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Identification of Soybean Mosaic Virus Resistant Soybean Genotypes using Gene-Linked Markers

Manu Maya Magar[1*], Ramesh Acharya[1], Laxman Aryal[2], Rajendra Darai[3], Resham Babu Amgai[1] and Jiban Shrestha[4]

[1]National Biotechnology Research Centre, Khumaltar, Lalitpur, Nepal
[2]National Wheat Research Program, Bhairahawa, Rupandehi, Nepal
[3]National Maize Research Program, Rampur, Chitwan, Nepal
[4]National Plant Breeding and Genetics Research Centre, Khumaltar, Lalitpur, Nepal
*Corresponding author's email: manu.bdnarc@gmail.com
*ORCID: 0000-0002-4140-8999

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ABSTRACT

Soybean is widely consumed legumes both in the form of food and feed. In the present scenario, the quality and quantity of soybean production is largely affected by different biotic factors, Soybean Mosaic Virus being one of the major biotic stress. Plant host resistance is an effective major to develop durable resistance from viral diseases, therefore understanding the molecular mechanism and identifying the resistant genotypes can contribute to resistance breeding in soybean. In 2020, ninety genotypes collected from Grain Legume Research Program, local collection and National Agricultural Genetic Resource Center were screened for the presence of resistant genes using gene linked molecular markers. Among them, three genotypes were identified with Soybean Mosaic Virus resistant loci, Rsv1 amplified by linked marker Satt114, four genotypes were identified with resistant loci, Rsv3 with linked marker Sat_424, and eight genotypes were identified with resistant loci, Rsv4 amplified by linked marker Satt558. Thus, showing expression of dominant resistance to some SMV strains. Interestingly, two genotypes (Co 175 and TGx1990-55) possessed both *Rsv1* and *Rsv3* loci thus showing potential of resisting broad spectrum SMV resistance to all SMV strains. Therefore, this study has identified potential genotypes that can be used as parental materials for resistant gene pyramiding in soybean breeding for SMV resistance.

Keywords: Soybean, Soybean mosaic virus, Host resistance, Molecular markers

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INTRODUCTION

Soybean (*Glycine max* L., 2n =2x = 40) is grown widely around the globe for its oil and protein. In Nepal it is third important grain legumes after lentil and black gram, which is cultivated from mid hills to terai and inner terai region of the country at elevation ranging from 500 to 1500 masl. It is commonly used for food as tofu, soya milk, nugget, roasted beans or pulse splits. Recently, it has gained popularity in poultry industry as a source of protein for poultry feed, thus motivating farmers to produce more soybean grains and grown them as monocrop. It is grown in 25,758 ha producing 32,178 Mt. with a productivity of 1.25 Mt ha^{-1} (MOALD 2021). Beside, soybean oil is one of the major import commodity in Nepal (4628 Million rupees) (MOALD 2021), which poses an urgent need to increase soybean production to substitute import in soybean products and fulfill the demand of poultry industry. However, the quality and quantity of soybean grain production is affected by various biotic factors including soybean mosaic virus (SMV) as a major constraint (Poudel and Khanal 2018, Darai et al 2016). SMV is endemic in most of the soybean growing areas causing yield losses of up to 90%, reducing nitrogen fixation, seed size, and quality under sever outbreaks (Wang et al 2001).

SMV is *Potyvirus* commonly transmitted to soybean through infected seeds, aphid vector or via tissue damage (Usovsky et al 2022). It causes mottling, leaf curling, green vein banding, stunting, reduction of pods at maturity leading to seed coat mottling, reduced seed number, and seed size (Gunduz et al 2004). At the same time, effect of G x E in the disease response of genotypes is huge. The use of naturally occurring plant host resistance has been considered as most effective in controlling viral diseases in plants (Wang et al 2001). Therefore, identification of genetic resistance and pyramiding multiple resistant genes can overcome these constraints (Shi et al 2008).

