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MAPPING GENE FOR BACTERIAL BLIGHT (*RPG4* LOCUS) IN SOYBEAN

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Abstract

Rpg4 gene in soybean. *Rpg4* also confers resistance to *Pseudomonas syrinagae* pv. *tomato* strains expressing the avirulence gene-*avrD*. The genetic linkage studies reported here were undertaken to advance the construction of the classical genetic map of soybean. Hybridizations were made in the green house and the F_2 generation was produced and classified. Data were tested by chi-square for single-factor segregation and recombination estimates were computed with computer program, Linkage-1. The *Rpg4* locus is controlling bacterial blight resistance to *Pseudomonas syrinagae* pv. *glycinea* race 4 segregated independently of the *y12, y23, fr1, y13, pb, y9, fr2, Fr3, w1, w4* and *y10*. The chi-square for the *Rpg4* and *y9* indicated linkage. The recombination frequency between the *Rpg4* and y9 loci was estimated at 29.2 + 8.5 cM.

Introduction

Soybean is diploid (2n = 2x = 40), having 20 chromosome pairs (Palmer & Kilen, 1987). Bacterial blight of soybean incited by *Pseudomonas syringae* pv. *glycinea* (Coerper) Young, Dye & Wilkie (*Psg*) occurs world wide (Dye *et al*., 1980; Cross *et al*., 1966). Primarily, the bacterium infects cotyledons and later seedlings. Leaves are mostly attacked by this organism because it exists epiphytically on leaf surfaces. Infected young leaves are frequently stunted and chlorotic. Lesions on leaves are initially small, angular and water-soaked. Lesions are also found on stems, petioles, and pods. Seeds become infected and a slimy bacterial growth may develop, which causes tremendous yield losses (Sinclair, 1982; Tisselli *et al*., 1980).

The avirulance gene D is the only bacterial gene for which information is available on how avirulence genes interact with resistant genes of plants. The cloned *avr*D gene functioned in *Psg* cells to elicit the hypersensitive response (HR- a resistance reaction) in only those cultivars of soybean carrying the disease resistant gene *Rpg4* (Keen & Buzzell, 1991; Farhatullah *et al*., 1996).

The "gene-for-gene" model (Flor, 1942) has been extended beyond race-cultivar interactions to include interactions between plant pathogens and "nonhost(s)". For example, the tomato pathogen *Pseudomonas syringae* pv. tomato (*Pst*) possesses multiple avirulence genes that when expressed in *P. syringae* pv. *Glycinea* (*Psg*), induce an HR in various cultivars of soybean (Kobayashi *et al*., 1989; Farhatullah *et al*., 1996 and 2010). This interaction was a true gene-for-gene interaction (Keen & Buzzel, 1991). These studies focus on the location of the *Rpg4* locus on the classical soybean genetic map.

Materials and Methods

All genetic stock i.e., PI290136(fr2), PI424078(Fr3), L69(y9), L72(w4), Minsoy(fr1,Pb), T161(y10), T225H(y18), T230(y13), T233(y12), T288(y23) and T295(ms,pb) was obtained from the Soybean Genetic Type Collection maintained by the USDA at the University of Illinois at Urbana, IL. Gene symbols i.e. Fr, Ms, Pb, W, and Y refer to various loci that condition root fluorescence, male-sterility, pubescence tip, and chlorophyll deficiency traits in seedlings respectively as defined by Palmer and Kilen, 1987 (Table 1).

All genetic stocks were resistant (*Rpg4/Rpg4*), except Minsoy (*rpg4/rpg4*). These were hybridized with susceptible male sterile-ms (*rpg4/rpg4*) parents. Susceptible ms segregantes were derived from a cross between resistant ms stock T295H (resistant carrying ms6 and ms2 locus (Palmer, 2000; Palmer & Skorupska, 1990) and Acme (susceptible).

All hybridizations were conducted in the greenhouse. The F_1 seeds were planted in large pots. The F_2 seeds were single-plant threshed from individual F_1 plants.

For evaluating pubescence tip (sharp *Pb*- and blunt *pbpb*), a compound microscope (100X) was used. For observation of the root fluorescence trait, F_2 seed were germinated on paper and roots of 4-d-old seedlings were examined under an ultraviolet light in the dark according to the method of Delannay & Palmer (1982). For chlorophyll deficiency conditioned by the *y9* and *y13* loci, green and yellow phenotypes and purple hypocotyl color (w4) were scored in the greenhouse. These families were then shifted to the growth chambers for *Rpg4* assays.

