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An unusual polymorphic locus useful for tagging *Rps1* resistance alleles in soybean

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Abstract The *Phytophthora* root and stem resistance locus *Rps1* has been mapped to linkage group *N* of the USDA-ARS soybean molecular map, approximately 2 cM from locus A071-1. To determine if A071-1 polymorphisms exist that distinguish and tag different *Rps1* alleles, germplasms containing the seven *Rps1* alleles were screened with eight enzymes for pA071-detectable polymorphisms. Six enzymes revealed at least one polymorphic fragment. All six detected a polymorphism at A071-1 as determined by restriction fragment length polymorphism mapping, comparison to an EMBL3 clone containing locus A071-1, and Southern hybridization with probes specific for locus A071-1. Screening of the *Rps1* donors and 24 *rps1*- and 15 *Rps1*-containing U.S. soybean varieties showed that locus A071-1 exhibited three polymorphisms with each enzyme. The polymorphisms detected by one enyme did not always correlate with those detected by the other four, suggesting that multiple mutation events may be responsible for the different A071-1 polymorphisms. Although no combination of alleles distinguished *rps1*- and *Rps1*-containing genotypes, polymorphism at A071-1 made it possible to distinguish five groups of soybean germplasms. Thus, the unusual polymorphism of locus A071-1 should useful for following *Rps1* inheritance in many breeding programs.

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Introduction

Isozyme and restriction fragment length polymorphism (RFLP) analysis of different soybean (*Glycine max* L. Merr) varieties has revealed only low levels of genetic diversity in this cultivated species (Doyle and Beachy 1985; Griffin 1986; Apuya et al. 1988; Keim et al. 1989). The most likely cause for this is that only ten accessions contributed 88% of the northern germplasm, while only seven accessions contributed 70% of the southern germplasm, developed in U.S. soybean breeding programs (Delannay et al. 1983; Specht and Williams 1984). This low level of diversity has made it difficult to construct saturated molecular maps, to identify polymorphisms closely linked to genes of interest, and to identify polymorphisms that tag alleles of particular genes.

Infection of soybean by *Phytophthora sojae* causes significant yield loss due to damping-off of seedlings and yield reduction in mature plants. The gene *Rps1* has been used extensively by soybean breeders as a source of resistance to *P. sojae* infection. *Rps1* is unusually polymorphic, encoding six alleles that provide resistance to various races of *P. sojae* (Buzzell and Anderson 1992; T. Kilen, personal communication). Using near-isogenic lines and RFLPs, Diers et al. (1992b) were able to map *Rps1* to linkage group N of the USDA-ARS soybean molecular map, approximately 2 cM from the loci A071-1 and K418-1 and 6 cM from A280 (Fig. 1).

With the advent of molecular techniques, such as RFLP mapping and the polymerase chain reaction, plant breeders have begun using marker-assisted breeding, in which the inheritance of desired traits is followed genotypically rather than phenotypically. Because the *Rps1* gene has not been cloned, marker-assisted breeding based on direct tagging and differentiation of *Rps1*

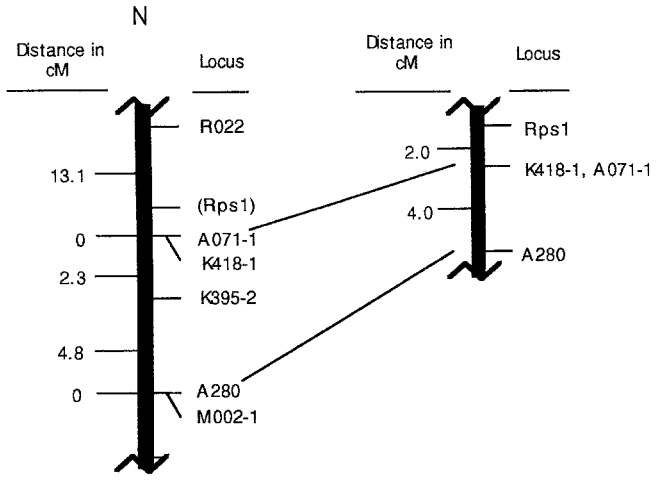

Fig. 1 Locations of locus A071-1 and *Rps1* on linkage group N of the USDA-ARS soybean molecular map. Only a portion of linkage group N is shown. The outset map showing the location of *Rps1* is based on the results of Diers et al., 1992b)