Identification of resistant genotypes and understanding the host resistance mechanism in soybean is essential to breed for SMV resistance. SMV isolates have been classified into seven strains as G1 to G7, where G1 is least virulent and G7 is most virulent (Cho and Goodman 1979.). At the same time, the host resistance in soybean is controlled by four nuclear genes identified as four independent loci, *Rsv1*, *Rsv3*, *Rsv4* and *Rsv5* (Cho and Goodman 1979, Widyasari et al 2020). Molecular markers linked to resistant genes are useful tools for identification and selection of specific resistant genes in soybean genotypes, which can be used for gene-pyramiding in resistance breeding (Usovsky et al 2022). Majority of SMV resistant genotypes carry single resistance genes (Karthikeyan et al 2018), however marker assisted pyramiding of multiple genes provides additional benefit to cope with wide range of SMV strains (Wang et al 2017, Shi et al 2009, Gunduz et al 2004). However, Nepalese soybean genotypes have yet to be explored for its genetic resistance to SMV. Therefore, in this study, we have used molecular markers linked to SMV resistant genes to identify SMV resistant soybean genotypes from soybean gene pool available in Nepal, thus they can be used to develop SMV resistant varieties through marker assisted resistance breeding.

MATERIALS AND METHODS

Collection of genotypes

A total of 90 soybean genotypes were collected from Grain Legume Research Program, Nepalgunj; National Plant Breeding and Genetic Research Center, Khumaltar; and National Agriculture Genetic Resources Center, Khumaltar and local growers in 2020 (Table 1).

Table 1. List of soybean genotypes collected from different sources for this study.

S.N.	Genotypes	Source of collection	S.N.	Genotypes	Source of collection
1	G-7959	GLRP	46	PUJA	GLRP
2	Vio (Blpuro)	GLRP	47	SBO-115	GLRP
3	TGx1889-11F	GLRP	48	G-8754	GLRP
4	TGx1989-68FN	GLRP	49	F778817	GLRP
5	SBO 103	GLRP	50	COLL#5SIURE	GLRP
6	TGx1993-4FN	GLRP	51	TGx1990-21F	GLRP
7	TGx1990-18F	GLRP	52	Chatewan-9	GLRP
8	G-4508	GLRP	53	TGx1989-41F	GLRP
9	Co 175	GLRP	54	TGx1990-10F	GLRP
10	G-1871	GLRP	55	TGx1990-55F	GLRP
11	TGx1991-78F	GLRP	56	TGx1991-10F	GLRP
12	TGx1995-5FN	GLRP	57	G758	GLRP
13	C 2017	GLRP	58	TGx1988-3F	GLRP
14	IARS 87-1	GLRP	59	IPBSY-178	GLRP
15	TGx1935-10F	GLRP	60	CO 169	GLRP
16	TGx1987-42F	GLRP	61	TGx1989-20F	GLRP
17	Cina-2	GLRP	62	TGx1903-1	GLRP
18	CO 164	GLRP	63	TGx1805-31F	GLRP
19	TGx1990-51F	GLRP	64	TGx1990-3F	GLRP
20	TGx1989-21F	GLRP	65	TGx1987-62F	GLRP
21	NGRC16	NAGRC	66	Co 176	GLRP
22	GC8234GC13	GLRP	67	TGx1990-4JF	GLRP
23	AGS-367	GLRP	68	TGx1925-1F	GLRP
24	TGx1990-5F	GLRP	69	TGx1990-97F	GLRP
25	G-1873	GLRP	70	G-8586	GLRP
26	G-757	GLRP	71	NGRC34	NAGRC
27	AGS 378	RARS, Lumle	72	AGS371	GLRP
28	Lamjung Local-2	Besisahar, Lamjung	73	TGx1904-4F	GLRP

