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Major gene resistance to *Phakopsora pachyrhizi* in *Glycine canescens*, a wild relative of soybean

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Summary. An analysis of the genetic basis of resistance to the soybean rust pathogen *Phakopsora pachyrhizi* in a set of differential lines of the wild plant *Glycine canescens* showed that resistance was dominant and controlled by genes with major phenotypic effects. Single resistance genes were detected in six of seven host lines. In the seventh line, two independently inherited genes for resistance were present. Intercrossing of most of these lines showed that some of these genes were inherited independently. In other cases, no F_2 segregation was observed, implying that the resistance factors involved in the cross were closely linked or allelic. In yet other cases, distorted F_2 segregation ratios were detected. It is suggested that these were generally caused by the wide nature of the crosses between morphologically and biochemically dissimilar accessions. At least four distinct resistance loci were detected in these lines of *G. canescens*. They have been given the tentative gene symbols Lr_1, Lr_2, Lr_3 and Lr_4.

Key words: Race specific resistance – Allelism – Linkage – Segregation – Wide crosses

Introduction

Phakopsora pachyrhizi Syd., the causal agent of soybean rust, is a widespread and damaging pathogen found throughout the tropical and sub-tropical regions of the world (Bromfield 1984). To date, attempts to control this pathogen through the use of cultivars carrying resistance genes with major phenotypic effects have proved unsuccessful. Extensive surveys of soybean germplasm collections (Hymowitz et al. 1976; Yang 1977; Bromfield 1980; A. Tschanz, personal communication) have uncovered only a few lines that are highly resistant. Unfortunately, however, even these lines have generally proved susceptible to at least some Asian races of *P. pachyrhizi* (A. Tschanz, personal communication). Overall, there appears to be a general paucity of race-specific resistance to *P. pachyrhizi* in *Glycine max* and its wild progenitor, *G. soja*.

An alternative source of resistance to *Phakopsora pachyrhizi* is that offered by the perennial wild relatives of *Glycine max* (Burdon and Marshall 1981 a, b; Burdon 1986, 1987). A number of accessions of several of these wild species have already been used to construct a series of sets of differential hosts for use in the documentation of racial variation in *P. pachyrhizi* (Burdon and Speer 1984). Preliminary results concerning two of the host lines in the *Glycine canescens* F.J. Herm. differential set indicated that this resistance is race-specific in nature and is inherited in a normal Mendelian manner. The present paper provides an analysis of the genetic basis of resistance to *P. pachyrhizi* in the six *Glycine canescens* lines used in that differential set (plus one other line), and by means of a series of intercrosses, investigates the relationships of these different resistant factors to one another.

Materials and methods

Germplasm

The six lines of *Glycine canescens* used in the differential set (designated 1112.1, 1113.1, 1123.1, 1232.2, 1302.1, 1340.1 and an additional line 1341.1) were originally developed by selecting individual plants from material in the CSIRO Division of Plant Industry *Glycine* sp. germplasm collection (Table 1). Uniformity of infection type reaction to selected races of *Phakopsora pachyrhizi* was tested over many generations.

Disease resistance screening

The isolates of *Phakopsora pachyrhizi* used in this study were collected from various species of wild *Glycine* and cultivated

Table 1. The origin and infection type response of the seven lines of *Glycine canescens* to six races of *Phakopsora pachyrhizi*

Accession no.[a]	Geographic origin	Resistant to pathogen races
1112.1	Condoblin, N.S.W.	R1, R2, R3[b], R4[b]
1113.1	Condoblin, N.S.W.	R1, R2, R5
1123.1	nr Young, N.S.W.	R1, R3
1232.1	Cobham Lake, N.S.W.	None
1232.2	Cobham Lake, N.S.W.	R1–R6
1302.1	Bobadah/Nymagee, N.S.W.	R1, R2, R4
1340.1	Murray Downs Station, N.T.	R1–R5
1341.1	Alice Springs, N.T.	R1–R6