alleles is not possible. However, it may be possible to indirectly tag *Rps1* alleles by tagging alleles of a linked marker locus. In this study, we have examined polymorphism at the linked A071-1 locus in soybean germplasms containing different *Rps1* alleles. We report that (1) locus A071-1 exhibits an unusually high level of polymorphism, (2) polymorphism at A071-1 distinguishes five groups of *Rps1* donor germplasms, and (3) many *rps1*- and *Rps1*-containing U.S. soybean varieties differ at locus A071-1, making molecular tagging of *Rps1* inheritance by A071-1 polymorphisms feasible in many breeding programs.

Materials and methods

Plant material

The USDA-ARS RFLP mapping population has been described by Diers et al. (1992a). Seed for the *Rps1* donors 'Williams' (*rps1*) (Moots et al. 1983), 'Mukden' (*Rps1-a*) (Bernard et al. 1957), 'Sanga' (*Rps1-b*) (Lam-Sanchez et al., 1968), 'Harrel' (*Rps1-b*), 'Arksoy' (*Rps1-c*) (Mueller et al. 1978), PI103091 (*Rps1-d*) (Buzzell and Anderson 1992), PI172902 (*Rps1-?*) (T. Kilen personal communication) and 'Kingwa' (*Rps1-k*) (Bernard and Cremmens 1981), and for the U.S. soybean varieties listed in Table 2, was obtained for the USDA Soybean Germplasm Collection (Randy Nelson, USDA-ARS, University of Illinois, Urbana-Champaign/Ill.). The *Rps1* donors were grown in the field during the summer of 1992. The various U.S. soybean varieties were grown either in the field or the greenhouse. The *Rps1* allelic status of the U.S. soybean varieties was determined from information available on the Germplasm Resoources Information Network (USDA-ARS-PSI-NGRL, Beltsville, M.D.) or provided by the plant Variety Protection Office (Beltsville, M.D.). In some instances, information was provided only for resistance to *P. Sojae* race 1. Because all *Rps1* alleles confer resistance to this race, and variety exhibiting sensitivity to race 1 was scored as containing the *rps1* allels.

Preparation of DNA and Southern hybridization

DNA was prepared from leaf material as described by Keim et al. (1988). Restriction enzyme digestion, electrophoresis, and Southern transfer and hybridization were as described by Sambrook et al. (1989). After hybridization, membranes were rinsed in 1 × SSC, 0.1% SDS, 60 °C, and washed in 0.5 × SSC, 0.5% SDS, 60 °C (low stringency) or 0.1 × SSC, 0.1% SDS, 65 °C (high stringency) before autoradiography. The probe pA071 was obtained from the *Pst*I library of random soybean genomic fragments described by Keim and Shoemaker (1988). The probe was labelled with ^{32}P-dCTP (NEN-Dupont, Boston, Mass.) by the random priming method (Feinberg and Vogelstein 1983). Probes a10, a11, and a14 were isolated from an EMBL3 clone containing locus A071-1 and purified by electrophoresis in SeaPlaque low-melting-temperature agarose (FMC, Rockland/Me.).

Isolation of a genomic clone containing locus A071-1

An EMBL3 genomic library (Clontech, Palo Alto, Calif.), constructed from 'Williams 82' DNA partially digested with *Mbo*I, was screened with pA071. Positive clones were analyzed as described by Sambrook et al. (1989). Clones containing locus A071-1 were identified by the presence of the polymorphic 3.8-kb *Taq*I fragment from Williams 82. Clones were confirmed to contain A071-1 by mapping an *Eco*RI polymorphism detected by probe a14 (see Fig. 3). Probe a14 detected only a single fragment in five digests of the USDA-ARS *G. max* and *G. soja* parents indicating that this sequence was present only once in the genome. Locus a14 co-segregated with A071-1 in the USDA-ARS mapping population (data not shown). Therefore, the presence of the 3.8-kb *Taq*I fragment and RFLP mapping of a contiguous polymorphismn confirmed that both EMBL3 clones contained the A071-1 locus.