S.N.	Genotypes	Source of collection	S.N.	Genotypes	Source of collection
29	TGx1987-86F	NPBGRC	74	TGx1990-57F	GLRP
30	Tompomas	NPBGRC	75	TGx1987-62F	GLRP
31	Tanahu Seto	Dumritar, Tanahu	76	272W	GLRP
32	TGx1890-114FN	GLRP	77	TGx1890-124FN	GLRP
33	TGx1987-10F	GLRP	78	P1368055	GLRP
34	200525 (Rampur)	GLRP	79	V5(Blpur-5)	GLRP
35	Kavre	GLRP	80	NGRC82	NAGRC
36	PI94159	GLRP	81	NGRC5	NAGRC
37	CN9125	GLRP	82	NGRC1	NAGRC
38	TGx1485-1D	GLRP	83	NGRC60	NAGRC
39	TGx1890-106FN	GLRP	84	NGRC88	NAGRC
40	LS77-16-1	GLRP	85	NGRC64	NAGRC
41	PK739	GLRP	86	NGRC75	NAGRC
42	TGx1987-10F	GLRP	87	NGRC67	NAGRC
43	TGx1990-40F	GLRP	88	NGRC72	NAGRC
44	CO163	GLRP	89	NGRC73	NAGRC
45	TGx1990-38F	GLRP	90	NGRC81	NAGRC

DNA extraction

Seed of collected genotypes were germinated in sterile soil in laboratory condition and sampled for genomic DNA extraction at 3 to 4 leaf seedling stage. Fresh leaves from three seedlings were bulked from each genotypes and DNA was extracted using modified CTAB method (Doyle 1991) to obtain higher quantity and purity of DNA. The extracted DNA were resuspended in 1X TE buffer and quantified using 1% agarose gel electrophoresis as well as UV-UIs spectrophotometer (Quawell, Q5000).

PCR amplification and gel electrophoresis

The DNA solution of each genotypes were amplified with gene-linked primers (Table 2) in Mygene L series thermo-cycler (Long-Gene scientific instrument co. LTD). The reaction mixture contains about 50-60 ng of template DNA, 2x master mix (Promega Corporation, USA), 0.5 µM of each Primer (Macrogene Inc South Korea), with additional 2mM $MgCl_2$ for specific amplification (Himedia laboratories Pvt. Ltd, India), 0.2mM dNTP Mix (Promega Corporation, USA), 0.2U of Taq polymerase (Promega Corporation, USA). The PCR cycle was programmed as an initial denaturation step of 4 min at 94 ℃, followed by 35 cycles at respective annealing temperature of each primer (Table 2) for 45 sec, extension at 72 ℃ for 35 sec, followed by final extension at 72 ℃ for 5 min.

The PCR products obtained from each cycle were evaluated in 1.5% agarose gel using 1X TAE buffer (40 mM Tris-acetate, 2 mM EDTA at pH 8.5) and run for 90 min at 80 V with 100 bp and 50 bp DNA ladder (Invitrogen). The amplified fragments were stained with Ethidium bromide and visualized in Gel Documentation System (VWR®Genosmart 2, UK).

Table 2: List of molecular markers linked to SMV resistant genes with linked genes and their chromosomal location.

S. N.	Gene	Chromosome	Primer	Forward sequence	Reverse Sequence	Tm (°C)	Product size (bp)	References
1	*Rsv1*	13	Satt114	GGGTTATCCTC CCCAATA	ATATGGGATG ATAAGG	63.2	145	(Shi et al 2008)
2	*Rsv3*	14	Sat_424	CAACCTGTATT CCACAAAAAA TCTCACC	GCGCCCCAAT TTGACTATAA ATAA	63.6	200	(Shi et al 2008)
3	*Rsv4*	2	Satt558	CTCACACCCTT TCATTATCTA	AAATCGCGCA TCTAAATT	62.4	230, 240	(Hayes et al 2000)