Specific elicitor preparation: The specific elicitor (SE) was isolated to a high degree of purity from *Psg* transformed with *avr*D (Keen *et al*., 1990), called syringolides (Smith *et al*., 1993; Midland *et al*., 1993). Syringolides are glycolipids produced by Gram-negative bacteria expressing *Pseudomonas syringae* carrying an active allele of *avr*D. The syringolides mediate gene-for-gene complementarity, inducing the hypersensitive reaction (HR) only in those soybean plants carrying the *Rpg*4 disease resistance gene (Slaymaker & Keen, 2004; Farhatullah *et al*., 1996; Keen & Buzzell, 1991) but not *rpg4*. The syringolides elecitors are at least 1000 times more active in *Rpg*4 than in *rpg4* cultivars (Okinaka *et al*., 2002)

Elicitor application: Fully expanded primary leaves of soybean seedlings at the 4-leaf stage were infused with a concentration of 125 units of SE/ml of water. The blunt needle mounting end of a disposable 1ml plastic syringe was pressed on to the lower surface of the leaf and approximately 20-25 µL per site of SE were administered by applying pressure to the plunger. After infusion, seedlings were placed in a controlled environment chamber with an 18h photoperiod at a constant temperature of 25^{o}c and 90% relative humidity for 48h (Keen *et al*., 1990).

Reaction of elicitor:

A standard procedure as reported by Keen *et al*., (1990), was used to assess the disease reaction. Assays of *Rpg4* locus genotype (HR versus nonHR) were conducted.

Classical mapping: Chi-square tests, tests of linkage, and recombination values were calculated using the Linkage-1 program, which utilizes the maximum likelihood method (Suiter *et al*., 1983).

Table 1. Parental genotypes used in crosses for genetic linkage tests of *Rpg4* locus in soybean.

Genes¶	Parents											
	290136	**PI424078**	**L69*f***	**L72§**	**Minsoy**	**ms!**	**T161**	**T225H**	**T230**	**T233**	**T288**	**T295**
Fr1					*fr1*							
Fr2	*fr2*											
Fr3		*Fr3*										
Ms6												*ms*
Pb					*Pb*							*Pb*
Rpg4	*Rpg4*	*Rpg4*	*Rpg4*	*Rpg4*	*rpg4*	*rpg4*	*Rpg4*	*Rpg4*	*Rpg4*	*Rpg4*	*Rpg4*	*Rpg4*
W4				*w4*								
Y9			*y9*									
Y10							*y10*					
Y12										*y12*		
Y13									*y13*			
Y18								*y18*				
Y23											*y23*	

¶Gene designations for morphological traits are according to Keen & Buzzell (1991); Palmer & Kilen (1987, 1990)
f L69-4318, § L72-1138, ! derived from cross of T295H x Acme

Results and Discussion

Although considerable progress is being made in constructing the molecular maps of soybean [*Glycine max* (L.) Merr). The soybean linkage map, composed of nearly 250 named morphological, physiological, and protein markers, have been assigned gene symbols and 68 genes of these traits have been associated with 21 classical linkage groups (CLG) or linkage fragments (Thorson *et al*., 1989; Palmer & Kiang, 1990; Palmer & Shoemaker, 1998; Cregon *et al*., 1999; Devine, 2003). Soybean cultivars carrying resistance genes specific to avirulence genes both in the soybean (*Psg*) and tomato pathogen (*Pst*).

In this study linkage of *Rpg4* locus was tested with 11 known marker loci. Single locus segregations for all marker loci were according to the expectation but the segregation at *Rpg4* locus deviated from the expected 3:1 at the $p<0.01$ level for one cross (#7) and for two crosses (#5 and #9) the deviation from 3:1 approached significance at the $p<0.05$ level. In all three of these cases there was an excess of nonHR segregates. There was also a small but significant difference from the expected 3:1 segregation ratio for the pooled data for the *Rpg4* locus where the observed segregation of 70.9%:29.1% deviates significantly from the expected 75.0%:25.0% ($p<0.005$). The simplest explanation for an excess of nonHR individuals is that HR genotypes (*Rpg4/*----) tend to be occasionally misclassified as nonHR. In these experiments misclassification was probably exacerbated by weak SE (as based on the reaction on control HR plants) that was used for evaluating the progeny of crosses #3, #6, and #7 involving root fluorescence genes. In all three of these F_2 segregations the proportions of nonHR segregates were 29.5%, 30.3%, and 35.4%, respectively, and the latter case, as noted above, was significant at the $p<0.05$ level (Table 2).