[a] CSIRO Division of Plant Industry *Glycine* sp. germplasm accession number
[b] Intermediate reaction

soybean (*G. max*) growing along the eastern Australian coast (Burdon and Speer 1984). Before use, each isolate was purified (derived from a single uredial pustule) and maintained separately on *G. max*. Resistance screening was carried out on individual *G. canescens* seedlings at the 2–4 trifoliate leaf stage by dusting fresh, dry urediospores of a single race of *P. pachyrhizi* onto the leaves. The seedling plants were then sprayed with water and kept at 100% relative humidity overnight. Subsequently, the inoculated plants were held under a 18°/24°C ± 3°C night/day regime in a naturally lit glasshouse. Approximately 14 days later, disease symptoms were fully developed and the infection type responses of individual seedlings to particular pathogen races were assessed.

Infection type responses were graded as follows (Burdon 1987):

Infection type	Symptoms
;	Necrotic flecks only, no sporulation;
1	Minute uredia surrounded by large necrotic or chlorotic zones;
2	Small uredia with some associated necrotic or chlorotic tissue;
3	Large profusely sporulating uredia, little or no necrosis or chlorosis.

Where disease symptoms overlapped two infection types a combination of symbols was used.

Crosses

Like *G. max*, *G. canescens* produces both cleistogamous and chasmogamous self-fertile flowers. In making crosses, the larger chasmogamous flowers borne in racemes were used. Young flowers were emasculated prior to the dehiscence of anthers and pollination was immediately affected. The female flowers were then covered overnight to protect the exposed stigma and ovary against drying.

Initially all lines (1112.1, 1113.1, 1123.1, 1232.2, 1302.1, 1340.1 and 1341.1) were crossed with 1232.1, a universally susceptible line. In all cases, 1232.1 was the maternal parent. Subsequent to the analysis of segregating F_2 families derived from these crosses, most of the original lines were intercrossed with each other to produce an almost complete half-diallel. In the case of 1112.1 × 1232.2, reciprocal crosses were made.

The hybrid nature of putative crosses was confirmed through comparisons of morphological characters, infection type responses and phenotypic patterns of isozymes extracted from young leaves (Burdon 1986).

Where segregation for resistance and susceptibility was observed among F_2 seedling families, examples of seedlings showing different infection type responses were saved and used to generate F_3 seedling families. Segregation patterns among these families were used to confirm the genetic basis of the observed phenotypic differences.

Results

Crosses with the universal susceptible 1232.1

The results of the F_2 analysis of seedlings derived from crosses between individual members of the differential set and the universal susceptible line 1232.1 are shown in Table 2. F_2 seedlings derived from hybrids between 1232.1 and the lines 1112.1, 1123.1, 1302.1, 1232.2 and 1341.1, all showed segregation ratios (3 : 1) indicative of the presence, in the original parental lines, of a single dominant gene for resistance. Hybrids between 1232.1 and 1123.1 were difficult to produce and had low fertility. As a result, only 68 F_2 seedlings were assessed. The segregation ratio observed with respect to line 1113.1 was compatible with the presence of two independently inherited, dominant genes for resistance. Each of these genes apparently conditioned the same infection type response (necrotic fleck with no sporulation) as all resistant individuals were phenotypically identical.

In many of the crosses involving these differential host lines, several separate batches of seedlings were assessed at different times using different avirulent pathogen races. χ^2 tests for heterogeneity among the results obtained showed no significant differences.

Assessment of F_2 seedlings derived from crosses between 1232.1 × 1340.1 produced some anomalous results. When pathogen race R1 was used to assess segregation patterns, a 3 : 1 ratio of resistant to susceptible individuals was observed. On the other hand, when races R2 and R4 were initially used, many more susceptible individuals were detected and the results best fitted a 9 : 7 ratio of resistant to susceptible individuals. Subsequent additional testing of 130 F_2 seedlings of this cross with race R1 confirmed the earlier detected 3 : 1 ratio. However, additional testing of 118 F_2 seedlings with race R4 detected an extremely good fit to a 3 : 1 ratio (89R[;], 29S[3]) as opposed to the previous 9 : 7 ratio.