Results

Examination of donor germplasms containing different *Rps*1 alleles for pA071-detected polymorphism

As a first step in determining whether donor germplasms containing different *Rps1* alleles were polymorphic at locus A071-1, DNA from Williams (*rps1*), Mukden (*Rps1-a*), Sanga and Harrel (*Rps1-b*), Arksoy (*Rps1-c*), PI103091 (*Rps1-d*), PI172902 (*Rps1-?*), and Kingwa (*Rsp1-k*) was digested with the restriction enzymes *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Hae*III, *Hin*dIII, *Ssp*I, and *Taq*I and probed with pA071. *Dra*I and *Ssp*I did not reveal any polymorphism. The remaining six enzymes revealed from one to three fragments that were polymorphic among the eight *Rps1* donor germplasms (Table 1). Two enzymes, *Taq*I and *Hae*III, seemed to reveal a single polymorphic locus with three polymorphisms (Fig. 2 A and B, respectively); *Taq*I fragments of 3.0, 3.8, and 4.4 kb, and *Hae*III fragments of 3.5, 3.8 and 4.6 kb. *Bgl*II also appeared to reveal a single polymorphic locus (4.5 kb). *Hin*dIII produced two potentially co-dominant polymorphisms of 2.4 vs 2.3 kb and 1.4 vs 1.3 kb. The pattern of polymorphisms was more complicated for *Eco*RV or *Eco*RI. Most of the germplasms seemed to contain a single polymorphic locus with at least three polymorphisms; *Eco*RI fragments of 6.0, 2.8, and 2.5 kb, and *Eco*RV fragments of 4.7, 2.7, and 2.5 kb. However, Kingwa (*Eco*RI), and Arksoy and PI103091 (*Eco*RV) each displayed several polymorphic fragments, making it impossible to determine which fragment correspon-

Table 1 Polymorphisms detected by pA071 and a10, a11 in donor germplasms containing different *Rps1* alleles

Germplasm	*Bgl*II		*Eco*RI		*Eco*RV		*Hae*III		*Hin*dIII		*Taq*I	
	pA071	a10 a11[a]	pA071	a10 a11	pA071	a10 a11	pA071	a10 a11	pA071	a10 a11	pA071	a10 a11
Williams (*rps1*)	4.5[a]	13	2.8	2.8	4.7	9.5	4.6	4.6	2.4 1.4	10	3.0	3.0
Mukden (*Rps1-a*)	–	13	2.8	2.8	4.7	9.5	3.5	3.5	2.4 1.3	10	3.0	3.0
Sanga (*Rps1-b*)	–	13	2.8	2.8	4.7	9.5	4.6	4.6	2.4 1.3	10	3.0	3.0
Harrel (*Rps1-b*)	–	13	2.8	2.8	2.5	9.5	4.6	4.6	2.4 1.3	10	3.0	3.0
Arksoy (*Rps1-c*)	–	5.6	6.0	6.0	4.7 2.7 2.5	2.5	4.6	4.6	2.4 1.3	2.4	3.8	3.8
PI103091 (*Rps1-d*)	–	17	2.5	2.5	4.7 2.0	2.0	3.8	3.8	2.3 1.4	2.3	4.4	4.4
PI172902 (*Rps1-?*)	–	13	2.8	2.8	4.7	9.5	4.6	4.6	2.4 1.3	10	3.0	3.0
Kingwa (*Rps1-k*)	4.5	5.6	6.0 5.4 2.5	6.0	2.5	2.5	4.6	4.6	2.4 1.4	2.4	3.8	3.8

[a] Probes a10 and a11 contain the A071-1 locus of Williams 82
[b] Numbers indicate size of polymorphic fragments in kilobases

ded to the polymorphic locus detected in the other germplasms. In total, pA071 detected 19 polymorphic fragments among the *Rps1* donor germplasms (*Bgl*II 4.5 kb; *Eco*RV 2.0, 2.5, 2.7, and 4.7 kb; *Eco*RI 2.5, 2.8, 5.4, and 6.0 kb; *Hea*III 3.5, 3.8, and 4.6 kb; *Hin*dIII 1.3, 1.4, 2.3, and 2.4 kb; *Taq*I 3.0, 3.8, and 4.4 kb).

