Identification and sequence analysis of a RAPD marker tightly linked to the *Rsv1* locus in soybean

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Abstract 'Williams' and three near-isogenic lines (isolines) with soybean mosaic virus (SMV) resistance were used to identify a random amplified polymorphic DNA (RAPD) marker linked to the locus conferring resistance to SMV. A RAPD marker was found to be present in Williams and absent in three isolines of Williams with SMV resistance. Three F_2 populations developed from the crosses of Williams x L81-4420, Williams x L92-8151, and Marshall x Kanro were produced to verify the linkage between the fragment and SMV resistance gene. A 1,000 base pair fragment, designated OPN-11_{1000}, generated by the primer OPN-11 was located approximately 1.2 ± 1.2 cM in repulsion phase and 1.8±1.3 cM in coupling phase from *Rsv1*, a gene for resistance to SMV. In a survey of other germplasm, OPN-11_{1000} was found linked with either *Rsv1* or *rsv1*. F_3 families derived from F_2 resistant plants were used to confirm the F_2 genotypes. Using this marker, homozygous and heterozygous SMV resistant plants could be easily distinguished based on the fragment intensity. By cloning and sequencing the fragment, the RAPD marker was converted to a SCAR marker. This SCAR marker can be used reliably for marker-assisted selection of *Rsv1* locus, map-based cloning or further SNP marker development

Key words: Soybean mosaic virus · RAPD marker · SCAR marker · Soybean (*Glycine max* [L.] Merr.) · Linkage

Introduction

Soybean mosaic virus (SMV) is a disease that occurs widely in the soybean (*Glycine max* [L.] Merr.) production areas of the world. SMV can cause loss of yield and degradation of seed quality. Cho and Goodman (1979) classified isolates of SMV present in soybean germplasm into seven strains (G1-G7) based on the reaction of eight soybean cultivars to the virus strains. Among the strains, G1 is the least virulent and G7 is the most virulent. Genetic resistance is an essential part of the control of this disease (Cho and Goodman 1982).

In soybean, resistance to SMV is controlled by independent, single, dominant genes. Four loci for SMV resistance have been reported in soybean (Kiihl and Hartwig 1979; Buzzell and Tu 1984, 1989; Chen et al., 1993; Buss et al., 1995; Hayes et al., 2000). Based on genetic studies of resistance to SMV, Kiihl and Hartwig (1979) assigned the gene symbol *Rsv* (now *Rsv1*) to the allele in PI 96983 (originally from Korea) and rsv-t (now Rsv1-t) to the allele in 'Ogden'. Buzzell and Tu (1984) found a dominant gene that was different from *Rsv1* in crosses involving the SMV resistant Japanese cultivar Raiden (PI 360844) and the Canadian cultivar 'Harcor', and assigned it the gene symbol *Rsv2*. Further study (Buss et al., 1995) indicated that Raiden and L88-8431, a SMV resistant isoline of Williams with Raiden as the donor parent, had an *Rsv1* allele and not *Rsv2*. The source of *Rsv2* is currently unknown, but is presumably from Harcor. Buzzell and Tu (1989) assigned a third locus, *Rsv3*, for stem tip necrosis reaction in the cultivar Columbia. Chen et al. (1991) studied the allelic relationship of SMV resistance in PI 96983, Ogden, 'York', 'Marshall' and 'Kwangkyo' (PI 406710) among which the latter four were used by Cho and Goodman (1979) in classifying SMV strains. They found that SMV resistance in each line had a single dominant gene conferring resistance to the G1 strain. The

alleles in these cultivars were located at a common locus, and the gene symbols *Rsv1-y, Rsv1-m*, and *Rsv1-k* were assigned to the alleles in York, Marshall, and Kwangkyo, respectively. Chen et al. (1993) found two independent loci for SMV resistance in PI 486355 (SS74185 from South Korea), and one of the loci is *Rsv1* and other was referred to as *Rsv4* in the report of Hayes et al. (2000).

