Australian Journal of Crop Science AJCS
AJCS 8(1):27-34 (2014) ISSN:1835-2707

Screening of soybean [*Glycine max* (L.) Merr.] genotypes for yellow mosaic virus (YMV) disease resistance and their molecular characterization using RGA and SSRs markers

Bhupender Kumar[1, 2], Akshay Talukdar[2*], Khushbu Verma[2], Vanishree Girmilla[3], Indu Bala[4], Sanjay Kumar Lal[2], Kanwar Pal Singh[2], Ramesh Lal Sapra[2]

[1]Cummings's Lab., Directorate of Maize Research, Pusa Campus New Delhi-12, India
[2]Division of Genetics, Indian Agricultural Research Institute, New Delhi-12, India
[3]Directorate of Sorghum Research, Hyderabad, India
[4]Molecular Cytogenetics and Tissue Culture Lab., Department of Crop Improvement, CSK Himachal Pradesh Agricultural University, Palampur 176 062, India

***Corresponding author: akshayassam@yahoo.co.in**

Abstract

Yellow mosaic virus (YMV) is a major disease of soybean, which can cause up to 80 % yield loss in severe cases. Chemical or cultural control of YMV is neither economical nor environment-friendly. Deployment of genetic resistance is considered to be the effective way to control it. Therefore, present study was conducted to identify stable sources of resistance for YMV disease, and their molecular characterization. A 500 soybean germplasm lines, collected from different parts of the world were screened for YMV disease reaction at YMV hotspot consecutively for 3 years (2007-09). It could identify 96 genotypes, comprising 48 each of resistant and susceptible genotypes that showed stable disease reactions over the years. Soybean 'R' gene-based primer pair linked with YMV variation in *Vigna mungo* failed to differentiate the YMV resistant and susceptible soybean genotypes. Genetic diversity panel of the 96 soybean genotypes was analyzed with 121 simple sequence repeat (SSR) markers, of which 97 were polymorphic (80.16% polymorphism). Total of 286 normal and 90 rare alleles were detected, with a mean of 2.36 and 0.74 alleles per locus, respectively. The mean of polymorphism information content (PIC) was 0.32, which ranged from 0.06 to 0.75. UPGMA cluster analysis grouped the genotypes into three major clusters which were further sub-grouped into 6 sub-clusters. Patterns of grouping were supported by the principal component analysis (PCA). The pair-wise genetic similarity values as calculated by Jaccard's coefficients, ranged from 0.39 to 0.95 with an overall mean of 0.65. The findings of the study thus strongly indicated the need for broadening the genetic base of the present Indian soybean cultivars, and also suggested the use of exotic collections that were found resistant to YMV for genetic enhancement of soybean.

Keywords: *Glycine max*; yellow mosaic virus; simple sequence repeats; resistance gene analogue; genetic diversity.
Abbreviations: YMV_Yellow mosaic virus; SMV_Soybean mosaic virus; MYMIV_Mungbean yellow mosaic India virus; GBNV_ Groundnut bud necrosis virus; SSR_Simple sequence repeats; RGA_resistance gene analogue; PCR_Polymerase chain reaction; PIC_ polymorphism information content; UPMGA_Unweighted paired group method using arithmetic averages; PCA_Principal components analysis;

Introduction

Soybean [*Glycine max* (L.) Merrill] (2n = 40), the major oil seed crop of the world including India, is susceptible to about fifty different diseases caused by virus. Yellow mosaic virus (YMV), Soybean mosaic virus (SMV) and Groundnut bud necrosis virus (GBNV) are the major viral diseases of soybean in India (Lal et al., 2005). Yellow mosaic virus (YMV) is serious and widespread disease of soybean in the Northern India, parts of South India, Sri Lanka, Bangladesh, Pakistan and Thailand (Bhattacharyya et al., 1999). At first, it was observed in North India in the early 1970s and since then it was being spread at alarming proportions. Its expansion towards central India or the hub of the country for soybean cultivation might be fatal to the soybean industry. Secondly, nearly all the major varieties grown in the central India are susceptible to YMV. The magnitude of yield loss due to YMV in soybean has been reported to be as high as 80 per cent (Nene, 1972). Therefore, there is an urgent need to tackle the disease before it causes serious damage to the soybean industry.

YMV is transmitted by white fly (*Bemisia tabaci*); therefore, control of this disease is indirectly related to the control of its vector. Its chemical or cultural control has not been found to be economical and environmental friendly. Only deployment of genetic resistance has been proved the way of its control or management. However, identifying the source of genetic resistance is challenging task as screening is impaired by many factors like irregular appearance and uneven density of vector in field along with other climatic factors which affects disease development. Moreover, it is not durable and largely overcome by newer strains of the virus. Hence, it is required to identify and deploy stable and durable sources of resistance towards the YMV disease in soybean. Wild relatives of soybean could be potential source of resistance. However, high linkage drags along with partial sterility are the major hindrances to use these wild relatives in

