Identification of genetically diverse genotypes for photoperiod insensitivity in soybean using RAPD Markers

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ABSTRACT

Most of the Indian soybean varieties were found to be highly sensitive to photoperiod, which limits their cultivation in only localized area. Identification of genetically diverse source of photoperiod insensitive would help to broaden the genetic base for this trait. Present study was undertaken with RAPD markers for genetic diversity estimation in 44 accessions of soybean differing in response to photoperiod sensitivity. The selected twenty-five RAPD primers produced a total of 199 amplicons, which generated 89.9 % polymorphism. The number of amplification products ranged from 2 to 13 for different primers. The polymorphism information content ranged from 0.0 for monomorphic loci to 0.5 with an average of 0.289. Genetic diversity between pairs of genotypes was 37.7% with a range of 3.9 to 71.6%. UPGMA cluster analysis placed all the accessions of soybean into four major clusters. No discernable geographical patterns were observed in clustering however; the smaller groups corresponded well with pedigree. Mantel's test ($r = 0.915$) indicates very good fit for clustering pattern. Two genotypes, MACS 330 and 111/2/1939 made a very divergent group from other accessions of soybean and highly photoperiod insensitive that may be potential source for broadening the genetic base of soybean for this trait. [*Physiol. Mol. Biol. Plants* 2008; 14(4) : 369-375] E-mail : ikrps@yahoo.com

Key words : Genetic diversity, Photoperiod, RAPD, Soybean, UPGMA

INTRODUCTION

Soybean is the first largest oil seed crop in India and played a major role in up-liftment of socio-economic status of Indian farmers. Indian soybean has to be globally competitive in present era of opening up of world trade and liberalized economy. India produced 5.60 mt of soybean in an area of 6.0 mha during 2001-2002 with an average of 0.93 t/ha and contributed 3.17 % of total global production (Singh *et al.*, 2006). Soybean producing regions in India range from the lower Himalayan Hills and Northern Plain in the north to the Deccan Plateau in south. The soybean varieties cultivated in these areas were developed through

separate breeding programmes, because most of the Indian soybean varieties (> 95%) were found to be highly sensitive to photoperiod that limits their cultivation in only localized area (Bhatia *et al.*, 2003). The photoperiod response is a major criterion, which determines the latitudinal adaptation of a soybean variety (Hartwig 1970; Hartwig and Kiihl, 1979).

Identification of diverse genotypes is the prerequisite for improvement of any trait in the crop plants. Further more, monitoring the genetic variability within gene pool of elite breeding material could make crop improvement more efficient by the direct accumulation of favored alleles. DNA markers are being increasingly utilized in cultivar development, quality control of seed production, measurement of genetic diversity for conservation and management, varietal identification and intellectual property protection (IPP). Recent studies have used molecular markers to help in identification of genetically diverse genotypes to use in crosses in cultivar

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improvement programme. These studies have more success than conventional selection programme in producing productive lines from plant introduction/exotic lines crosses with elite lines.

There are several reports of using molecular markers for evaluation of soybean germplasm. RAPD markers have been shown to be a simple and effective means to evaluate variability in crops. RAPD are well suited for diversity studies because they are technically simple, non radioactive, relatively inexpensive, and require small quantity of DNA (Chen and Nelson, 2005). Previously, RAPD markers were used for evaluation of genetic diversity in soybean (Thompson and Nelson, 1998; Thompson *et al.*, 1998, Brown-Guidera *et al.*, 2000; Li *et al.*, 2001; Li and Nelson, 2001, 2002; Chen and Nelson, 2005; Singh *et al.*, 2006). In the present study, a concerted effort was made firstly to study the genetic relationships among the screened photoperiod insensitive and sensitive accessions of soybean germplasm belonging to different countries and secondly, identification of genetically diverse source of photoperiod-insensitive genotypes using RAPD markers for their further utilization in breeding programme for development of soybean cultivars.