The identification of resistance sources and their incorporation into modern cultivars is a major approach in disease resistance breeding. The use of molecular markers can facilitate the identification and pyramiding of resistance genes in cultivar development and it is also a good approach for isolating target genes (Tanksley et al., 1995). Linkages between molecular markers and disease resistance genes have been extensively reported in soybean (Byrum et al., 1994; Yu et al., 1994; Mudge et al., 1997), Using an F2 population from PI 96983 x 'Lee 68', Yu et al. (1994) identified one SSR marker, SM 176, and two RFLP markers, pA186 and pK644a, closely linked to the *Rsv1* locus. *Rsv3* and *Rsv4* loci have been mapped on the linkage group (LG) B2 and D1b of soybean genetic linkage map (Cregan et al, 1999; Hayes et al., 2000; Jeong et al., 2002). Initially, we reported linkage between a RAPD marker, OPN-11_{1000} and *Rsv1* locus (Li et al., 1998) by using near-isogenic lines of Williams and three mapping populations. In 2003, Zheng et al. confirmed the linkage with different mapping populations and associated the RAPD marker with SSR markers on LG-F of the soybean genetic map (Cregan et al., 1999), which is the same region that Yu et al (1994) mapped *Rsv1* allele. In this study, we report an approach of identifying RAPD markers linked to a SMV resistance gene in soybean using near-isogenic lines (isolines) as well as the clone sequence of the fragment.

Materials and Methods

Plant materials

L78-379, L88-8431, and L92-8151 are BC5 derived SMV resistant isolines with the cultivar Williams as the recurrent parent and PI 96983, Raiden (PI 360844), and PI 486355 as the donor parents, respectively (Bernard et al., 1991). All lines identified with a PI prefix are accessions from the USDA Soybean Germplasm Collection housed at the University of Illinois, Urbana, IL. Williams is susceptible to all known strains of SMV. L92-8151 has one of the two dominant resistance genes in PI 486355 (Chen et al., 1993), but it was unknown which resistance gene was transferred.

After a marker putatively linked with the *Rsv1* locus was found using Williams and these isolines, crosses were made between lines L92-8151 and L81-4420 and the susceptible recurrent parent Williams in the greenhouse during the winter of 1995-1996 to confirm this linkage. L81-4420 is an isoline of Williams that has *Rps1-k* conferring resistance to Phytophthora root rot from the cultivar Kingwa in addition to *Rsv1* from PI 96983 (Bernard et al., 1991). F_1 plants from each cross were grown in the field in 1996. The reaction to SMV-G1 was determined for 94 F_2 plants from Williams x L81-4420, and 88 F_2 plants from Williams x L92-8151 in the greenhouse during the winter of 1996-1997 to test the putative linkage between the RAPD fragment and the *Rsv1* locus. The DNA pattern of each individual F_2 plant and four F_1 plants from each cross was characterized with the selected primer. The F_3 families derived from part of the resistant plants in the F_2 populations were used to confirm the genotypes in the F_2 populations during the summer of 1997 either in the greenhouse or an aphid-proof cage in the field.

Thirty-five ancestors of modern U.S. soybean cultivars (Gizlice et al., 1994) as well as selected lines with known resistance and susceptibility to SMV were characterized with the selected primer. Based on these data, the cross between Marshall (carrying the *Rsv1-m* allele with the OPN-11_{1000} fragment) and Kanro (having the *rsv1* allele without the fragment) was made in the summer of 1997 to test if the fragment was linked to the *Rsv1* allele in coupling phase in some lines.

SMV reaction

Inoculum was prepared from fresh leaves of Williams that had been inoculated with the G1 strain of SMV. Infected leaves were ground in a 0.05 M K2HPO4 buffer solution (PH 7.0) with a clean mortar and pestle. Carborundum powder (600 mesh) was added to the inoculum. All the plants of parents, F_1 and F_2 were inoculated by rubbing inoculum on the unifoliolate leaves with a pestle. About two weeks after inoculation, each plant was classified for resistance or susceptibility to SMV. The classification was confirmed ten days later. Ten plants of Williams were inoculated each time to verify the effectiveness of the inoculation.

DNA isolation and RAPD assay

Unifoliolate leaf samples were bulked from 10 plants for each line. For F_1 plants and F_2 populations, leaf samples were taken from each individual plant at the unifoliolate leaf stage before inoculation with SMV. The cetyltrimethylammonium bromide (CTAB) protocol with slight modification was used for DNA isolation (Keim et al., 1988).