breeding programme. Therefore, a large scale screening is needed to identify stable source of resistance among the cultivated types to accelerate the breeding programme. The basic requirements for any crop improvement programme are the genetic variation for the trait of interest. Narrow genetic base due to its highly self pollinated nature is also an important obstruct to get higher potential of the soybean genotypes (Min et al., 2010). Assessment of the extent of genetic variability is fundamental for breeding, conservation of germplasm and future hybridization programme. Earlier, only morphological traits were used for assessing the genetic diversities. However, morphological characterization alone does not reliably portray the genetic diversity and relationships among the genotypes due to environmental interactions and largely unknown genetic control (Reif et al., 2005). Molecular markers, on the other hand, being DNA-based and independent from environment, assess the genetic diversity in a more precise way. A number of molecular approaches are now available for such analysis, which has proved to be genetically informative and useful for genotype identification and genetic diversity assessment (Wang et al., 2006; Nichols et al., 2007). Among the molecular markers, simple sequence repeat (SSR) or microsatellite markers are being used widely (Yoon et al., 2009; Guo et al., 2010). SSR markers are PCR-based, co-dominant, robust, reliable and are highly reproducible, with greater discriminatory ability than RFLP and RAPD markers. Screening of resistance genes with molecular markers is important for identification of gene-based markers which can further fasten the breeding programme. Basak et al. (2004) screened the *Vigna mungo* genotypes showing segregation for YMV symptoms with 24 sets of soybean 'R' gene-based RGA markers and found only one pair which differentiated the resistant from the susceptible ones. The present study is therefore, conducted to identify stable sources of YMV resistance and, to assess the genetic diversity among genotypes using molecular marker techniques to identify suitable genotypes for genetic amelioration of soybean.

Results and Discussion

Variation for YMV disease reaction

After continuous screening of the soybean genotypes for three years (2007-2009) under field condition, 96 genotypes comprising 48, each of resistant and susceptible were found showing stable reactions for YMV disease (Table 1).

The disease score of the resistant genotypes had 0 or 3, while that for susceptible genotypes had 7 or 9. From 96 genotypes, 21 genotypes *viz.,* UPSM534, DS9712, DS9814, DS9817, DS9819, DS9801, PK1169, PK1223, PK1347, PK292, PK1041, PS1042, SL525, SL633, SL46, SL528, SL637, SL444, SL459, EC9467 and EC456549 exhibited complete resistance with score '0' consistently for the three years, while score for other resistant genotypes was 3. Similarly, 31 genotypes *viz.*, SKAF 106, SKAF 635, SKAF 750-1, SKAF 2202, SKA 2008, SKAF 415, JS335, JS (SH) 93-01, EC 458354, UPSL 534, EC 472229, EC 472095, EC 472126, EC 113397, EC 472101, IC 244409, EC 472211 and EC 456626 etc. were showing high susceptibility for three years (2007-2009) with disease score '9', while rest of the susceptible genotypes were with disease score '7' (Table 1). Screening of the soybean genotypes for YMV disease under field conditions depicted the existence of genetic variations for YMV responses. However, degree of responses (i.e. resistance and susceptibility) was found to vary from year to year. From a total of 500 genotypes only 96 genotypes could be identified that showed resistance or susceptible reactions consistently over the three years of screening. This implies the need for stringent screening through creation of disease pressure even in the hot-spot. It would reduce the chances of reading disease escape as disease resistant. It has been observed that the varieties released by the North-Indian institutes were generally resistant to YMV, whereas the South-Indian varieties as well as the exotic germplasm except a few from Taiwan, USA and New Guinea were susceptible. This might be because of the fact that YMV disease is still not very prominent in Southern India. However, in Northern India, because of severity, resistance to YMV has become an important criterion for identification and release of the varieties. Most of the exotic genotypes were susceptible for the disease, indicating their less adoption for races of virus prevalent in India. The genotypes with prefix SKAF were found to be highly susceptible for the disease. At molecular and phenotypic level too, these were highly similar and were close to JS335, the very popular variety released from Jabalpur, Indore (Madhya Pradesh) and also highly susceptible to YMV. This indicates that the SKAF series and JS335 might have originated from similar genetic background.

Genotyping with SSR and RGA based markers

A diversity panel of 96 genotypes comprising 48, each of resistant and susceptible genotypes was constituted to represent the variations from different parts of the world. It was screened with 4 RGA and 121 SSR markers. Molecular profile of the genotypes depicted with Satt538 primer set has been mentioned in the Fig. (1a). Usharani et al. (2004) studied various isolates of YMV of soybean from India and found more than 89% identity with mungbean yellow mosaic India virus (MYMIV), which causes YMV disease in other legume crops. In this study, primer pair RGA 1F-CG/RGA 1R, which was designed from the 'R' gene of soybean and were reported to be polymorphic in urdbean (*Vigna mungo)* for YMV variation (Basak et al., 2004), appeared to be monomorphic between the resistant and susceptible genotypes of soybean (Fig. 1b). Similarly other three sets of RGA primer pairs designed for the same locus *viz*., RGA1F-TC/RGA 1R, RGA1F-CC/RGA 1R and RGA1F-TG/RGA 1R (Basak et al., 2004), were also unable to differentiate the resistant from susceptible soybean genotypes. It indicates the involvement of different gene (s) for conferring YMV resistance in urdbean and soybean. So, different sets of markers have to be designed in soybean for effective breeding against YMV disease.

Study of polymorphism using SSR markers

Analysis of genetic polymorphism with 121 SSR markers picked-up from across the genome (6 markers/ chromosome) resulted in the detection of high polymorphism (Supplementary table 1). Out of 121 markers, 97 were polymorphic indicating the polymorphism to be 80.20%, which is very high (Table 2). However, distribution of polymorphism was not uniform across the genome, as some of the linkage groups (LGs) like, A_2, B_2 and A_1 were found with more than 95% of polymorphic markers as compared with LGs H, L and C2, where the percentage was less. The average polymorphism information content (PIC) for the 97 SSRs markers was 0.32 with a range of 0.06 to 0.75 (Supplementary table 1). The lowest PIC was found with the

Table 1. List of 96 genotypes used to constitute the panel for genetic characterization using SSR and RGA markers. Genotypes with 0 and 3score are considered as resistant to YMV disease whereas with score 7 and 9 were considered as susceptible.