RESULTS

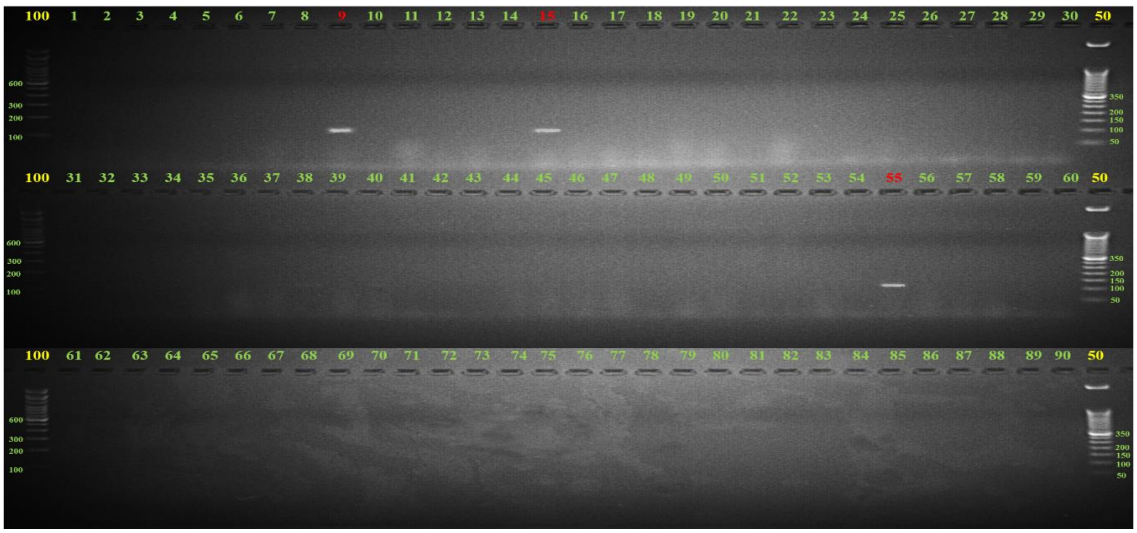
Molecular maker analysis in 90 Nepalese soybean genotypes showed a distinct pattern of resistance at genetic level (Figure 1). Among 90 genotypes only three genotypes (Co 175, TGx1935-10F, and TGx1990-55) amplified SSR marker Satt114 linked to *Rsv1* gene showing banding pattern of size 145 bp (Figure 1a). Similarly, four genotypes (Co 175, LS77-16-1, TGX1990-55, and Co 176) amplified Sat_424 marker linked to

Rsv3 gene showing banding pattern of size 200 bp (Figure 1b). Beside eight genotypes (TGX1990-18F, IARS 87-1, GC8234GC13, PI94159, TGX1485-1D, PUJA, F778817, and TGX 1805-31F) amplified Satt558 linked to *Rsv4* gene showing bands at 230-240 bp. Interestingly, two genotypes (Co 175, and TGX1990-55) were found to have combination of both *Rsv1* and *Rsv3* genes (Table 3).

Table 3: Nepalese genotypes carrying different SMV resistant genes

Primer	Gene loci	Genotypes	Number of genotypes
Satt114	*Rsv1*	Co 175, TGx1935-10F, TGx1990-55	3
Sat_424	*Rsv3*	Co 175, LS77-16-1, TGx1990-55, Co 176	4
Satt558	*Rsv4*	TGx1990-18F, IARS 87-1, GC8234GC13, PI94159, TGx1485-1D, PUJA, F778817, TGx1805-31F	8
Satt114 + Sat_424	*Rsv*1+*Rsv3*	Co 175, TGx1990-55	2