MATERIALS AND METHODS

Plant Materials

One thousand soybean genotypes assembled from India, USA, Hungary, Philippines and Taiwan were screened for sensitivity to photoperiodism measured as days to flowering in ambient as well as in extended photoperiod (17 hrs) created artificially at the Experimental Farm of National Research Centre for Soybean, Indore (22.4° N), India for two years (2002-03 and 2003-04). Each genotype was sown in single row plots of 1 m long with 0.6 m row to row and 5 cm plant-to-plant distance. Recommended package of practices for the region were followed. The average photoperiod from planting to mean days to flowering under ambient conditions was 13.2 hrs. Extended photoperiod of 17 hrs was created by providing lighting with 40-watt incandescent bulbs at a height of 3 feet above the crop canopy and the bulbs were connected to an automatic timer. Data on the appearance of all the phenological stages (Fehr *et al.*, 1971) was recorded for all the genotypes grown under ambient and extended photoperiods. The degree of sensitivity to photoperiod among the genotypes was determined on the basis of number of day's delay in flowering under extended photoperiod as compared to ambient

photoperiod (Bhatia *et al.*, 2003). Out of the 1000 genotypes, 15 were found to show different degree of photoperiod insensitivity and remaining 985 were sensitive. In the present experiment 44 genotypes, comprising 15 genotypes showing different degree of photoperiod insensitivity and 29 sensitive genotypes were selected for analysis using RAPD markers. The place of origin and number of days delay in flowering from ambient to extended photoperiod is given in Table 1. Ten leaves, one each from ten plants of 44 soybean genotypes were collected and DNA was isolated by the method described by Doyle and Doyle (1990).

DNA amplification

Arbitrary decamer primers from Operon Technologies Inc., Alameda, CA, USA were dissolved in sterilized T₁₀E₁ (10mM Tris-Cl, 1mM EDTA, pH 8.0) to a concentration of 15 ng/μl. Twenty five primers from Operon kits (13 from AA, 7 from B, 1 from E and 4 from F series) were used for RAPD amplification as described by Williams *et al.* (1990). Amplification was carried out in a 25μl reaction volume containing 1X PCR assay buffer (50 mM KCl, 10 mM Tris-Cl, 1.5 mM MgCl₂), 200 μM of each dNTPs, 0.6 μM primer, 0.5 units Taq polymerase (Bangalore Genei Pvt. Ltd Bangalore, India) and 30 ng of DNA template (conc. approximately 15 ng/μl). The amplification reaction was carried out in a peltier controlled thermal cycler (M J Research, Model PTC-200). The first cycle consisted of denaturation of template DNA at 94°C for 4 min. followed by primer annealing at 37°C for 1 min. and primer extension at 72°C for 2 min. In the next 43 cycles, the period of denaturation was reduced to 1 min while the primer annealing and the primer extension time remained as in the first cycle. The last step was primer extension at 72°C for 8 min. PCR products were separated on a 1.5 % agarose gel containing ethidium bromide using 1X TAE buffer. The sizes of the amplified fragments were determined by using DNA size standards (1 kb DNA ladder, MBI Fermentas, Lithuania). DNA fragments were visualized and photographed using gel documentation system (Gene Genius, Bio Imaging System, Synaptics Group, UK). To test the reproducibility of the profiles, the reactions were repeated at least twice.

Data Scoring and Statistical analysis

The RAPD products were scored as present (1) or absent (0) for each primer – genotype combination. The data entry was done into a binary data matrix as discrete variables. The Jaccard's similarity coefficient values (Jaccard, 1908) for each pairwise comparison between

Table 1. Genotypes and cultivars, their country of origin, days delayed in flowering (mean of 2002-03 and 2003-04) of the 44 soybean genotypes/cultivars