S.N	Genotype	Source	YMV score	S.N	Genotype	Source	YMV score	S.N	Genotype	Source	YMV score	S.N	Genotype	Source	YMV score
1	UPSM 534	Pantnagar	0	25	PK 1225	Pantnagar	3	49	SKAF 106	Mandsaur	9	73	EC 472239	Taiwan	7
2	SL 633	Ludhiana	0	26	PKV 25	Akola	3	50	SKAF 635	Mandsaur	9	74	DS 2006	Delhi	7
3	DS 9817	Delhi	0	27	SL 46	Ludhiana	0	51	SKAF 750-1	Mandsaur	9	75	DS 2009	Delhi	7
4	DS 9819	Delhi	0	28	SL 427	Ludhiana	3	52	SKAF 2202	Mandsaur	9	76	DS 2011	Taiwan	7
5	DS 9821	Delhi	3	29	PK 1347	Pantnagar	0	53	SKA 2008	Mandsaur	9	77	EC 458354	USA	9
6	PK 292	Pantnagar	0	30	SL 525	Ludhiana	0	54	SKAF 415	Mandsaur	9	78	PK 7427-B	Pantnagar	7
7	DS 9720	Delhi	3	31	PS 1042	Pantnagar	0	55	M 135	India	7	79	EC 471427	Taiwan	9
8	EC 9467	USA	0	32	SL 432	Ludhiana	3	56	EC 113397	N.Guinea	9	80	PK 1080	Pantnagar	7
9	PK 1041	Pantnagar	0	33	SL 459	Ludhiana	0	57	EC 389392	Taiwan	9	81	UPSL 534	Pantnagar	9
10	SL 528	Ludhiana	0	34	MAUS 164	Parbhani	3	58	G 2130	Taiwan	7	82	IC 244409	India	9
11	PS 1392	Pantnagar	3	35	SL 444	Ludhiana	0	59	G 2132	Taiwan	7	83	JS(SH) 93-01	Sehore	9
12	PS 1394	Pantnagar	3	36	DS 9801	Delhi	0	60	EC 439617	Taiwan	7	84	JS 335	Jabbalpur	9
13	SL 710	Ludhiana	3	37	EC 458356	USA	3	61	EC 472095	Taiwan	9	85	G 2144	Taiwan	7
14	EC456549	USA	0	38	EC 456597	USA	3	62	EC 472101	Taiwan	9	86	EC 472171	Taiwan	9
15	SL 637	Ludhiana	0	39	EC 471276	Taiwan	3	63	EC 472103	Taiwan	9	87	EC 472211	Taiwan	9
16	EC439618	Taiwan	3	40	EC 471784	Taiwan	3	64	EC 472118	Taiwan	9	88	EC 472229	Taiwan	9
17	EC439619	Taiwan	3	41	EC 471809	Taiwan	3	65	EC 472126	Taiwan	9	89	EC 456580	USA	7
18	EC 44303	Taiwan	3	42	DS 9814	Delhi	0	66	EC 472127	Taiwan	9	90	EC 456626	USA	9
19	DS 9712	Delhi	0	43	DS 9820	Delhi	3	67	EC 472145	Taiwan	9	91	JS 96	Jabalpur	9
20	PK 1169	Pantnagar	0	44	EC 456574	USA	3	68	EC 472184	Taiwan	7	92	MAUS 162	Parbhani	7
21	PK 1241	Pantnagar	3	45	L 291	Taiwan	3	69	EC 472217	Taiwan	9	93	KG 83-1A	Kasbe Digraj	9
22	PK 1135	Pantnagar	3	46	EC 456554	USA	3	70	EC 472220	Taiwan	9	94	DS 9816	Delhi	7
23	NRC 1180	Indore	3	47	VLS 57	Almora	3	71	EC 472218	Taiwan	9	95	EC 457254	USA	7
24	PK 1223	Pantnagar	0	48	Himso 1598	Palampur	3	72	EC 472228	Taiwan	9	96	EC 456535	USA	7

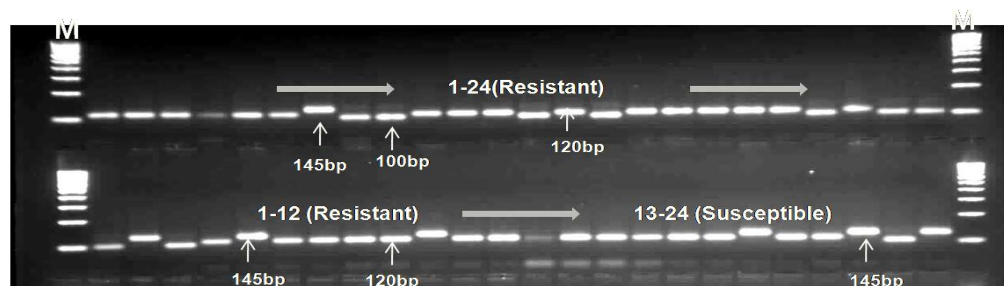

Fig 1a. Diversity among the soybean genotypes amplified with primer set Satt538 along with a 100bp ladder (M). There are three alleles of size 145bp, 120bp and 100bp distributed among the genotypes. The lane sequence in the gel corresponds to the identification of the genotypes as indicated in Table 1.