Two hundred and fifty decanucleotides of arbitrary sequence obtained from Operon Technologies (Alameda, CA) from the D, E, H, J, K, L, N, O, P, R, S, U, and Y sets were used to screen Williams and the isolines. The polymerase chain reaction (PCR) procedure reported

by Kresovich et al. (1994) was followed with slight modification on a Perkin-Elmer GeneAmp PCR System 9600 or 9700 (Perkin-Elmer Corporation, Norwalk, CT).

DNA samples were standardized to a uniform concentration (10 ng/µl) with a Perkin Elmer UV/VIS spectrometer (Perkin-Elmer Corporation, Norwalk, CT). The 25 µl reaction mix contained 5 µl template DNA, 1 X PCR buffer, 2.5 mM MgCl2, 0.25 mM dNTPs, 0.5 µM primer, and 0.13 µl Taq DNA polymerase. The amplification program consisted of 2 min at 94^0 C followed by 45 cycles of 1 min at 94^0 C, 5 min at 38^0 C and 2 min ramp to 72^0 C, and 2 min at 72^0 C. A final cycle of 72^0 C for 7 min was completed before the reaction mixtures were held at 4^0 C. Amplified products were electrophoresed on 1% agarose gels in 1 X TBE buffer at 96 V for approximately 2.5 hr. The gels were stained with ethidium bromide, viewed under ultraviolet light and photographed. Molecular size of the amplified products was estimated with a 100 bp DNA ladder (GibcoBRL, Life Technologies, Rockville, MD).

Analysis of Linkage

The chi-square statistic was used to test the segregation ratio of phenotypes and marker genotypes for the F_2 populations of the three crosses. The recombination frequency between the fragment and the *Rsv1* locus was determined with the program Linkage-1 (Suiter et al., 1983). For the linkage analysis, heterozygous and homozygous resistant F_2 plants were not distinguished. The F_3 families derived from part of the F_2 resistant plants were used to classify the genotypes of the F_2 plants and verify the heterozygous form of the RAPD fragment.

Cloning and sequencing PCR fragments

The RAPD fragments linked to the *Rsv1* allele were amplified from the cultivar Williams. The PCR fragments were excised from agarose gels, then extracted and purified by using QIAquick Gel Extraction Kit (Qiagene, Valencia, CA). The PCR fragments were used as a template for re-amplification with N11 primer for confirmation of the right fragment. The purified PCR fragments were then directly cloned into pCR®2.1-TOPO vector using TA cloning system (Invitrogen Corporation, Carlsbad, CA). Cloned fragments were sequenced at the Biotechnology Center at the University of Illinois at Urbana-Champaign by using universal-sequencing primers, M13forward (GTAAAACGACGGCCAGT) and M13reverse (AAC AGC TATGACCATG), respectively. Based on the sequence information, the SCAR primers were designed using Oligo 6.0 (Molecular Biology Insights, Inc.). Amplification of genomic DNA with SCAR primers was conducted by following the above protocol. The PCR program consisted of 2 min at 94^0 C followed by 35 cycles of 30 seconds at 94^0 C, 30 seconds at 60^0 C and 30 second at 72^0 C. Amplified PCR products were electrophoresed on 1% agarose gels in 1 X TBE buffer at 96 V.

Results and discussion

Of the 250 primers screened, 12 primers produced a polymorphic fragment among Williams and the isolines L78-379, L88-8431 and L92-8151. One fragment, OPN-11_{1000}, amplified by primer sequence 5`-TCGCCGCAAA-3`, was present in Williams and absent in the three isolines. This indicates a possible association between the marker and the SMV resistance

allele in repulsion phase. Only 12 of the more than 1000 fragments scored were polymorphic indicating a high degree of homogeneity among Williams and these isolines.