a



b

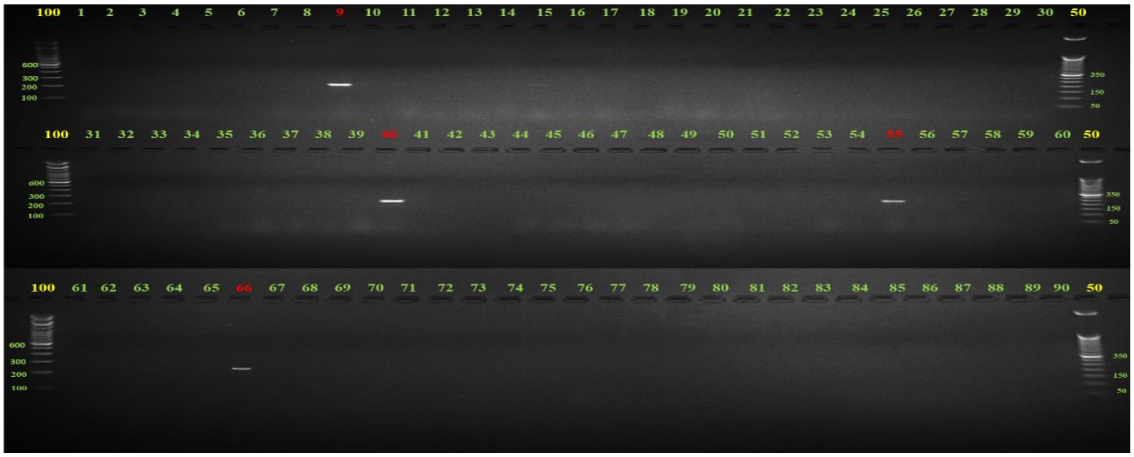


Figure 1: Agarose gel electrophoresis distinguishing the tolerant and susceptible lines identified by molecular markers (a) Satt114 linked to *Rsv1* gene and (b) Sat_424 linked to Rsv3 gene. The tolerant genotypes number are marked with red font color and susceptible genotype numbers are marked with green font color. The 50bp and 100bp DNA ladders used are marked with yellow font color at both edges of gel picture

DISCUSSION

Soybean has long been a popular crop consumed widely for its high protein content and good quality oil, along with additional variation in its food products. SMV infection is major virus disease affecting soybean production. It is transmitted rapidly by aphid significantly affecting seed quality and quantity (Steinlage et al 2002). Host resistance to SMV has been widely studied using molecular markers, which revealed the SMV

resistance had been conferred by single dominant gene in many studies (Karthikeyan et al 2018). However combining multiple resistant genes can resist multiple or all virus strains (Wang et al 2017, Usovsky et al 2022, Shi et al 2009, Gunduz et al 2004). *Rsv1*, *Rsv3*, *Rsv4* and *Rsv5* are major SMV resistant genes in Soybean. Therefore, identifying the possibility of detecting resistant gene in Nepalese soybean genotypes using molecular markers is essential to enhance resistance breeding in soybean. Molecular marker study identified three SMV resistant genes in different Nepalese soybean genotypes.

Among 90 soybean genotypes studied, *Rsv1* gene loci was detected in three genotypes (Co 175, TGx1935-10F, and TGx1990-55) amplified by Satt114 marker (Table 3 and Figure 1a). SSR marker Satt114 is tightly linked to *Rsv1* gene at genetic distance of 4.3 cM (Shi et al 2008). Similarly, independent *Rsv1* resistant alleles have also been reported in many soybean cultivars conferring resistance to SMV (Tucker et al 2009, Moon et al 2009), which can confer resistance to G1-G3 strains (Chen et al 1991). In addition, *Rsv1* loci in PI 96983 genotype was found resistant to all the SMV strains except strain G7 (Klepadlo et al 2017), which suggests that three genotypes identified with *Rsv1* in this study (Table 3 and Figure 1a) has potential to resist most of the SMV strains. *Rsv1* was first SMV resistant loci identified and mapped on chromosome 13 (Kiihl and Hartwig 1979). It is also most common SMV resistance loci found in soybean, which contains at least 10 alleles and confer resistance to only one or few SMV strains (Klepadlo et al 2017). Besides, the *Rsv1* has several alleles, which are expressed differently in different genotypes and exhibit resistance either due to complete or partial dominance to some SMV strains. Also genetic studies have revealed that alleles in homozygous condition are dominant while in heterozygous condition are necrotic (Chen et al 1994). However, differential resistance expressed in different genotypes shows significant effect of genetic background of genotype on expression of resistant loci.