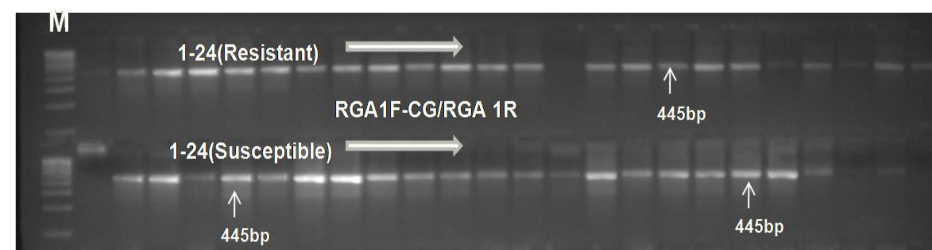

Fig 1b. RGA-based (RGA1F-CG/RGA1R) markers profile depicted in resistant and susceptible genotypes with a100bp ladder (M). The 445bp size allele was amplified uniformly in resistant as well as susceptible genotypes. The lane sequence in the gel corresponds to the identification of the genotypes as indicated in Table 1.

primer pairs Satt459 (0.06) and the highest was with the primers pairs AW734043 (0.75) and Satt301 (0.75). The markers having PIC ≥ 0.50 are shown in Fig. 2. Total of 286 alleles with a range of 1 to 6 and average of 2.36 alleles per locus were found across the population. The highest numbers of alleles (6) were found with marker Sat_156, which was followed by marker AW734043 with 5 alleles. There were 90 rare alleles, which ranged from 0 to 3 and average of 0.74 in the population (Supplementary table 1). There are sufficient variations identified with respect to YMV resistance among the cultivated as well as in the germplasm accessions that can be exploited in soybean improvement programme. However, at the molecular level, the genetic variation among the Indian genotypes was low. Most of the cultivars, released from a particular centre were genetically more similar which indicate their origin from same parent population. The number of alleles identified using 121 SSR markers, were markedly low in comparison to reports published by others. Li et al. (2008) studied genetic diversity of 1863 Chinese soybean landraces with 59 SSR loci and found an average of 19.7 alleles per locus. Guan et al. (2010) and Liu et al. (2011) reported an average of 16.2 and 7.14 alleles per locus, respectively. Incorporation of germplasm accessions and landraces in their studies has primarily contributed towards detection of more number of alleles per locus. The plant material of present study was consisted of more number of Indian released cultivars along with a few exotic germplasm (40 out of 96) collected from other parts of the world. Our studies were matching with Burnham et al. (2002) who also detected less allelic richness with average of 2.59 alleles per locus in his materials of 88 soybean genotypes (included cultivars and accession) screened for *Phytophthora* resistant and nearly close to the finding of Liu et al. (2011) who detected average alleles of 6.55 per locus. Secondly, since the purpose of this study is to evaluate soybean genotypes with special emphasis on YMV reactions, it leads to the rejection of many diverse accessions which were not showing consistent responses to YMV disease. This also led to less allelic richness in this study. The rare alleles found here were mostly from the exotic germplasm only. This again confined genetic uniformity among the Indian varieties used in study. It thus, signifies the need of using exotic germplasm as sources of YMV resistance for widening the genetic base, and also to introgress novel alleles available in the exotic germplasm.

Cluster analysis

The pair-wise genetic similarity values as calculated by Jaccard's coefficients, ranged from 0.39 (between G2144 and EC456597) to 0.95 (between SKAF635 and SKAF106) with an overall mean of 0.65. The resulting dendrogram indicated that all the genotypes could be differentiated and clustered into three major clusters (Fig. 3). Cluster I was the biggest one with 45 genotypes which was further divided into two sub-clusters with 33 and 12 genotypes, respectively. The sub-cluster I was consisting of the genotypes from Taiwan (24) and SKAF (6), which clustered separately as per their source of collection. The six genotypes with prefix SKAF *viz.,* SKAF106, SKAF635, SKAF750-1, SKAF2202 and SKAF2008 were collected from Sipani Krishi Anusandhan Farm (SKAF), Madhya Pradesh, a private group working in India were highly susceptible for YMV disease across the years. They had the highest level of genetic similarity among them and with JS335, a very popular variety of soybean but highly susceptible to YMV, implies that all of these might be sharing similar genetic background. Sub cluster II possessed 12 genotypes of diverse origin i.e. 8 from Taiwan, 3 from Delhi (DS2006, DS2009 and DS2011) and 1 from USA (EC458354). The cluster II contained 44 genotypes in three sub-clusters possessing 20, 5 and 19 genotypes, respectively. This cluster was predominantly carrying the Indian genotypes collected from Delhi, Pantnagar and Ludhiana with a couple of genotypes from USA and Taiwan. The last cluster (cluster III) had only 7 genotypes originated from India, USA and Taiwan (Fig. 3). Genotypes grouped in same cluster, might have originated from same base population and or same center and country (Supplementary table 2).

The Indian released cultivars did not exhibit much variation among themselves. Further, their clustering with the genotypes introduced from USA and a few genotypes from Taiwan was primary resulted, because most of the Indian cultivars have been developed from a few genotypes introduced in India during 1960s from USA. So as expected, these genotypes formed clusters with almost all the Indian genotypes. Principal components (PC) were analyzed to unearth the source of genetic variations in the genotypes. The PC-1 explained 15.8% of the total genetic variations, which includes mainly of exotic germplasm accession as compared with PC-2 (9.4%) and PC-3 (6.4%), covering most of the released cultivar from India. This supports the availability of sufficient variations in the exotic germplasm, which can be exploited for broadening of soybean genetic base in India. The patterns of grouping of genotypes towards the major components were supportive of the grouping based on UPGMA (Fig. 4).

Materials and Methods

Plant materials

For identification of stable source of resistance for YMV disease in soybean, initially 500 genotypes comprising both of germplasm accessions as well as release cultivars were used for field screening at New Delhi (hot spot location for YMV). To assess the genetic diversity and validation of RGA markers for YMV disease resistance, a diverse panel of 96 soybean genotypes, comprising 48, each of resistant and susceptible genotypes was selected from 500 germplasm lines (Table 1).

