) contention.

The present study elucidated a three-gene control for blackleg resistance in the F_3 derived from a cross between a susceptible *B. napus* cultivar ("Tower"), and a line from Roy's (1978, 1984) material ("R13") possessing JR resistance. Keri 1991, however, found only two genes controlling such resistance in resistant × susceptible *B. juncea* crosses. This discrepancy may be due to genetic differences in the isolates of *L. maculans* used, and/or the accession of *B. juncea* from which these genes were derived.

It is unclear whether all three genes detected in the present study were derived from the A or B genome of *B. juncea*. It is also possible that one or more of these genes came from the recurrent *B. napus* parent, "Wesroona". Roy (1978) reported that adult-plant blackleg resistance had been transferred from *B. juncea* to *B. napus*, possibly via recombination between the common A genome chromosomes of these species. JR resistance, however, was thought to be controlled by a gene(s) in the B genome (Roy 1984). Given the low level of homoeologous recombination between the chromosomes of the B and AC (*B. napus*) genomes (Attia et al. 1987a,b), the probability of obtaining recombination for three independently segregating blackleg resistance genes from *B. juncea* into *B. napus* in these hybrids appears to be extremely small. Therefore, a few (perhaps all) of the resistance genes detected in the present study may have come from the A genome of *B. juncea*, or from "Wesroona".

The possibility should be considered of the possible transfer of blackleg resistance gene(s) from *B. juneca* to *B. napus* by spontaneous disomic substitution of a chromosome(s) of *B. juncea* carrying such a gene in the *B. napus* complement. However, if this event occured, such a disomic substitution would confer homozygosity for that gene(s), which would therefore not exhibit any segregation. The statistically significant fits of observed F_3 segregation ratios with that expected for the proposed three-gene system thus precludes this possibility.

Roy (unpublished) noted that JR (seedling) resistance was expressed as a dominant trait in F_1 hybrids between *B. juncea* and *B. napus*. Adult-plant resistance of *B. juncea-B. napus* hybrids to *L. maculans* is also expressed as a dominant trait (Sacristan and Gerdemann 1986). However, if the blackleg resistance genes transferred from *B. juncea* are unique to the B genome (no equivalent loci in the A or C genomes), then the use of the term "dominance" may be inappropriate (Sacristan and Gerdemann 1986). As the action of the JR resistance gene(s) overrides the expression of genes for resistance/susceptibility in *B. napus* to *L. maculans*, terms such as "pseudo-dominance" (Sacristan and Gerdemann 1986) or "epistasis" may be more appropriate. Of the three resistance genes detected in the present study, only Bl_1 displayed pseudo-dominance or epistasis. However, whether Bl_1 came from the B genome of *B. juncea* and whether it accounts for JR resistance, *sensu* Roy (1984), cannot be determined conclusively from the results of the present study.

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