Identification of polymorphic fragments located at locus A071-1

Locus A071-1 (formerly A71) was mapped to linkage group N of the USDA-ARS genetic map as a polymorphism of the largest pA071-hybridizing *Taq*I fragment (3.0-kb *G. max* vs 4.4-kb *G. soja* band, Fig. 2A, Lanes M and S). This same *Taq*I fragment was polymorphic among the *Rps1* donors (Fig. 2A) indicating that the donors were polymorphic at A071-1. Two other enzymes, *Eco*RI and *Hae*III (Fig. 2B), produced the same polymorphisms in the USDA-ARS mapping parents as were observed among the *Rps1* donor germplasms. Segregation analysis in the USDA-ARS mapping population showed that these polymorphisms co-segregated with A071-1 (data not shown). Thus, the *Hae*III and *Eco*RI polymorphisms were also located either at, or very near, locus A071-1 on linkage group *N*.

To confirm that the *Eco*RI and *Hae*III polymorphisms were located at locus A071-1, lambda clones containing A071-1 were isolated from an EMBL3 genomic DNA library of Williams 82 DNA. Williams 82 is an isoline containing the *Rps1-k* allele (Moots et al. 1983) and the A071-1 locus from Kingwa (data not shown). Two clones containing the expected 3.8-kb pA071-hybridizing *Taq*I fragment were obtained. A restriction map of the genomic region contained in the clones is shown in Fig. 3. Both the polymorphic *Hae*III and *Eco*RI fragments present in Kingwa (Table 1) were present on the lambda clones, indicating that these polymorphic fragments are located at locus A071-1.

Comparison of Fig. 3 and Table 1 shows that, in addition to containing the polymorphic *Eco*RI and *Hae*III fragments, the lambda clones also contained *Hin*dIII and *Eco*RV fragments of the same size as polymorphic fragments observed in Kingwa, suggesting that these polymorphic fragments may also be located at locus A071-1. Because these polymorphisms were not present in the mapping population it was not possible to locate them to A071-1 by RFLP analysis. Therefore, a molecular approach was taken to determine if these polymorphisms were located at locus A071-1. It was reasoned that polymorphic fragments containing A071-1 should hybridize to probes containing portions of locus A071-1 under high-stringency conditions while fragments containing only related A071 sequences should hybridize less strongly or not at all. Therefore, fragments a10 and a11 (Fig. 3) were used to probe the *Rps1* donor DNAs (Table 1) under high-stringency conditions. Comparison of Fig. 2 B (*Hae*III digest probed with pA071) with Fig. 4 (*Hae*III digest probed with a10 and a11) shows that, as predicted, probes a10 and a11 were more specific in detecting the polymorphic *Hae*III fragment located at locus A071-1 than was probe pA071. In accordance with the segregation data and lambda clones, probes a10 and a11 detected the same polymorphic *Eco*RI, *Hae*III (Fig. 4), and *Taq*I fragments as pA071 in all *Rps1* donors. However, in the