Screening technique

Screening was done in the Experimental Field of the Division of Genetics, IARI, New Delhi which is hot-spot for YMV disease. 500 diverse soybean genotypes were screened for YMV disease during *kharif* 2007, using 0-9 scale (Lal et al., 2005). A set of 260 genotypes showing unambiguous resistance and susceptible reactions was selected and tested further during 2008. For uniform distribution of the vector across the field and to have disease pressure evenly, the resistance genotypes were intermingled with the susceptible ones. Further, JS335, a highly susceptible genotype was sown after every two rows to act as source of inoculums (spreader row). Based on disease screening for two years (2007 and 2008), 96 genotypes comprising 48, each of resistant and susceptible genotypes were identified showing consistent reaction for YMV disease over the year. Response of 96 genotypes towards YMV disease was further assessed during 2009 using the same screening plan under field conditions and was used to constitute the diverse panel for molecular characterization using SSRs and RGA markers.

Table 2. List of SSR markers found polymorphic during molecular characterization of soybean genotypes.

LG	Polymorphic SSR markers
A1	Satt211(95.96,a^2), Satt619 (69.21,a^2), Satt648 (59.18,a^4,b^2), Satt717 (51.95,a^2,b^1), Satt593 (25.56,a^2)
A2	Satt429 (162.3,a^3,b^2), Satt538 (159.03,a^3,b^1), Satt228 (154.11,a^2), Satt409 (145.57,a^4,b^2),Satt119 (92.43,a^4,b^2), Sat_199 (84.09,a^3,b^1), Satt187 (54.92,a^2,b^1), Satt177 (36.77,a^2)
B1	Satt453 (123.96,a^3,b^2), Satt665 (96.36,a^2), Satt197 (46.39,a^3,b^1), Satt251 (36.48,a^3), Sat_156 (35.00,a^5,b^3)
B2	Satt687 (113.61,a^2), Satt560 (97.92,a^3,b^1), Satt534 (87.59,a^4,b^3), Satt272 (71.68,a^3), Satt168 (55.20.a^2,b^1), Satt_287 (31.88,a^2), Satt467 (17.77,a^3,b^2)
C1	Satt180 (127.77,a^3,b^1), Satt524 (120.12,a^2,b^1), Satt646 (70.52,a^4,b^1), Satt396 (24.11,a^2,b^1), Sct_186(9.02), Satt565 (0.00,a^2)
C2	Satt202 (126.24,a^2,b^1), Satt460 (117.77,a^4,b^1), Satt170 (70.56,a^2), Satt432 (38.05,a^2,b^1), AW734043 (4.22,a^5,b^3)
D1a	Satt408 (106.69,a^4,b^2), Satt407 (99.59,a^2,b^1), Satt532 (49.07,a^3,b^1), Satt342 (48.14,a^2), Satt531 (40.87,a^2,b^1)
D1b	Satt459 (118.62,a^2,b^1), Satt600 (75.41,a^2,b^1), Satt290 (73.35,a^3,b^1), Satt266 (59.61,a^3,b^1), Satt558 (43.91,a^2), Satt698 (38.04,a^3)
D2	Satt386 (125.00,a^3,b^1), Satt186 (105.45,a^2,b^1), GMHSP179 (99.04,a^2), Satt301 (93.71,a^4,b^2), Satt543 (88.02,a^3,b^2)
E	Satt230 (71.31,a^2), Satt685 (56.70,a^4,b^2), Satt720 (20.80,a^2,b^1)
F	Satt522 (119.19,a^2,b^1), Satt554 (111.89,a^2,b^1), Satt335 (77.70,a^2), Satt114 (63.69,a^4,b^1)
G	Sct_187 (107.11,a^2,b^1), Satt612 (80.38,a^2), Satt288 (76.77,a^3,b^1), Satt566 (49.91,a^2)
H	Satt434 (105.74,a^2), Satt302 (81.04,a^2), Satt635 (4.88,a^3,b^2)
I	Sat_324 (84.48,a^3,b^1), Satt671 (72.09,a^2,b^1), Satt239 (36.94,a^3,b^2), Satt367 (27.98,a^2,b^1)
J	Sat_224 (75.13,a^4,b^1), Satt183 (42.51,a^2), Satt529 (41.90,a^3,b^1), Satt674 (15.95,a^3,b^2), Satt405 (12.41,a^2,b^1)
K	Satt196 (104.79,a^2), Satt260 (80.12,a^2,b^1), Satt240 (52.88,a^2,b^1), Satt337 (47.38,a^3,b^1), Sat_087 (4.85,a^3,b^1)
L	Satt373 (107.24,a^2,b^1), Satt006 (92.00,a^2,b^1), Sat_286 (87.42,a^3,b^1), Satt481 (54.57,a^3,b^1)
M	Satt308 (130.76,a^4), Satt618 (111.06,a^2), Satt463 (50.10,a^4,b^2), Satt435 (38.94,a^2), Sat_389 (0.00,a^2)
N	Satt022 (102.06,a^3), Satt255 (76.49,a^4,b^1), Satt549 (70.60,a^4,b^1), Satt530 (32.85,a^3,b^2)
O	Satt581 (106.63,a^2,b^1), Satt331 (93.37,a^2,b^2), Sat_282 (63.81,a^4,b^2), Satt347 (42.29,a^2,b^1), Satt653 (38.09,a^3)

-Figures within parentheses are the location (cM) in the soybean genome map (Cregan et al. 1999), - Figures of a^x and b^y in the parentheses represents 'x' as total number of alleles and 'y' as number of rare alleles depicted by marker in the diversity panel.
-LG-Linkage Group