Crosses between members of the differential set

The results of the analysis of F_2 segregation patterns among crosses between members of the differential set are given in Table 3. Fertile hybrids were produced between 15 of the 21 possible pair-wise combinations of *Glycine canescens* lines. Hybrids between 1112.1 and 1113.1, between 1123.1 and 1112.1, 1113.1, 1232.2 and 1340.1, and

Table 2. Patterns of segregation for resistance or susceptibility to *Phakopsora pachyrhizi* in F_2 seedling families derived from crosses between individual lines of the *Glycine canescens* differential set and the 'universal' susceptible line 1232.1

Male parent in cross[a]	Testing races[b]	Hetero-geneity[c] χ^2	Total no. of F_2 individuals	Observed no. F_2 individuals			Ratio of best fit	χ^2	Appropriate *P*
				R		S			
				;[d]	; 1⁻	2⁺ 3			
1112.1	R1, 2	6.78[(7)]	511	397	–	114	3 : 1	1.97	0.25–0.10
1113.1	R1, 2, 5	7.14[(7)]	419	385	–	34	15 : 1	2.49	0.25–0.10
1123.1	R1	–[e]	68	–	52	16	3 : 1	0.08	0.90–0.75
1232.2	R1, 2, 4	0.46[(4)]	448	340	–	108	3 : 1	0.19	0.75–0.50
1302.1	R1, 2, 4	3.98[(5)]	399	309	–	90	3 : 1	1.27	0.50–0.25
1340.1	R1	0.74[(3)]	228	173	–	55	3 : 1	0.09	0.90–0.75
	R2, 4	0.18[(4)]	202	124	–	78	9 : 7	2.17	0.25–0.10
	R4	–	118	89		29	3 : 1	0.01	> 0.90
1341.1	R1, 5	0.21[(1)]	110	84	–	26	3 : 1	0.11	0.75–0.50

[a] The 'universal' susceptible line 1232.1 was the female parent in all crosses
[b] Designation of races follows Burdon and Speer (1984)
[c] Superscript values in *parentheses* are the nos. of degrees of freedom when all independent assessments were combined
[d] Infection type responses, see text for details
[e] Only 68 seeds available, all tested simultaneously

Table 3. Patterns of segregation for resistance or susceptibility to *Phakopsora pachyrhizi* in F_2 seedling families derived from inter-crossing members of the *Glycine canescens* differential set

Cross	No of F_2 families	Testing races[a]	Hetero-geneity[b] χ^2	Total no. of F_2 individuals	Observed no. F_2 individuals				Ex-pected ratio[d]	χ^2	Appropriate *P*
					R			S			
					;[c]	; 1⁻	; 2⁻	2⁺ 3			
1. No segregation											
1112.1 × 1302.1	3	R1	0.00[(2)]	859	858	1	–	0	15 : 1	57.27	< 0.005
1340.1 × 1302.1	3	R1	0.00[(2)]	769	769	–	–	0	15 : 1	51.27	< 0.005
2. Segregation											
(a) Normal											
1113.1 × 1302.1	1	R2	–	369	320	41	–	8	63 : 1	0.88	0.50–0.25
1113.1 × 1340.1	2	R2	4.26[(2)]	759	648	106	–	(5)[e]	63 : 1	4.03	0.05–0.025
1232.2 × 1113.1	7	R2, 5	18.12[(12)]	872	816	45	–	11	63 : 1	0.51	0.50–0.25
1302.1 × 1123.1	3	R1	4.78[(10)]	358	263	71	–	24	15 : 1	0.13	0.75–0.50
1341.1 × 1113.1	2	R5	4.01[(2)]	628	525	–	96	7	63 : 1	0.82	0.50–0.25
1341.1 × 1302.1	2	R4	4.40[(2)]	428	347	64	–	17	15 : 1	3.79	0.10–0.05
(b) Distorted											
1112.1 × 1232.2	5	R2	8.60[(8)]	359	340	12	–	7	15 : 1	11.33	< 0.005
1232.2 × 1112.1	4	R2	5.74[(6)]	734	695	30	–	9	15 : 1	31.62	< 0.005
1232.2 × 1302.1	3	R2	1.40[(4)]	892	855	11	–	26	15 : 1	16.93	< 0.005
1232.2 × 1340.1	4	R3, 4	10.76[(10)]	351	327	16	–	8	15 : 1	9.45	< 0.005
1341.1 × 1112.1	1	R2	1.82[(2)]	514	476	32	–	6	15 : 1	22.66	< 0.005
1341.1 × 1123.1	3	R3, 5	2.39[(2)]	266	–	261	–	5	15 : 1	8.67	< 0.005
1341.1 × 1340.1	2	R1	2.46[(2)]	470	411	53	–	7	15 : 1	18.82	< 0.005
1341.1 × 1340.1	2	R1	0.59[(2)]	380	358	16	–	9	15 : 1	9.94	< 0.005