F_2 populations from two crosses between Williams and the isolines L81-4420 and L92-8151 were used to test the cosegregation between OPN-11_{1000} and the locus conferring SMV resistance. When all plants from the F_2 populations were scored for the presence or absence of the OPN-11_{1000} fragment, a less intense fragment was found to be present in some SMV resistant individuals (Fig. 1). This faint fragment occurred in approximately two-thirds of the SMV resistant plants. To verify if the F_2 individuals with a faint fragment were heterozygous, DNA from four F_1 plants of each cross was assayed for the fragment. All F_1 plants expressed a faint fragment similar to the one in the F_2 populations. Therefore, the faint fragment was considered to be caused by individuals heterozygous at the *Rsv1* locus. All plants in the F_2 populations were classified as having an intense, faint or absent OPN-11_{1000} fragment. The analysis of the F_2 data revealed that the SMV reaction phenotypes fit a 3 resistant: 1 susceptible ratio (Table 1) and the OPN-11_{1000} fragment fit a 1 intense: 2 faint: 1 absent ratio (Table 2). Linkage analysis of F_2 data of both crosses revealed that the RAPD fragment, OPN-11_{1000}, and *Rsv1* were closely linked (1.2±1.2 cM) in repulsion phase (Table 3). To verify the linkage between the fragment and the locus, and the relationship between the faint fragment and heterozygousity, 59 F_3 families derived from SMV resistant F_2 plants were tested for phenotypic segregation of SMV resistance. The result confirmed that plants with the faint fragment were heterozygous (data not shown).

Thirty-five ancestors of modern U.S. soybean cultivars (Gizlice et al., 1994) and some cultivars with known resistance to SMV were classified for the presence or absence of the OPN-11_{1000} fragment (Table 4). In five of 11 resistant cultivars, the fragment was present. In 13 of 31 susceptible cultivars, the fragment was absent. These data indicate that crossovers between the fragment and the *Rsv1* locus are present in these lines. The result was similar to the report by Yu et al. (1994) using RFLP and SSR markers. Yu et al. (1994) reported that the susceptible cultivar Williams had the same size SSR fragment linked to the *Rsv1* locus (0.5 cM) as three resistant isolines (L78-379, L81-4420, and L84-2112), PI 96983, and Marshall. L83-529, L88-8431, 'Buffalo', 'Dorman', and Raiden had different sizes of fragment from above lines. In our research, however, the OPN-11_{1000} fragment was present in Marshall and Williams, and absent in L78-379, L81-4420, L88-8431, and PI 96983 (Table 4).

Based on the results from the assay of the U.S. soybean ancestral lines, the cross of Marshall x Kanro was made. The linkage of a marker to a resistance gene in coupling phase may be more useful for marker-assisted selection. The results from this population were consistent with the previous data. The intense, faint, and absent fragment pattern fit a 1:2:1 ratio (Table 2, Fig. 2). The F_2 phenotypic data of SMV resistance also fit a 3 resistant: 1 susceptible ratio (Table 1). Linkage analysis indicated that the RAPD fragment, OPN-11_{1000}, was also closely linked (1.8 ± 1.3 cM) in coupling phase with the *Rsv1* locus (Table 3).

Previous research has reported that RAPD markers are dominant (Haley et al., 1993; Oh et al., 1994; Poulsen et al., 1995; Myburg et al., 1998) and there has been no report of a less intense fragment from a heterozygous locus. In this study, homozygous and heterozygous

individuals could be easily distinguished based on the intensity of the fragment. The probable reason is that the heterozygous individual only has one copy of the amplifiable region near the *Rsv1* locus. Therefore, after the PCR amplification, the heterozygous individuals will have only half the amount of amplified DNA product compared to the homozygous individuals.

The location of this RAPD marker at less than 2 cM from the *Rsv1* locus would make it effective in marker-assisted selection and map-based cloning for SMV resistance gene. The finding of heterozygous form of the fragment may also be useful as a reference for other dominant RAPD markers linked to the genes or QTL in marker-assisted selection. The fact that the OPN-11_{1000} fragment has an allelic frequency of approximately 0.5 in both the SMV susceptible and resistant lines that were characterized could complicate the use of this marker in some situations but it will also facilitate finding two parental lines that are polymorphic for this fragment.