Rsv3 was first identified as dominant gene in OX686 with necrotic effect to G1 strain of SMV (Tu and Bussell 1987), and it contains at least six alleles and was mapped on chromosome 14 (Jeong et al 2002). In this study, *Rsv3* was detected in four genotypes (Co 175, LS77-16-1, TGX1990-55, and Co 176) among 90 genotypes screened for SMV resistance amplified by Sat_424 marker (Table 3 and Figure 1b). The molecular marker Sat_424 is closely linked to *Rsv3* loci at genetic distance of 1.5 cM in J05 soybean genotype (Shi et al. 2008). *Rsv3* detected in soybean genotypes; L29, Ox686, and Harosoy conferred resistance to more virulent SMV strains G5-G7, while susceptibility to less virulent SMV strains G1-G4 (Buss et al 1999, Buzzell and Tu 1989, Gunduz et al 2004, Maroof et al 2010). This shows that *Rsv3* containing genotypes can be potential resistant genotypes for more virulent SMV strains.

In this study, *Rsv4* was detected in eight genotypes (TGX1990-18F, IARS 87-1, GC8234GC13, PI94159, TGX1485-1D, PUJA, F778817, and TGX 1805-31F) among 90 genotypes showing double bands at 230 bp and 240 bp amplified by Satt558 marker (Table 3). Initially, it was identified in PI 486355 (Ma et al 1995) and observed to confers broad spectrum dominant resistance to all SMV strains (Chen et al 1993, Ma et al 1995, Buss et al 1997, Gunduz et al 2004). In addition, *Rsv4* was also identified as dominant SMV resistant gene in V94-5152, PI 88788, and Beeson (Shakiba et al 2013, Buss et al 1997, Ma et al 2002). It is mapped on chromosome 2 and is tightly linked to Satt558 marker at genetic distance of 7.8 cM (Hayes et al 2000). This shows that eight soybean genotypes identified with Rsv4 genes in this study are potential candidates to confer broad spectrum resistance to all SMV strains.

Most of the SMV resistance in soybean are governed by single resistant gene (Usovsky et al 2022). However, multiple resistant gene combinations can reduce the vulnerability of different SMV strains posing resistance breakdown and can contribute to durable and broad-spectrum resistance. We have also identified two genotypes (Co 175 and TGx1990-55) carrying both *Rsv1* and *Rsv3* genes in this study (Table 3). Similarly, combination of *Rsv1* and *Rsv3* conferring resistance to all the SMV strains with complementary reaction has been reported in different studies (Gunduz et al 2002, Zheng et al 2006, Shi et al 2008). This shows, that these two genotypes with *Rsv1Rsv3* SMV resistant gene combination can resist all SMV strains thus providing complete resistance against SMV in soybean.

CONCLUSION

Plant host resistance is well known to control virus resistance in plants. At the same time host resistance contributed by combination of multiple resistant genes can enhance durability and coverage of multiple virus strains. Marker assisted pyramiding of SMV resistant genes, *Rsv1* linked to Satt114 marker in Co 175, TGx1935-10F, and TGx1990-55; *Rsv3* linked to Sat_424 marker in Co 175, LS77-16-1, TGx1990-55, and Co 176; and *Rsv4* linked to Satt558 marker in TGx1990-18F, IARS 87-1, GC8234GC13, PI94159, TGx1485-1D, PUJA, F778817, and TGx1805-31F, identified in this study are potential genotypes to be used in gene pyramiding for SMV resistance. In addition, two genotypes (Co 175 and TGx1990-55) with both *Rsv1* and *Rsv3* can be utilized both as resistant parent in SMV resistant breeding as well as developed as SMV resistant variety

following rigorous phenotyping and characterization of yield related traits. Thus this study has identified SMV resistant genotypes in Nepalese soybean gene pool and provided a strong foundation for SMV resistance breeding in soybean.

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AUTHORS' CONTRIBUTION

Manu Maya Magar: Conceptualized, designed and conducted experiment; wrote the draft and manuscript
Ramesh Acharya: Wrote the draft and manuscript
Laxman Aryal: Collected the germplasm
Rajendra Darai: Conceptualized the experiment and collected germplasm
Resham Babu Amgai: Data interpretation
Jiban Shrestha: Manuscript revision and finalization.

CONFLICTS OF INTEREST

The authors express no conflict of interest.

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