Despite the statistically deviation from the expected 3:1 segregation at the *Rpg4* locus, the fitness of data to the most likely alternative genetic explanation (a 9 HR:7 nonHR ratio) is even worse. This alternative is rejected for all 10 segregations (overall) at an extremely high level of significance (pooled $X^2_{1df}[9:7] = 104.39$; $p<0.001$). Importantly, deviations observed from the expected 3:1 ratio are not large, and the Linkage-1 program used for these analyses can detect linkage and calculate recombination distances even when single-locus segregations are disturbed (Suiter *et al*., 1983). The results of linkage analysis are presented in Table 3. For 11 of 12 loci, X^2_{1df} linkage was not significant at the $p<0.05$ level, but for cross #5, X^2_{1df} linkage=9.72 was significant at the $p<0.005$ level, with a recombination distance of 29.2 cM $\pm$ 8.5 cM (Table 3). Thus, the *Rpg4* and *y9* loci are on the same chromosome, although not tightly linked.

Table 2. Genetic analysis of F_2 segregations for morphological traits from crosses between nonHR lines and a male sterile HR line (T295H).

Gene	Cross	Genotypic¶ classes		X^2_{1df}§	*P*↑	Genotypic classes		X^2_{1df}§	*P*↑	SumΩ
		A-	*aa*			R-	rr			
y12	1	100!	40	0.95	0.329	109	31	0.61	0.434	140
y23	2	82	27	0.00	0.956	79	30	0.37	0.543	109
fr1	3	89	23	1.19	0.275	79	33	1.19	0.275	112
y13	4	85	21	1.52	0.217	74	32	1.52	0.217	109
pb	3	79	33	1.19	0.275	85	27	0.05	0.827	112
y9	5	79	34	1.56	0.212	76	37	3.61	0.057	113
fr2	6	97	35	0.16	0.688	92	40	1.98	0.159	132
Fr3	7	109	49	3.05	0.081	106	52	5.27	0.022	158
w1	3	83	29	0.05	0.827	79	33	1.19	0.275	112
w4	8	89	51	3.05	0.081*f*	100	40	0.95	0.329	140
y10	9	122	37	0.25	0.615	109	50	3.52	0.060	159
y18	10	82	19	2.06	0.151	71	30	1.19	0.275	101

§ Chi-square tests for assuming monogenic ratio 3:1; *f* Chi-square test for assuming 9:7 ratio
↑ Probability of a greater chi-square; ¶ Common genotype designation for different genes, representing the respected gene in the row; Ω Sum of each locus in the row; *a* R- = *Rpg4*-; rr = *rpg4*/*rpg4*; ! Number of plants

Table 3. Linkage and linkage analysis of *Rpg4* locus from F_2 populating in soybean.

Linkage group	Gene	Cross	Genotypic classes¶				Sum	X^2_{1df}§	*P*↑	R	±	SE*f*
			A	B	C	D						
I	*y12*	1	79!	30	21	10	140	0.27	0.61	0.473	±	0.065
VIII	*w1*	3	57	22	26	7	112	0.53	0.46	0.451	±	0.075
VIII	*y23*	2	61	18	21	9	109	0.61	0.44	0.448	±	0.076
XII	*fr1*	3	65	14	24	9	112	1.30	0.25	0.433	±	0.076
XII	*y13*	4	57	17	28	4	106	1.54	0.21	0.389	±	0.081
XIV	*Pb*	3	60	25	19	8	112	0.02	0.88	0.491	±	0.066
XIV	*y9*	5	46	33	30	4	113	9.72	0.00	0.292	±	0.085
Unknown	*fr2*	6	70	22	27	13	132	1.06	0.30	0.439	±	0.070
Unknown	*Fr3*	7	77	29	32	20	158	2.01	0.16	0.419	±	0.065
Unknown	*w4*	8	61	28	39	12	140	3.87	0.06	0.479	±	0.042Ω
Unknown	*y10*	9	81	28	41	9	159	1.13	0.29	0.433	±	0.068
Unknown	*y18*	10	57	25	14	5	101	0.13	0.72	0.460	±	0.078

¶ Class designations as per Allard, 1956; § Chi-square tests for independence assuming 9:3:3:1 ratio; Ω Chi-square tests for independence assuming 27:21:9:7 ratio; ↑ Probability of a greater chi-square; *f* Recombination value ± standard error; ! Number of plants

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