In order to develop a reliable and specific marker for *Rsv1* locus, the RAPD marker was converted into a SCAR marker. The RAPD fragment, OPN-11_{1000}, was amplified from both Williams and Marshall using OPN11 primer. After the PCR fragments were excised from agarose gels and purified, the re-amplification with N11 primer confirmed that anticipated fragments were excised from gels. Only the fragment amplified from Williams was used for further cloning for SCAR marker development. Five clones containing the anticipated insert of the PCR fragment were sequenced bi-directionally by using M13 forward and M13 reverse primers, respectively. By aligning all clone sequences, a consensus sequence was generated (Fig. 3), which actually consisted of 992 bp. The sequence has been deposited into Genebank with ID AY547310 (http://www.ncbi.nlm.nih.gov)

Based on the clone sequence, the SCAR primers were designed as 1U20-Forward-Primer (5'- TCGCC GCAAA CTCAC AGGAC-3') and 970L23-Reverse-Primer (5'- TCGCC GCAAA TTAAA GATCT TCA-3'). Using SCAR primers, PCR was performed with seven soybean lines (Table 5), which are the parents of the mapping populations. With this SCAR marker, amplicons were generated for all of those soybean lines and two marker alleles were observed. One marker allele with approximately 992 bp was generated from Williams (susceptible) and Marshall (resistant), and another allele approximately 1050 bp was generated from L81-4420, L78-379, L88-8431 and Kanro (Fig. 4 and Table 4). It can be inferred that this SCAR marker became a codominant marker after conversion, which could allow us to easily select *Rsv1* heterozygous individuals in breeding selection process.

Soybean isolines have been widely used to study the effects of specific genes and to identify the linkage between DNA markers and the transferred gene. In this study, the SMV resistance genes in three isolines from different sources did not have the OPN-11_{1000} fragment, but it was present in the recurrent parent Williams. L78-379, L88-4341, and L92-8151 all have a SMV resistant allele at the *Rsv1* locus. Based on these data the allele for SMV resistance transferred from PI 486355 to L92-8151 is at the *Rsv1* locus. Other sources have been identified in which the OPN-11_{1000} fragment is linked in coupling phase to SMV resistance alleles.

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Table 1. Reaction of F_2 plants from three crosses to strain G1 of soybean mosaic virus.

Cross	F2 plants[a]		Expected ratio	$\chi 2$ (3:1)	P value
	Resistant	Susceptible			
	no.				
Williams x L81-4420	68	24	3:1	0.23	0.63
Williams x L92-8151	64	24	3:1	0.14	0.71
Marshall x Kanro	76	31	3:1	0.70	0.40

[a] Plants were inoculated and classified in the greenhouse.

Table 2. Segregation of the OPN-11_{1000} fragment in three F_2 populations.

Cross	F_2 Plants	Marker genotype			χ^2 (1:2:1)	P value
		MM	Mm[a]	mm		
	no.					
Williams x L81-4420	84	23	40	21	0.29	0.59
Williams x L92-8151	84	21	43	20	0.07	0.79
Marshall x Kanro	107	25	53	29	0.17	0.68

[a] The Mm genotype had the faint fragment phenotype.

Table 3. Chi-square test for independent segregation of *Rsv1* and OPN-11_{1000} and recombination frequencies in F_2 populations.

Cross	Plant genotype	Marker genotype			χ^2	Recombination frequency
		MM	Mm[a]	mm		
		no.				
Williams x L81-4420	*Rsv1*____	0	39	21	79.23[b]	1.16±1.17
	rsv1rsv1	23	1	0		
Williams x L92-8151	*Rsv1*____	0	42	20	78.94[b]	1.17±1.18
	rsv1rsv1	21	1	0		
Marshall x Kanro	*Rsv1*____	25	51	0	97.65[b]	1.79±1.29
	rsv1rsv1	0	2	29		

[a] The Mm genotype had the faint fragment phenotype.