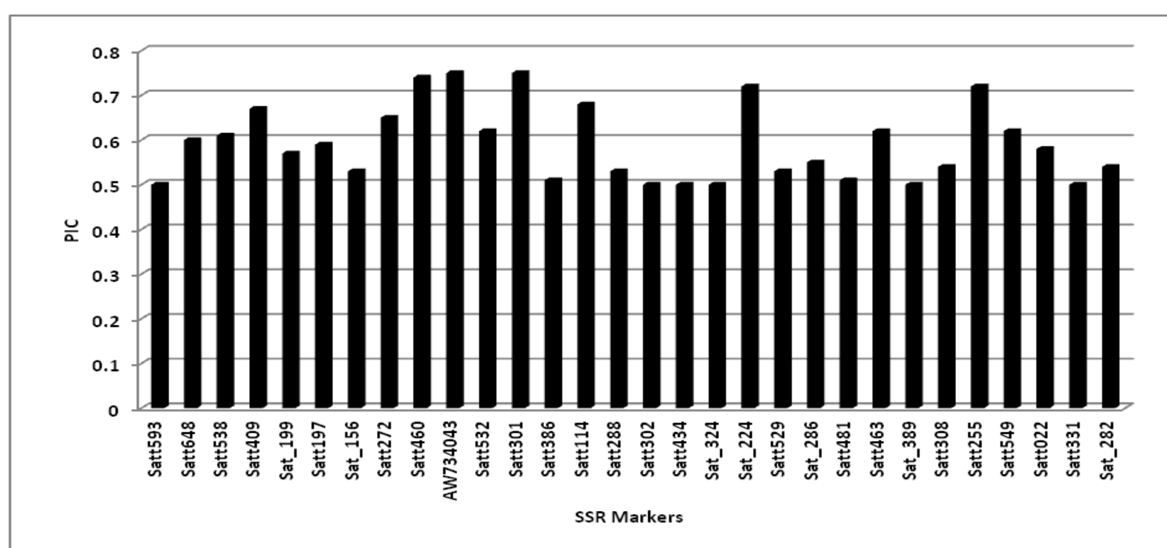

Fig 2. SSR markers found with high PIC value (≥0.50) among 97 polymorphic markers after screening of genetic diversity panel consisting of 96 soybean genotypes**.**

Molecular analysis

Genomic DNA from a pooled sample of 5 plants was isolated using CTAB method of Saghai-Maroof et al. (1984) with minor modification. Fresh bulk leaf sample of two grams, collected from five young and healthy plants was taken and frozen in liquid nitrogen (-196^{0}C). It was then, crushed into a fine powder using mortar and pestle. The powdered tissue was transferred to 2.0 ml micro centrifuge tube and 1500 µl of extraction buffer was added to it. This mixture was vortexed well and incubated in water bath at 65^{0}C for one hour with intermittent shaking. Following incubation, 600µl chloroform-isoamyl alcohol (24:1) was added to the micro tube and was centrifuged at 12,000 rpm for 20 minutes at 4^{0}C. The upper aqueous layer was transferred to fresh micro centrifuge tube and was purified, adding 5µl volume of RNAase. The contents were incubated and centrifuged. The upper aqueous layer was transferred to fresh micro centrifuge tube and 1/6th volume of chilled 100% ethanol was added. The whole content was mixed gently and all micro centrifuges were kept at -20^{0}C for one hour to get the DNA precipitated. It was followed by centrifugation at 10,000 rpm to get the DNA pellet. The DNA pellet was then washed two times with 70% ethanol and dried under vacuum. Finally dried pellet was dissolved in 1ml TE (100 mM Tris-Cl, 10 mM EDTA, pH 8.0). The genomic DNAs were checked in 0.8% agarose electrophoresis and were quantified using Spectrophotometer by taking the readings at wavelength of 260nm and 280nm for checking the quality and quantity of DNA in the stock samples. A set of 121 SSRs primers (Supplementary table 1), spanning across the soybean genome was selected for the study. The primer sequences and

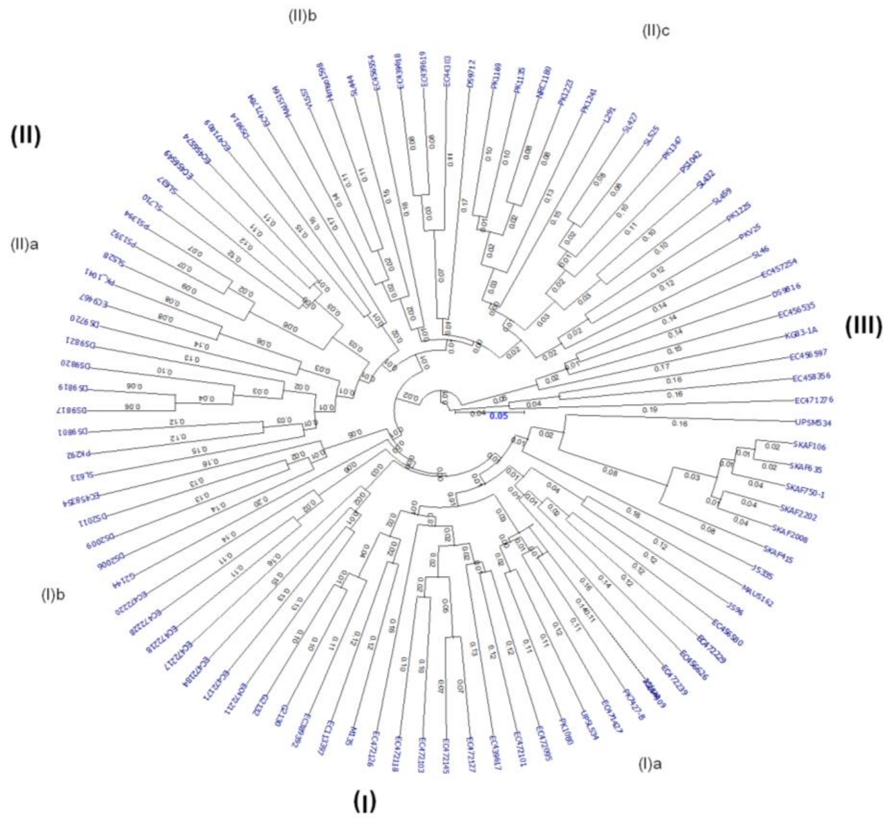

Fig 3. Dendrogram of 96 genotypes derived using UPGMA cluster analysis according to Jaccard's coefficient, values on nodes indicate dissimilarity between the genotypes based on SSR data.