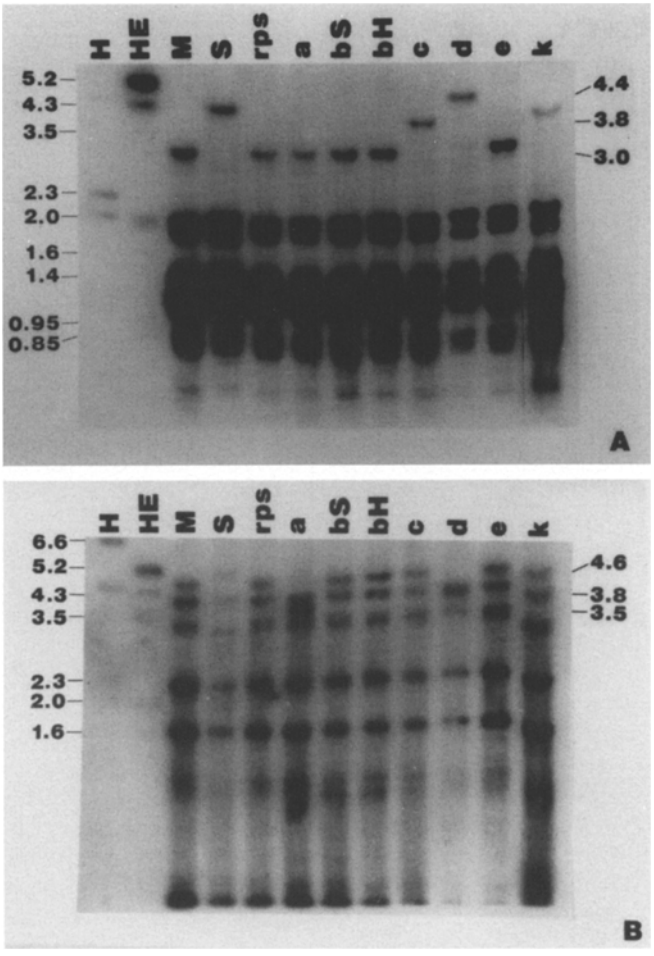

Fig. 2 A,B Screening of donor germplasms containing different *Rps1* alleles for polymorphism at locus A071-1. **A** *Taq*I digest and **B** *Hae*III digest. *M*, G. max; *S*; G. soja, *rps*, Williams; *a*, Mukden (*Rps1-a*); *bS*, Sanga (*Rps1-b*); *bH*, Harrel (*Rps1-b*); *c*, Arksoy (*Rps1-c*); *d*, PI103091 (*Rps1-d*); *e*, PI172902 (*Rps1-?*); and *k*, Kingwa (*Rps1-k*). Size markers are lambda DNA digested with *Hin*dIII (*H*) and *Hin*dIII and *Eco*RI (*HE*). Numbers to the right are the sizes of the polymorphic fragments in kilobases

*Bgl*II, *Eco*RV and *Hin*dIII digests, probes a10 and a11 revealed polymorphic fragments in several of the germplasms that were not detected by pA071 (Table 1). Instead, probes a10 and a11 identified new co-dominant polymorphic patterns at A071-1 which had previously been obscured by co-migrating pA071-hybridizing bands. For all three enzymes the new polymorphic fragment correlated with a fragment known to be present at locus A071-1 based on the A071-1 EMBL3 clones (Fig. 3). Thus, the A071-1 locus appeared to be polymorphic for *Hin*dIII, *Eco*RV, and *Bgl*II, as well as for *Eco*RI, *Hae*III, and *Taq*I.

Degree of A071-1 polymorphism in *Rps1* donors and in *rps1*- and *Rps1*-containing U.S. soybean varieties

The *Rps1* donors, and 24 *rps1*- and 15 *Rps1*-containing U.S. soybean varieties were screened with a10 and a11 for locus A071-1 *Taq*I, *Hae*III, *Eco*RI, and *Eco*RV polymorphisms (Table 2). All polymorphisms observed in the *Rps1* donor germplasms were also present in the U.S. soybean varieties, and no new polymorphisms were detected. However, a new combination of polymorphic fragments was observed for the *Rps1-a* variety, Illini (3.0 kb *Taq*I, 3.8 kb *Hae*III fragments). Comparisons of the polymorphic patterns for varieties with the same *Phytophthora* allele show that A071-1 polymorphisms was not consistent across genotypes. Instead *rps1*- and *Rps1-a*-containing varieties each displayed three polymorphic patterns. Also, no single A071-1 polymorphism, or combination of polymorphisms, characterized either the sensitive or resistant phenotype. Instead, the *rps1*-containing genotypes exhibited the same polymorphic patterns found in *Rps1*-containing varieties. Only two *Rps1-a* genotypes, Mukden and Illini, exhibited unique polymorphic patterns. Tabulation of the polymorphisms present in *rps1* varieties showed that one