[b] Significant at the 0.01 level of probability

Table 4. Reaction to strain G1 of soybean mosaic virus (SMV) and the form of the OPN-11$_{1000}$ fragment in 35 U.S. soybean ancestors of modern cultivars and other selected lines.[a]

Entry	Reaction to SMV (G1)	OPN-11$_{1000}$	Entry	Reaction to SMV (G1)	OPN-11$_{1000}$
FC 31745	S[b]	0[c]	Jackson	S	1
PI 180501	S	0	Korean	S	1
PI 360955B	S	0	Lee	S	1
PI 438471	S	0	Lincoln	S	1
PI 438477	S	0	Manitoba Brown	S	1
Bansei	S	0	Perry	S	1
Dunfield	S	0	Ralsoy	S	1
Jogun	S	0	Rampage	S	1
Kanro	S	0	S-100	S	1
Mandarin (Ottawa)	S	0	Williams	S	1
Mukden	S	0	FC 33243	R	0
Richland	S	0	PI 71506	R	0
Roanoke	S	0	PI 88788	R	0
PI 80837	S	1	PI 96983	R	0
PI 240664	S	1	CNS	R	0
A.K. (Harrow)	S	1	Peking	R	0
Arksoy	S	1	Haberlandt	R	1
Capital	S	1	Marshall	R	1
Flambeau	S	1	Ogden	R	1
Illini	S	1	Tokyo	R	1
Improved Pelican	S	1	York	R	1

[a] Data of reaction to G1 of SMV for some of U.S. soybean ancestors in this table are from Wang (1996)

[b] R = resistance and S = susceptibility to G1 strain of SMV

[c] 1 = presence and 0 = absence of fragment

Table 5. Estimated marker allele size amplified with the SCAR marker for *Rsv1* among parents of the mapping populations used in this research.

Line	Fragment size (Estimate based on size standard)
L81-4420	1050
L78-379	1050
L88-8431	1050
Williams	992
Marshall	992
Kanro	1050

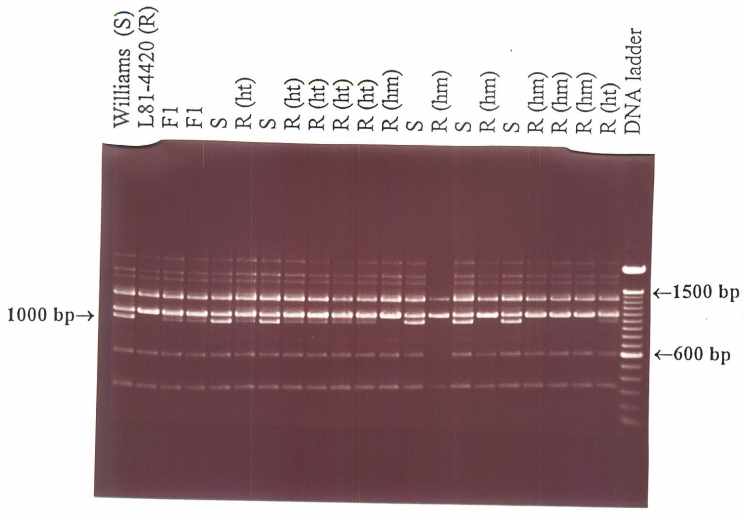

Fig. 1 PCR amplification products from the primer OPN-11 for the parents, and F_1 and F_2 plants from the cross of Williams and L81-4420 (linkage in repulsion phase). R = resistant; S = susceptible; ht = heterozygous genotype at the *Rsv1* locus; hm = homozygous genotype at the *Rsv1* locus.