[a] Designation of races follows Burdon and Speer (1984)
[b] Superscript values in *parentheses* are the nos. of degrees of freedom when all independent assessments were combined
[c] Infection type responses, see text for details
[d] Expected ratio of resistant to susceptible individuals assuming independent segregation of known resistance factors
[e] Susceptible infection type apparently characterized by more chlorosis than normal. See text for details

Table 4. Patterns of segregation for resistance or susceptibility to *Phakopsora pachyrhizi* in F_3 seedling families of three crosses involving lines of *Glycine canescens*

Cross	F_2 phenotype	No. of F_3 families	Heterogeneity χ^2	F_3 phenotype		Ratio of best fit	χ^2	Appropriate P
				R	S			
1232.2 × 1340.1	R	4	–	119	0	–	–	–
		5	2.22	219	14	15 : 1	0.02	0.90–0.75
		6	5.17	157	41	3 : 1	1.95	0.25–0.10
1302.1 × 1123.1	R	2	–	110	0	–	–	–
		4	1.05	155	9	15 : 1	0.16	0.75–0.50
		1	–	19	4	3 : 1	0.71	0.50–0.25
1341.1 × 1112.1	R	7	–	364	0	–	–	–
		3	4.65	185	13	15 : 1	0.03	0.90–0.75
		4	0.91	123	34	3 : 1	0.94	0.50–0.25

between 1112.1 and 1340.1 were either not obtained or failed to reproduce.

The F_2 segregation patterns of the pair-wise combinations that produced fertile F_1 hybrids indicated that these crosses could be divided into two broad categories. The first group contains the two crosses in which there was no obvious segregation for resistance and susceptibility. These crosses were: 1340.1 × 1302.1 and 1112.1 × 1302.1. All 769 F_2 seedlings of the cross 1340.1 × 1302.1 had exactly the same infection type response (;), while in the other cross a few F_2 individuals showed a small amount of sporulation (;1$^-$). The second group of 13 crosses contains those in which segregation was apparent among the F_2 seedlings. In five of these crosses (1113.1 × 1302.1, 1232.2 × 1113.1, 1302.1 × 1123.1, 1341.1 × 1113.1 and 1341.1 × 1302.1), the frequency of susceptible individuals in F_2 segregating families fitted that expected if the resistance factors known to be present in each parental line were assumed to be inherited independently. A sixth cross, 1113.1 × 1340.1, probably also fits such a model although the number of susceptible individuals observed was marginally low ($0.05 > P > 0.025$) and the infection type (1$^+$ 2) of the observed susceptible segregants was distinguished from a fully susceptible reaction by a greater degree of chlorosis than normal.

In the remaining six crosses, although susceptible individuals were found among the F_2 progeny, their frequency differed significantly from that predicted by an independent inheritance model.

The genetic basis of the observed F_2 phenotypic differences in resistance was confirmed by progeny testing a random sample of F_3 seedlings derived from resistant F_2 plants (Table 4). In all cases, these families of F_3 seedlings were either uniformly resistant or segregated for resistance and susceptibility. For each set of families derived from a particular cross, data were pooled only if χ^2 heterogeneity values were non-significant. In all cases, three classes of resistant phenotypes were detected among the F_3 families derived from resistant F_2 individuals. In one of these classes, all F_3 individuals were uniformly resistant while the other two segregated for resistance and suceptibility. In one of the latter groups, the frequency of resistant to susceptible individuals fitted a 15 : 1 ratio. The other fitted a 3 : 1 ratio. These results confirmed those from the F_2 seedling test (Table 3), indicating the presence of two dominant resistance genes.