S. No.	Collection Id.	Variety or original identity Cultivar	Country of origin of germplasm collection	Classification	Genetic distance	
					MACS330	111/2/1939
1.	EC 333904	PI 404177	USA	HS	70.0	71.3
2.	AGS 16	-	Taiwan	HS	56.9	55.7
3.	EC 251770	Pershing	USA	HS	65.3	65.8
4.	EC 333920	LAKOTA	USA	MS	68.1	68.9
5.	EC 325114	2426/85	Hungary	MS	69.1	68.2
6.	EC 232075	ROMEA	Philippines	MS	67.8	70.7
7.	EC 333919	Hedgson	USA	HS	66.7	67.9
8.	EC 325100	1145/84	Hungary	LS	55.9	57.7
9.	EC 325106	1146/84	Hungary	MS	60.7	63.3
10.	EC 333880	Evans	USA	MS	65.0	63.7
11.	EC 175321	G-1594	Taiwan	HS	68.9	69.7
12.	EC 378783	-	USA	HS	57.2	55.4
13.	EC 333922	PI 437418	USA	LS	56.9	59.7
14.	EC 251402	S-100	China	MS	67.9	67.9
15.	EC 325159	111-3-250	Hungary	HS	68.7	65.9
16.	EC 358004	PI 437883	USA	HS	53.2	57.9
17.	EC 242091	30229-11-11	Philippines	HS	60.0	62.5
18.	EC 34101	Dun-NunII-2-15	Hungary	I	68.2	70.3
19.	EC 325118	1158/84	Hungary	I	50.0	56.1
20.	MACS 330	Cultivar	India	I	-	-
21.	EC 325097	111/2/1939	Hungary	I	-	-
22.	LSb 1	Cultivar	India	LS	68.9	70.0
23.	EC 333897	Maple Arrow	USA	I	67.9	69.2
24.	EC 333925	PI 437428A	USA	HS	67.4	69.6
25.	EC 333867	Harosoy	USA	HS	68.9	70.3
26.	EC 325115	1172/84	Hungary	HS	65.4	67.1
27.	EC 325117	1165/84	Hungary	HS	67.3	68.7
28.	Type 49	Cultivar	India	HS	68.1	68.3
29.	Hardee	Cultivar	India	HS	70.3	71.6
30.	Co1	Cultivar	India	HS	69.0	67.4
31.	JS80-21	Cultivar	India	HS	66.4	65.4
32.	GS1	Cultivar	India	HS	67.9	67.4
33.	NRC 37	Cultivar	India	HS	66.7	65.4
34.	PK 472	Cultivar	India	HS	68.7	69.7
35.	MAUS 32	Cultivar	India	HS	67.3	68.0
36.	Indirasoya-9	Cultivar	India	HS	65.7	65.5
37.	MACS 58	Cultivar	India	HS	62.4	65.4
38.	MACS124	Cultivar	India	HS	63.3	68.0
39.	MACS13	Cultivar	India	HS	62.5	64.8
40.	EC 103336	CES-434	Philippines	HS	49.3	62.5
41.	EC 291448	PI 90-763	USA	HS	57.0	62.9
42.	EC 251446	Jackson	USA	HS	62.1	65.3
43.	EC 333912	PI 424489A	USA	MS	65.6	66.7
44.	Samarat	Cultivar	India	HS	62.7	65.3

*I = Photoperiod insensitive; LS = Low sensitive; MS = moderately sensitive; HS = highly sensitive

cultivars were calculated and similarity coefficient matrix was constructed. This matrix was subjected to unweighted pair-group method for arithmetic average analysis (UPGMA) to generate a dendrogram using average linkage procedure. All these computations were carried out using NTSYS-PC software (Rohlf, 1998). Genetic distances were calculated by subtracting the similarity indices from 1 and multiplying the result by 100 as described by Ude *et al.*, (2003). The polymorphism information content (PIC) was calculated for each marker as $1 - \sum p_{ij}^2$ where p_{ij} is the frequency of the j^{th} allele of i^{th} marker (Weir, 1990).

RESULTS AND DISCUSSION

Polymorphism analysis

Forty-four soybean genotypes differing in response to photoperiod sensitivity were characterized using 25 RAPD primers. These primers generated a total of 199 amplification products, among which 173 were found to be polymorphic (Table 2). These resulted in 89.9% polymorphism. The number of amplification products ranged from 2 (OPE18) to 13 (OPF4). The molecular weight of the generated band in the present study ranged from 250bp to 2500bp. This result is in agreement of previous report of Chen and Nelson (2005). The RAPD primers used in the present study produced polymorphic amplification products, however, the extent of percent polymorphism varied with each primer (50 -100%). Seven primers produced bands, all of which were found to be polymorphic. The RAPD profile obtained with primers OPAA 7 and OPB 02, respectively. The PIC scores range from 0.0 for monomorphic band to 0.50 with an average

of 0.289. Similar results were also reported by earlier workers in soybean (Thompson *et al.*, 1998; Li and Nelson, 2001; Chen and Nelson, 2005; Singh *et al.*, 2006).