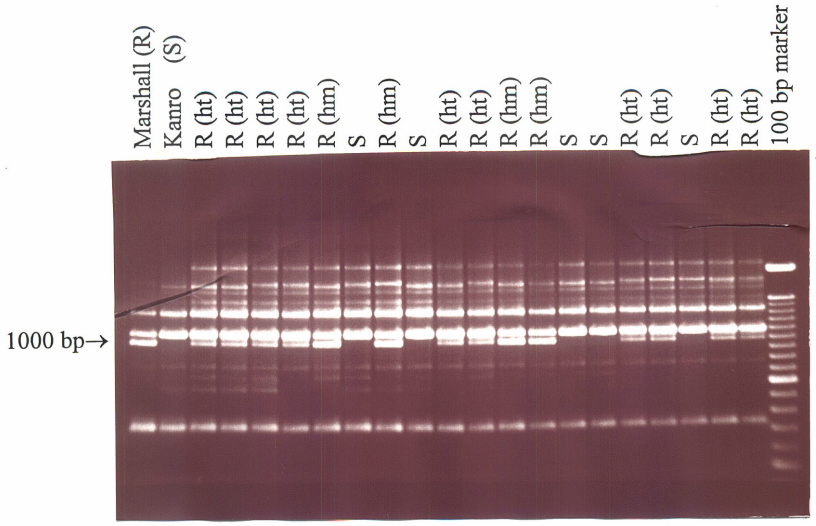

Fig. 2 PCR amplification products from the primer OPN-11 for the parents and F_2 plants from the cross of Marshall and Kanro (linkage in coupling phase). R = resistant; S = susceptible; ht = heterozygous genotype at the *Rsv1* locus; and hm = homozygous genotype at the *Rsv1* locus.

Fig. 3. Consensus sequence of the amplified fragment from Williams with OPN11, which is tightly linked to *Rsv1* locus in soybean

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TCGCC GCAAA CTCAC AGGAC TTGGG GACAT GGCTT TTCAG GGTCA ACAAC          50
GACTT TTGCC TTTTT AAAAA CGAAA AAACA AAAAA TACAA TACAA CTTGT          100
ACAGA TAAAG CCATT TATGT AAATT GTGAC TTTCA ATTCA ATCTA TAAAA          150
TTCTT CAACA GGTCA ATAGT CACAC ATCTT TAACC ATCCA AATTA TTTAT          200
TATAG ATCCA AAGGC TATTA AATAT CACAA TCATA CCTAA ACCAC ATGCT          250
GCAAA GTTTA AAAAC AATAT GTATT GCTTT GTACA CAGGT GAATC ATTTA          300
ATAGA TGATT CACCT TAGAA TTTAC CTATT TAATT AAATG GTTTC CCATC          350
CCCCT CCCTT CCCCT CTTTT CTTCC TAATC TATTT AAATT TTTAA ATTAA          400
ATAAA ATAAT TCTTT ATTAC CCAGT TTTAA AGTTT CTTTG GATTG GTCAT          450
GCTCT ATCAA TGAAG TAAAA GTTAA TACGA TAGAA GAGGA AAATG CCCCA          500
CATGC ACAAT CTAAT TTCCA CAAAT AAAGA AAAAT AAAAA TAATG AAAAG          550
AACCA CACTT CAAAA GTCAT GAAAT CAATA TGAAA ATGAT ACACA ATTTT          600
TGTAT AAGAG AACAT GAAAT TAAAA AAAAA AACAT ACAAT AAGGG AAGTT          650
TATAT ATATT ATCCT AATTG CCAAA TCATC TCTCG CAAGT GCAAG GTTGG          700
GAGGA AGCAG ACGCC ACTTC GTTGT GACAA CAACT TAATG GTGGA AATTA          750
AAGAG GCTAG AGGTA GTTTG TGCAT AAAGG CTTTA TGCGT AGCAG ATAGG          800
AAATT CTTAT TGGAT AATTA TGTTT TTATT TCTTG AATTT TCATT TTTAA          850
TCTTT AAATA AAAAA TTCAC TGATC TTGAT TCTTA GATGT TTTCA TCCGT          900
TAACA TTTTT AGTCT CTTTC ATCAA ATGCA CCATT AGTTG ATGAT GTGAC          950
GATTG ATAGG AGGGC CACGT GAAGA TCTTT AATTT GCGGC GA                   992
```

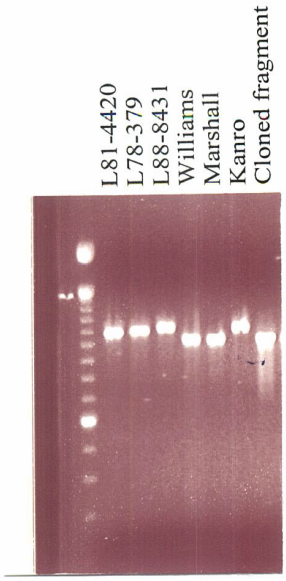

Fig. 4 PCR amplification products using the primers of SCAR marker for the parents of mapping populations. Two marker alleles were observed among five soybean lines with the SCAR marker