Discussion

Resistance to the pathogen *Phakopsora pachyrhizi* in the seven lines of *Glycine canescens* was found to be conditioned by race-specific genes with major phenotype effects. In six of the seven lines studied, single genes were detected while in the seventh, two independently inherited genes were present. In most instances, the frequency of resistant and susceptible individuals in segregating F_2 families indicated that inheritance followed a normal Mendelian pattern (Table 2). However, in the case of line 1340.1, inconsistent results were obtained when F_2 seedlings were challenged with one particular pathogen isolate (R4). In this instance, ratios of 9 : 7 and 3 : 1 resistant to susceptible individuals were obtained on different occasions. However, the latter ratio was consistently obtained with pathogen race R1; without further evidence, the 9 : 7 ratio result should be treated with caution.

When the seven lines of *G. canescens* were intercrossed two broad categories of crosses could be distinguished – those in which F_2 seedling families showed no obvious segregation for resistance and susceptibility and those in which such segregation was apparent. The three crosses 1112.1 × 1302.1, 1113.1 × 1112.1 and 1340.1 × 1302.1 fell into the former category. 859, 497 and 769 F_2 seedlings were scored in these crosses, respectively, and no fully susceptible individuals were encountered despite an expected number of 53.69, 7.76, and 48.06 (assuming independent genes). One explanation for this

lack of susceptible segregants is that the resistant factors present in the original parental lines are allelic or at least closely linked.

In all the remaining crosses, F_2 seedling families all segregated for resistance and susceptibility. However, these crosses can be further subdivided into those in which observed segregation ratios were in accord with those predicted from the number of resistance genes detected in the parental lines (Table 3) and those which deviated significantly from the predicted ratios. The frequency of resistant to susceptible individuals among F_2 families of the crosses 1232.2 × 1113.1, 1302.1 × 1123.1, 1341.1 × 1113.1 and 1341.1 × 1302.1 all fit predictions of 3, 2, 3, and 2 independently inherited resistance genes, respectively.

In the crosses 1112.1 × 1232.2, 1232.2 × 1302.1, 1232.2 × 1340.1, 1341.1 × 1112.1, 1341.1 × 1123.1 and 1341.1 × 1340.1 the recovery of at least some fully susceptible individuals in the F_2 generation clearly indicates that resistance in the host lines of each pair is controlled by separate loci. However, in all cases the frequency of susceptible segregants was significantly less than expected. In the most extreme case involving crosses between 1112.1 and 1232.2, only 16 of 68 expected susceptible individuals were detected (Table 3). The observed low numbers of susceptible individuals in these crosses may result from chance, loose linkage, epistatic interactions, genetic background effects or from a combination of these phenomena. While none of these possibilities can be ruled out totally, it must be realized that many of the crosses produced were hybrids between morphologically and isozymically dissimilar lines of *G. canescens* collected from widely scattered natural populations (Burdon and Brown, unpublished data). Wide crosses of this nature often give rise to segregating families in which ratios are distorted as a result of selection at the gamete or zygote stage (for example, achlorophyllous mutants were often observed). Without evidence to the contrary, this explanation seems the most parsimonious. Indeed, the possibility of even loose linkage between the resistance factors in these different *G. canescens* lines is further reduced by the segregation data reported for F_3 seedling families (Table 4). Individuals from the crosses 1232.2 × 1302.1 and 1341.1 × 1112.1, heterozygous for resistance at the F_2 stage, all produced F_3 progeny that segregated in complete agreement with that expected of freely recombining systems.