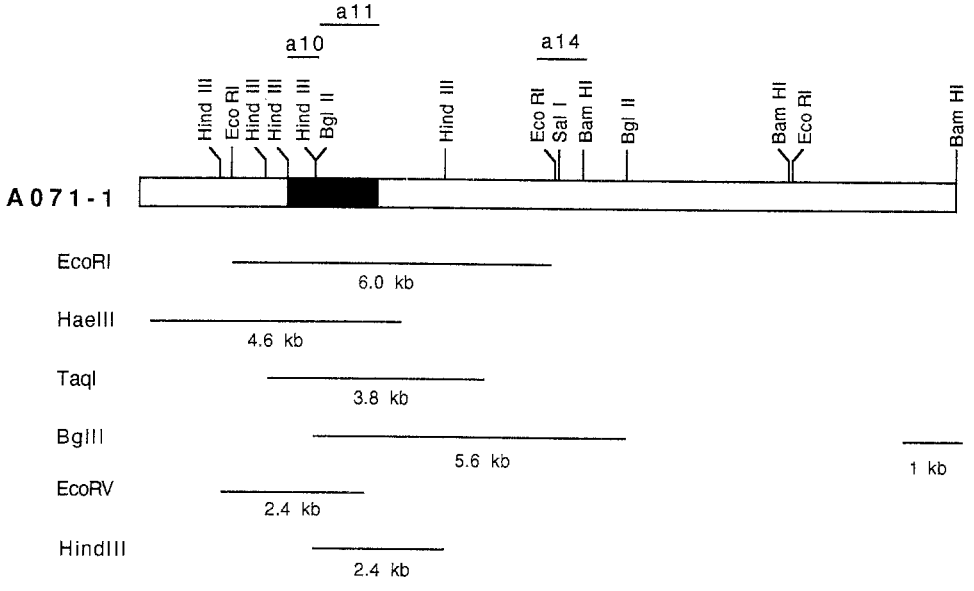

Fig. 3 Restriction map of the region containing the Kingwa A071-1 locus present in two overlapping EMBL3 clones. The location of fragments polymorphic between different *Rps1* donors is indicated by *lines* under the map. The position of the A071-1 locus is indicated by the *black box*. The position of the probes a10, a11, and a14 are indicated by *lines* above the map

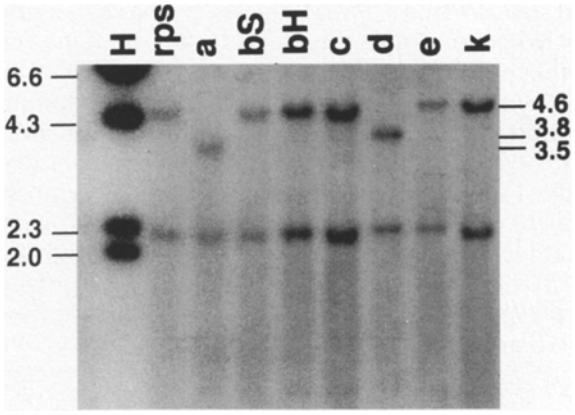

Fig. 4 Screening of *Hae*III-digested *Rps1* donor DNAs with probes a10 and a11. *rps*, Williams; *a*, Mukden (*Rps1-a*); *bS*, Sanga (*Rps1-b*); *bH*, Harrel (*Rps1-b*); *c*, Arksoy (*Rps1-c*); *d*, PI103091 (*Rps1-d*); *e*, PI172902 (*Rps1-?*); and *k*, Kingwa (*Rps1-k*). Size markers are lambda DNA digested with *Hin*dIII (*H*). Numbers to the right are the sizes of the polymorphic fragments in kilobases

polymorphism, or group of polymorphisms, predominated: the 3.0 kb *Taq*I, 2.8 kb *Eco*RI, and 9.5 kb *Eco*RV polymorphisms occurred in 52%, and the 4.6-kb *Hae*III fragment occurred in 79% of the germplasms. This was similar to the frequency observed within the *Rps1* donors: the 3.0 kb *Taq*I, 2.8 kb *Eco*RI, and 9.5 kb *Eco*RV polymorphisms occurred in 57% and the 4.6 kb *Hea*III fragment occurred in 72% of the germplasms. Within the *Rps1* varieties, the frequency was greatly skewed with the 3.0 kb *Taq*I, 2.8 kb *Eco*RI, and 9.5 kb *Eco*RV and the 4.6 kb *Hae*III polymorphisms occurring in 93% of the germplasms. This may be due to the fact that all of the *Rps1-a* varieties except A.K. Harrow and Illini have Blackhawk (which contains these polymorphisms) in their pedigree and potentially obtained their *Rps1-a* allele from this variety (Bernard et al. 1988).