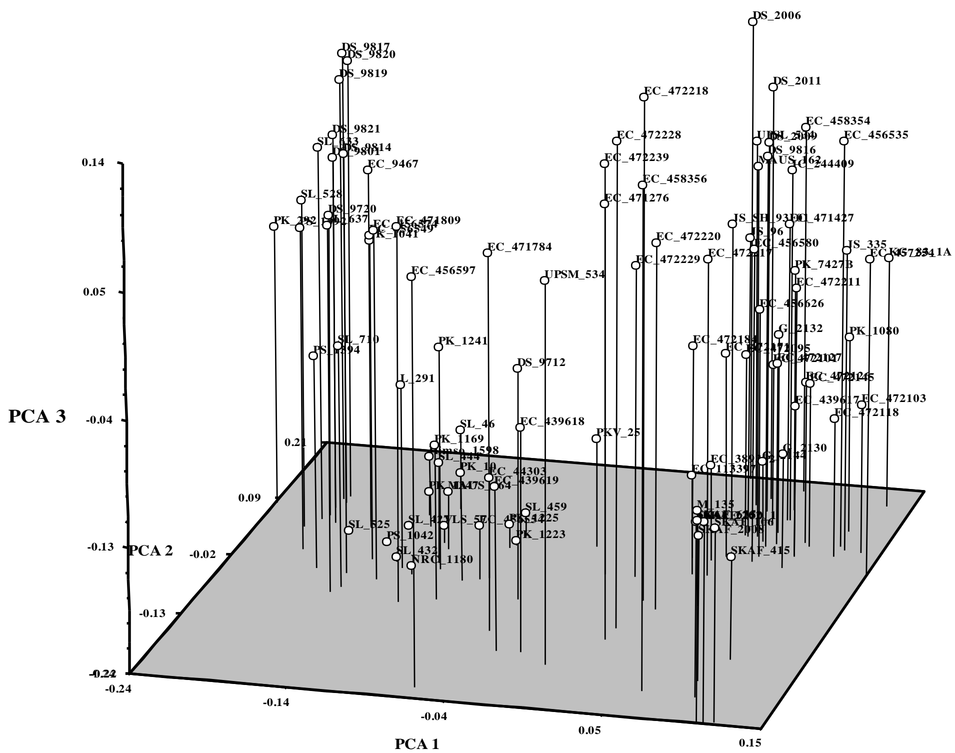

Fig 4. Grouping of 96 soybean genotypes on the basis of the first three principal coordinates. PC-1 explained 15.8% of the total genetic variations, which is mainly covering of most the germplasm accessions.

related information were obtained from Cregan et al. (1999) and http://www.soybase.org. Four RGA primers pairs derived from soybean 'R' gene and screened in *Vigna mungo* YMV (Basak et al., 2004) were also validated in 96 soybean genotypes comprising 48 each of resistant and susceptible for YMV disease. The primers were synthesised locally (Sigma Alderich Bangalore). The PCR amplification cycle consisted of initial denaturation at 94^0C for 2 min followed by 39 cycles consisting of denaturation, primer annealing and extension at 94^0C, 45-55^0C and 72^0C, respectively for 1 minute each. The final extension step was performed at 72^0C for 7 min. PCR was carried out in a volume of 20µl, containing 50 ng of template DNA, 1 µM of each forward and reverse primers, 1.5mM dNTPs, 1 U *Taq* polymerase and 1x of buffer with $MgCl_2$ in Applied Biosystem Thermocycler (Gene Amp^R PCR system 9700). The amplified products were resolved on 3% metaphor agarose gel along with 100 bp ladder (Bangalore Genei). The gel was run in 1x TBE buffer at a constant voltage of 70 V for about 4 h (until the tracking dye migrated to the end of the gel). The gel image was documented using a gel documentation system (AlphaImager® HP).

Marker data analysis

Scoring of the SSR alleles was performed manually in terms of positions of the bands relative to the ladder sequentially from the smallest to the largest-sized bands. Diffused bands or bands revealing ambiguity in scoring were considered as missing data and designated as 'V' in comparison with 'A' for the presence of the most frequent alleles followed by 'B' for the second and 'C' for the third most frequent alleles etc. Genotypes showing two allelic bands with equal intensity were considered as heterozygous for the locus. The polymorphism information content (PIC) was determined as described by Senior and Heun (1993), given as PIC = 1-$\sum P_{ij}^2$, where P_{ij} is the frequency of jth allele at i^{th} locus summed across all alleles in the locus. Alleles with frequency of less than 0.20 were considered as rare alleles and such allele representing a particular genotype was considered as unique allele for that genotype. Jaccard's coefficient (J) (Jaccard, 1908) was used to calculate the genetic similarities among pair wise comparison of genotypes based on SSR data, as follows: $J = N_{11} / (N_{11} + N_{10} + N_{01})$ where N_{11} is the number of bands present in both genotypes; N_{10} is the number of bands present in one genotype (lane) and N_{01} is the number of bands present in the other genotype. Grouping of genotypes was done using GGT 2.0 software (Berloo, 2008) to produce an agglomerative hierarchical classification by employing Unweighted Paired Group Method using Arithmetic Averages (UPGMA). Genetic distances between the genotypes were calculated using 1- coefficient of genetic similarity. Principal Component analysis (PCA) was studied to support the pattern of genetic diversity.