Overall, these data indicate that at least four different resistance genes are present in the *G. canescens* differential set. The resistance found in 1232.2 is distinct from that found in all lines with which it was tested. The resistance factors present in lines 1112.1, 1302.1 and 1340.1 are either allelic or closely linked to one another; the two resistance factors found in 1113.1 are independent of each other and, while one of these may well be at the same locus as that in 1112.1 (lines 1112.1 and 1113.1 are from the same population; Table 1), the other is most probably independent of those found in all other lines. Finally, the relationship between 1232.2 and 1341.1 was not assessed, so it is not possible to say whether the resistance in 1341.1 is allelic to that in 1232.2 or at a separate locus.

It is proposed that these resistances be given the following tentative gene-locus symbols: Lr_1 (1232.2), Lr_2 (1112.1, 1302.1, 1340.1) and Lr_2 and Lr_3 (1113.1). Although the data available concerning the resistance in line 1123.1 are very limited, it is independent of that in line 1302.1 and, therefore, presumably from that in 1112.1 and 1340.1. If the resistances in 1232.2 and 1341.1 are at a common locus (and these lines are morphologically and isozymically closely related; Brown and Burdon, unpubished), then the resistance in 1123.1 can tentatively be assigned to a fourth locus Lr_4.

The relationship of the *Glycine canescens* resistance genes described here to the four genes for resistance to *Phakopsora pachyrhizi* characterized in *G. max* (Bromfield and Hartwig 1980; Hartwig and Bromfield 1983; Hartwig 1986) is unknown. To date, no fertile crosses have been made between *G. canescens* and *G. max*, although the resistance gene present in line 1232.2 is expressed in sterile F_1 hybrids between this line and *G. max* (Brown et al. 1985). Furthermore, the single gene nature of this and other observed resistance (Burdon 1987) and its major phenotypic effect suggests that *G. canescens* and many other wild perennial relatives of *G. max* are likely to be important future sources of genes for resistance to *Phakopsora pachyrhizi*.

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References

Bromfield KR (1980) Soybean rust: some considerations relevant to threat analysis. Prot Ecol 2:241–257

Bromfield KR (1984) Soybean rust. Monograph No 11. American Phytopathological Society, St. Paul, Minnesota

Bromfield KR, Hartwig EE (1980) Resistance to soybean rust and mode of inheritance. Crop Sci 20:254–255

Brown AHD, Grant JE, Burdon JJ, Grace JP, Pullen R (1985) Collection and utilization of wild perennial *Glycine*. Proc 3rd World Soybean Res Conf, Ames, 1984. Westview Press, Colorado, pp 345–352

Burdon JJ (1986) The potential of Australian native *Glycine* species as sources of resistance to soybean leaf rust (*Phakopsora pachyrhizi*). In: Nampompeth B, Subhadrabandhu S (eds) New Frontiers in Breeding Researches. Faculty of Agriculture, Kasetsart University, Bangkok, Thailand, pp 823–832

Burdon JJ (1987) Phenotypic and genetic patterns of resistance to the pathogen *Phakopsora pachyrhizi* in populations of *Glycine canescens*. Oecologia (Berlin) 73:257–267

Burdon JJ, Marshall DR (1981 a) Evaluation of Australian native species of *Glycine* for resistance to soybean rust. Plant Dis 65:44–45

Burdon JJ, Marshall DR (1981 b) Inter- and intra-specific diversity in the disease-response of *Glycine* species to the leaf-rust fungus, *Phakopsora pachyrhizi*. J Ecol 69:381–390

Burdon JJ, Speer SS (1984) A set of differential *Glycine* hosts for the identification of races of *Phakopsora pachyrhizi* Syd. Euphytica 33:891–896

Hartwig EE (1986) Identification of a fourth major gene conferring resistance to soybean rust. Crop Sci 26:1135–1136

Hartwig EE, Bromfield KR (1983) Relationship among three genes conferring specific resistance to rust in soybeans. Crop Sci 23:237–239

Hymowitz T, Carmer SF, Newell CA (1976) Soybean cultivars released in the United States and Canada. Int Agric Publ Intsoy Ser No 9, 31 pp

Yang CY (1977) Soybean rust in the eastern hemisphere. In: Ford RE, Sinclair JB (eds) Rust of soybean – the problem and research needs. Int Agric Pub Intsoy Ser No 12:22–33