Table 2 also shows that, for all but two genotypes, the polymorphisms detected by three enzymes (*Taq*I, *Eco*RI, and *Eco*RV) were correlated. For example, all germplasms containingg the 3.0 kb *Taq*I fragment also contained the 2.8 kb *Eco*RI and 9.5 kb *Eco*RV fragments. The *Hin*dIII polymorphisms showed a similar correlation with the *Taq*I polymorphisms (data not shown). However, only a partial correlation existed between the polymorphisms detected by *Taq*I, *Eco*RI, *Eco*RV, and *Hin*dIII, and and *Hae*III polymorphisms. For example, although the 3.5 kb *Hae*III polymorphism was found only with the 3.0 kb *Taq*I polymorphism, the 4.6 kb *Hae*III polymorphism was found with both the 3.0 and 3.8 kb *Taq*I polymorphisms, and the 3.8 kb *Hae*III polymorphism was found with both the 4.4 and 3.0 kb *Taq*I polymorphisms. Thus, five different *Taq*I-*Hae*III combinations of polymorphisms were observed: (4.4 kb *Taq*I, 3.8 kb *Hae*III), (3.8 kb *Taq*I, 4.6 kb *Hae*III), (3.0 kb *Taq*I, 4.6 kb *Hae*III), (3.0 kb *Taq*I, 3.8 kb *Hae*III), and (3.0 kb *Taq*I, 3.5 kb *Hae*III). Similar correlations existed for *Hae*III and the *Eco*RI, *Eco*RV, and *Hin*dIII polymorphisms. However, two exceptions to the *Taq*I-*Eco*RI-*Eco*RV correlation were observed: the *Eco*RI poly morphism in Perry (2.8 kb) and all polymorphisms in Corsoy (3.0 kb *Taq*I, 2.5 and 6.0 kb *Eco*RI, and 2.4 kb *Eco*RV). The possibility that these exceptions indicate two new rare polymorphic combinations is under investigation.

Table 2 Polymorphic patterns at A071-1 in germplasms of *Rps1* donors and U.S. soybean varieties (*nd*, not determined)

Susceptible varieties	*Taq*I[a]	*Hae*III[b]	*Eco*RI[c]	*Eco*RV[d]	Resistant varieties	*Rps*I allele	*Taq*I	*Hae*III	*Eco*RI	*Eco*RV
Williams	3	1	3	3	*Rps1 donor germplasms*					
Mandarin	3	1	3	3	Mukden	*a*	3	3	3	3
Manchu	2	1	2	nd	Sanga	*b*	3	1	3	3
Richland	3	1	3	nd	Harrel	*b*	3	1	3	3
Tokyo	3	1	3	3	Arksoy	*c*	2	1	2	2
Lincoln	3	1	3	3	PI103091	*d*	1	2	1	1
McCall	3	nd	3	3	PI172902	*e*	3	1	3	3
Lee	1	2	1	nd	Kingwa	*k*	2	1	2	2
Chippewa	nd	1	3	3	*U.S. varieties*					
Shelby	3	1	3	3	A.K. Harrow	*a*	3	1	3	nd
Kent	2	1	2	2	Illini	*a*	3	2	3	3
Adelphia	3	1	3	3	Blackhawk	*a*	3	1	3	3
Wayne	1	2	1	1	Evans	*a*	3	1	3	3
Amsoy	2	1	2	2	Harosoy 63	*a*	3	1	3	3
Hark	3	1	3	3	Clark 63	*a*	3	1	3	3
Ogden	2	1	nd	2	Century	*a*	3	1	3	3
Patoka	3	1	3	3	Beeson	*a*	3	1	3	3
PI71506	1	2	1	1	Calland	*a*	3	1	3	3
Clark	3	1	3	nd	A1564	*a*	3	1	3	3
Corsoy	3	1	2,3	2	A2943	*a*	3	1	3	3
Harosoy	3	1	3	3	Bonus	*a*	3	1	3	3
Perry	2	1	1	2	BSR201	*a*	3	1	3	3
Essex	1	2	1	1	Pella	*a*	3	1	3	nd
Adams	2	1	2	2	Williams 82	*k*	2	1	2	2

[a] *Taq*I: 1 = 4.4 kb; 2 = 3.8 kb; 3 = 3.0 kb
[b] *Hae*III: 1 = 4.6 kb; 2 = 3.8 kb; 3 = 3.5 kb
[c] *Eco*RI; 1 = 2.5 kb; 2 = 6.0 kb; 3 = 2.8 kb
[d] *Eco*RV: 1 = 2.0 kb; 2 = 2.4 kb; 3 = 9.5 kb

Discussion

This paper reports unusually-high polymorphism at the *Rps1*-linked locus A071-1 in *Rps1* donors and *rps1*- and *Rps1*-containing germplasms of U.S. soybean varieties.