Conclusion

The present study established that Indian soybean cultivars are having narrow genetic base which need to be enriched by the use of genotypes from exotic and wild type origin. Sufficient genetic variation was found with respect to YMV resistance in the genotypes studied. Use of exotic sources of resistance towards YMV disease management will contribute towards widening of the genetic base. Failures of RGA-based primers designed from the 'R' gene of soybean and were reported to be polymorphic in urdbean for YMV variation, indicated involvement of different gene (s) for conferring YMV resistance in urdbean and soybean. This study also confirmed the power of SSRs in characterizing and developing fingerprints of the soybean genotypes. The identified genotypes will be good starting materials for developing YMV resistant varieties. They can also be used as the parents for developing mapping populations to map the YMV resistant gene (s) in the genome.

Acknowledgements

First author sincerely acknowledges PG School, IARI, for providing the fellowship during the Post-Graduate study.

References

Berloo RV (2008) GGT 2.0: Versatile Software for Visualization and Analysis of Genetic Data. J Hered 99:232-236.

Basak J, Kundagrami S, Ghose TK, Pal A (2004) Development of Yellow Mosaic Virus (YMV) resistance linked DNA marker in *Vigna mungo* from populations segregating for YMV-reaction. Mol Breed 14:375–383.

Bhattacharyya PK, Ram HH, Kole PC (1999) Inheritance of resistance to yellow mosaic virus in interspecific crosses of soybean. Euphytica 108:157–159.

Burnham KD, Francis DM, Dorrance AE, Fioritto RJ, Martin SK (2002) Genetic diversity patterns among Phytophthora resistant soybean plant introductions based on SSR markers. Crop Sci 42:338–343.

Cregan PB, Jarvik T, Bush AL, Shoemaker RC, Lark KG, Kahler AL, Kaya N, VanToai TT, Lohnes DG, Chung J, Specht JE (1999) An Integrated Genetic Linkage Map of the Soybean Genome. Crop Sci 39:1464–1490

Guan RX, Chang RZ, Li YH, Wang LX, Liu ZX, Qiu LJ (2010) Genetic diversity comparison between Chinese and Japanese soybeans (*Glycine max* (L.) Merr.) revealed by nuclear SSRs. Genet Resour Crop Evol 57(2):229-242.

Guo J, Wang YS, Song C, Zhou JF, Qiu LJ, Huang HW, Wang Y (2010) A single origin and moderate bottleneck during domestication of soybean (*Glycine max*): implications from microsatellites and nucleotide sequences. Ann Bot 106(3):505-514.

Jaccard P (1908) Nouvelles researches sur la distribution florate. Bull. Soc. Vaudolse Sci Nat 44:223-270.

Lal SK, Rana VKS, Sapra RL, Singh KP (2005) Screening and utilization of soybean germplasm for breeding resistance against Mungbean Yellow Mosaic Virus. Soybean Genet News Lett. Vol. 32. http://soybase.org:8083/articleFiles/45.

Li Y, Guan R, Liu Z, Ma Y, Wang L, Li L, Lin F, Luan W, Chen P, Yan Z, Guan Y, Zhu L, Ning X, Smulders MJM, Li W, Piao R, Cui Y, Yu Z, Guan M, Chang R, Hou A, Shi A, Zhang B, Zhu S, Qiu L (2008) Genetic structure and diversity of cultivated soybean (*Glycine max* (L.) Merr.) landraces in China. Theor Appl Genet 117:857-871.

Liu M, Zhang M, Jiang W, Sun G, Zhao H, Hu S (2011) Genetic diversity of Shaanxi soybean landraces based on agronomic traits and SSR markers. Afr J Biotechnol 10(24):4823-4837.

Min W, Run-zhi L, Wan-Ming Y, Wei-jun D (2010) Assessing the genetic diversity of cultivars and wild soybean using SSR markers. Afr J Biotechnol 9(31):4857-4866.

Nene YL (1972) A survey of the viral diseases of pulse crops in India. G B Pant Univ Agric Tech, Pantnagar, India J Res Bull 4:191.

Nichols DM, Wang LZ, Pei YL, Glover KD, Diers BW (2007) Variability among Chinese *Glycine soja* and Chinese and north American soybean genotypes. Crop Sci 47:1289-1298.

Reif JC, Hamrit S, Heckenberger M, Schipprack W, Maurer HP, Bohn M, Melchinger AE (2005) Trends in genetic diversity among European maize cultivars and their parental components during the past 50 years. Theor Appl Genet 111:838-845.

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci, USA 81:8014-8018.

Senior ML, Heun M (1993) Mapping maize microsatellites and polymerase chain reaction confirmation of the targeted repeats using a CT primer. Genome 36:884-889.

Usharani KS, Surendranath B, Haq QMR, Malathi VG (2004) Yellow mosaic virus infecting soybean in northern India is distinct from the species infecting soybean in southern and western India. Cur Sci 86:845-850.

Wang L, Guan R, Zhangxiong L, Chang R, Qiu L (2006) Genetic Diversity of Chinese Cultivated Soybean Revealed by SSR Markers. Crop Sci 46:1032–1038.

Yoon MS, Lee J, Kim CY, Kang JH, Cho EG, Baek HJ (2009) DNA profiling and genetic diversity of Korean soybean (*Glycine max* (L.) Merroll) landraces by SSR markers. Euphytica 165:69-77.