We found that all the studied primers produced polymorphic amplification products (89.9%), which were relatively higher than previous reports. Li and Nelson (2005) and Singh *et al.* (2006) reported 56 % and 53.9% polymorphism in their study, respectively. Thompson *et al.* (1998) observed a low level (36%) of DNA variation among 35 soybean genotypes after analysis with RAPD primers. Conflicting reports on the extent of observed polymorphism in soybean in different studies could be attributed to the nature of the genetic material under investigation and sequences of the primers or probes. The high degree of polymorphism in our study compared to other reports is due to the more diverse material, which belongs to five different countries and different set of RAPD primers with high PIC value.

Genetic Relationships

Jaccard's pair wise similarity estimates between genotypes were calculated. The average genetic diversity among the accessions was 37.3 % with a range of 3.9 (between 1145/84 and 2426/85) to 71.6 % (between Hardee and EC 325097). Average genetic distance (GD) observed in this study was in agreement with earlier findings (Alvernaz *et al.* 1998; Singh *et al.* 2006). Brown-Guedira *et al.*, (2000) have reported an average GD of 32.0 % with the range of 8.0 to 76.0 % in genetic diversity analysis of soybean introduction and North American ancestors using RAPD markers.

Fig. 1. RAPD profiles of soybean genotypes obtained with primers OPAA 7 (A) and OPB 02, respectively. Serial number of the genotypes corresponds to that given in the table 1. M = Molecular weight marker.

Table 2. Random oligonucleotide primers used to amplify DNA from 44 soybean genotypes and number of total bands, polymorphic bands their range of molecular weight (bp), average polymorphism information content (PIC) scored with each primer

S.No.	Name of the primer	Band range (bp)	Total No. of bands	No. of Polymorphic bands	Average PIC
1.	OPF 4	350-2500	13	12	0.214
2.	OPF 6	450-1800	6	5	0.231
3.	OPF 3	450-1500	6	5	0.312
4.	OPF 20	350-1000	7	6	0.235
5.	OPAA9	300-900	8	6	0.276
6.	OPAA7	500-1600	7	6	0.286
7.	OPAA18	400-1500	10	9	0.272
8.	OPAA15	400-1300	9	6	0.206
9.	OPAA01	350-1400	8	6	0.315
10.	OPAA02	500-2000	8	6	0.851
11.	OPAA03	1000-1500	4	4	0.288
12.	OPAA04	600-1500	9	8	0.334
13.	OPAA06	600-1100	3	3	0.394
14.	OPAA11	400-2500	8	7	0.290
15.	OPAA10	400-2500	9	9	0.229
16.	OPAA17	150-2000	10	9	0.299
17.	OPAA19	600-1900	10	10	0.316
18.	OPB02	450-1450	6	5	0.295
19.	OPB03	400-1600	10	9	0.328
20.	OPB06	400-2000	10	8	0.358
21.	OPB11	600-1100	5	5	0.245
22.	OPE18	1400-1900	2	1	0.267
23.	OPB01	400-2500	9	7	0.321
24.	OPB04	300-1900	10	10	0.265
25.	OPB12	250-1600	12	12	0.292

The cluster analysis based on UPGMA classified 44 soybean genotypes into four major clusters which were designated as a, b, c, and d (Fig. 2). The dendrogram indicated that 75 % of the 44 soybean genotypes grouped between 75 to 95 % similarity coefficients. No discernable geographical patterns were observed in clustering pattern; however, the smaller groups corresponded well with known pedigree. Mantel's test (0.915) indicated very good fit for the clustering pattern. Cluster 'a' comprised of two soybean genotypes one

each from India and Hungary. This cluster constituted most divergent genotypes in respect to photoperiod response and showed no delay in flowering in extended photoperiod meaning there by they were photoperiod insensitive. They showed 65 % genetic diversity from rest of the soybean genotypes used in the present study.

The cluster 'b' was composed of 14 genotypes from India, 9 from USA, 4 from Hungary, two each from

Fig. 2. Dendrogram of different genotypes of soybean constructed using UPGMA based on 25 RAPD primers. Scale on below is Jaccard's coefficient of similarity. The major clusters are indicated on right margin.