Previous reports have indicated a very low level of polymorphism at most soybean loci. Keim et al. (1989) reported that, of 17 loci screened in 58 accessions, only 2 exhibited more than two alleles, and for those loci that did exhibit three alleles, the third alleles was rare. Doyle and Beachy (1985) studied variation in repeat length and restriction enzyme site location in the genes encoding the 18:25 rDNA gene repeat in both wild progenitor and cultivated soybean. They found no variation in more than 40 accessions and no differences between the two species. In our laboratory, RFLP analysis of genotypes that contributed $>88\%$ of northern soybean germplasm (Delannay et al. 1983; Specht and Williams 1984) showed that only 11 of 217 loci exhibited more than two alleles (L. Lorenzen, unpublished data). In contrast, A071-1 exhibited three alleles for six enzymes and, thus, represents an unusually polymorphic soybean locus.

The reason for the unusually-high polymorphism at locus A071-1 has not yet been determined. Recently, simple sequence repeats (SSR) have also been shown to exhibit a higher-than-normal level of polymorphism in soybean. In this case, polymorphism is generated by the expansion or contraction of a series of di- or tri-nucleotide repeats (Akkaya et al. 1992; Morgante and Olivieri 1993). However, changes in the number of SSR repeats would not seem to account for the polymorphism at A071-1 because such polymorphisms result in a decrease or increase in the size of all restriction fragments encompassing the repeat. Also, the size variation between A071-1 polymorphisms is larger than the observed for SSR alleles (thousands vs tens of bases). That six restriction fragments containing locus A071-1 were simultaneously polymorphic suggests that the polymorphisms are due to a single genetic rearrangement, as was observed previously for two soybean loci by Apuya et al. (1988). However, the *Hae*III polymorphisms did not completely correlate with the polymorphisms detected by the other enzymes, suggesting either that this enzyme detects a second independent mutation at locus A071-1 or that similarly-sized *Hae*III fragments have been produced by different mutations. In the light of the low degree of polymorphism in the soybean genome, these results suggest that there may be something unusual about this region of linkage group N that results in a higher frequency of mutation. It is interesting that the linked locus, *Rps1*, also displays an unusually-high level of polymorphism (seven alleles). Whether the high level of polymorphism at locus A071-1 and *Rps1* is due to the same or different phenomena will require further study.

The discovery that the A071-1 locus near the *Rps1* gene is highly polymorphic extends the ability of soybean breeders to molecularly tag resistance to *Phytophthora* root rot. Whereas previously, only a single *Taq*I polymorphism was reported that allowed distinction between the *Rps1-c*, *Rps1-k* group and the *rps1* polymorphism from Williams (Diers et al. 1992b), the new alleles reported here extend molecular tagging to *Rps1-a* and *Rps1-d* from Mukden and PI103091, respectively. Our results indicate that whereas some donors that contained the same *Rps1* allele also contained the same A071-1 polymorphism (Sanga and Harrel), other genotypes containing the same *Rps1* allele did not (Mukden, Illini, and A. K. Harrow). Therefore, polymorphism at A071-1 may not be useful for identifying a particularl *Rps1* allele across germplasms. However, the partial independence of A071-1 polymorphisms allowed *Rps1* donor germplasms and U.S. soybean varieties to be devided into five groups that differed at locus A071-1. Breeding programs involving parents from any two of these groups could employ molecular monitoring of polymorphism at A071-1 to tag *Rps1* inheritance. Thus, the high degree of polymorphism at locus A071-1 increases the probability the donor (*Rps1*) and recurrent (*rps1*) parents differing at this locus can be identified for marker-assisted breeding programs.

Because locus A071-1 appears to be unusually polymorphic, may be the site of multiple independent mutation events, and is linked to a major disease resistance gene, this region of the soybean genome is one of particular interest. Further study of A071-1 should provide a model system for developing molecular means of tagging disease resistance genes in soybean and also provide some insight into the mutational events that generated germplasm polymorphism and that have shaped the structure of the soybean genome.

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