Philippines and Taiwan and one genotype from China. This cluster was again sub divided into 7 sub-clusters. The sub cluster 'b-V' comprised of 9 Indian soybean cultivars, which were corresponded well with known pedigrees (Fig. 2). It is evident from dendrogram that MACS124 and MACS58, a sister line developed from cross JS2 x Improved Pelican were grouped together with 86% similarity. GS1 a variant of Punjab1 grouped with NRC37, as Punjab1 is one of the parents of NRC37. Clustering of JS80-21, Indira Soya and MAUS 32 in the same sub-group is obvious as Indira Soya and MAUS32 are variants of JS80-21. Sub-cluster b-VII comprised of 3 soybean genotypes originated from USA. Clustering of Indian and USA soybean genotypes to gather in the present study is obvious as Indian soybean cultivars were either introduction from USA or single cross of USA and Indian land races (Karmakar and Bhatnagar, 1999). Grouping of soybean genotypes of USA with Hungarian genotypes in the present study was in agreement of the results of Brown-Guedira *et al.*, (2000). The USA cultivars like Jackson, Evans and Pershing grouped in different sub-clusters in the present study. Ude *et al.*, (2003) reported similar result in soybean. Amongst the 32 soybean genotypes in this cluster, 24 were highly sensitive, 4 were moderately sensitive and 2 were low sensitive while two cultivars, Maple Arrow and Dun-Nun-II-2-15 showed insensitivity to photoperiods. Cluster 'c' consisted of 5 genotypes from USA, 2 from Hungary and one from Philippines.

The closest genotypes of this study 1145/84 and 2426/83 grouped together with 96% similarity. The two Hungarian genotypes 1158/84 (photoperiod insensitive) and 1172/84 (highly sensitive) grouped to gather in cluster 'd' with 55% similarity while this group showed 48% similarity with the genotypes of cluster 'b' and 'c'. No discernable geographical patterns of variation were found in this study. However, lines included in the study were not selected to accurately represent the major regions of soybean production and many of the entries were products of modern breeding programmes. Griffin and Palmer (1995) assumed that the long history of domestication and commerce of soybean in Asia has contributed to the dispersion of alleles throughout the region, lessening the influence of geography on patterns of variation among Asian soybean accessions. Similar results were also reported by Brown-Guedira *et al.*, (2000) after evaluation of genetic diversity of soybean introductions of different origin and North American ancestor using RAPD and SSR markers. The genetic clustering pattern based on UPGMA of RAPD data was not comparable with classification of photoperiod sensitivity in the present study. This result was in agreement as reported by earlier workers (Cui *et al.*, 2000; Li *et al.*, 2001) in soybean.

More than 95 percent of Indian soybean cultivars are photoperiod sensitive that limits their cultivation in only localized area. Therefore, separate breeding programmes are being carried out at national levels for development of location / zone specific soybean cultivars. In the present study 44 soybean genotypes evaluated for identification of genetically diverse source for photoperiod insensitivity using RAPD markers. Among the five-photoperiod insensitive genotypes two genotypes MACS330 and 111/2/1939 (EC325097) showed no delay in flowering in extended photoperiod in two successive years (Table 1). The RAPD based genetic distance clearly formed a very divergent group (Fig. 1) and genetic distances of these genotypes with others are given in the Table 1. Thus, molecularly identified photoperiod insensitive genotypes have high potential in soybean improvement programme for development of cultivars with wide adaptability. The Hungarian soybean genotypes (111/2/1939) and Hardee were identified as photoperiod insensitive and highly sensitive genotypes, respectively. In addition they are genetically very divergent (sharing of common bands was only 28.7 %; and genetic distance was 71.3 %) (Table 1, Fig. 2). These two genotypes would be ideal parents for development of mapping population. As they are very divergent, it may be possible that many markers can be mapped into

the soybean genome to locate corresponding chromosomal regions, where the photoperiod insensitivity genes are situated.

It can be concluded that this study will aid breeders interested in selecting genetically diverse source for photoperiod insensitivity for broadening the genetic base as well as for development of mapping population for identification of linked markers for marker aided selection for this trait in soybean.

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