Annals of Agrarian Science xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Annals of Agrarian Science

journal homepage: www.elsevier.com/locate/aasci

Pair-wise combinations of RAPD primers for diversity analysis with reference to protein and single primer RAPD in soybean

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ARTICLE INFO

Keywords:
Diversity
Glycine max
RAPD
PAGE
Soybean

ABSTRACT

Both RAPD and protein markers were to estimate genetic diversity present in eight varieties of soybean. Of the nineteen random primers used fourteen produced 83 bands 62 being polymorphic generating polymorphism of 74.69% level. Similar level of (72.58%) polymorphism was generated by 12 pair-wise combinations of primers. Five of the paired combinations did not generate banding patterns. Two of the paired combination (OPC-5 + OPC-15) and (OPC-5 + OPC-17) produced unique banding patterns for all the eight varieties. The bands generated by paired combinations of primers were common to one of the primers in 21% cases however 79% of them were found novel and can be used to reveal additional polymorphism. The polymorphism generated by SDS-PAGE remained very less compared to RAPD analysis. The estimation of pairwise similarity coefficient using Jaccard's similarity index based on RAPD revealed approximately 70% similarity among the genotype studied that is diversity being 30% only. Average similarity shown by SDS-PAGE was much higher (81%) than RAPD based similarity estimates. The clustering based on UPGMA analysis of RAPD profiling separated all the varieties whereas SDS-PAGE grouped three varieties together at 100% similarity. Thus, it was concluded that the material though comes from diverse sources but had narrow genetic base.

Introduction

Cultivated soybean, *Glycine max* (L.) Merr. belongs to family Leguminosae, subfamily Papilionoideae is grown primarily for protein and oil [1]. It is an important crop in world food trade containing 40% protein and 20% oil. The importance of the conservation and utilization of diversity is well understood as the function and sustainability of ecosystems are dependent on their biological diversity [2,3]. In order to keep cultivation of crop at economically viable level and realize full potential of crop's productivity regular varietals improvements are prerequisite. Hence, the studies on diversity are essential to determine the genetic distance among genotypes and to identify groups with similar genetic backgrounds for conserving, evaluating and utilizing germplasm for hybridization [4]. The extent of genetic diversity in can be assessed through protein and genetic markers. Genetic diversity and the pattern of variation in soybean have been evaluated with seed protein by various researchers [5,6]. However, a few studies indicated that cultivar identification is less reliable with the SDS-PAGE method [6] Evaluation of genetic diversity with molecular markers can distinguish the individual accessions' rapidly virtually with no environmental influence. One of the methods based on polymerase chain reaction (PCR) is a rapid DNA amplification technique, called RAPD (Random Amplified Polymorphic DNA). RAPD markers have been used for numerous applications in plant molecular genetics research despite having disadvantages of poor reproducibility and not generally being associated with gene regions [7,8]. RAPD, being a multi locus marker with the simplest [9] and fastest detection technology, have been successfully employed for determination of intra-species genetic diversity in several grain legumes [10]. RAPD markers have been used for genetic diversity analysis in soybean [11–13]. Recently, the pattern of genetic diversity among 92 genotypes of soybean from 5 different origins/sources was analyzed using 10 polymorphic RAPD markers [14]. The reports of various researchers in RAPD based study in soybean indicated the use of single primer (single primer act as both forward and reverse). However, scanty information is available regarding the performance of different primers used for primer combinations in RAPD reaction. Therefore in present study along with single primers pair-wise combinations of primers were also used to produce new markers by combining two primers in single RAPD reactions [7,15].

Peer review under responsibility of Journal Annals of Agrarian Science.
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https://doi.org/10.1016/j.aasci.2018.03.002
Received 15 December 2017; Received in revised form 23 March 2018; Accepted 25 March 2018
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Table 1
List of single primers and polymorphic amplicons generated.

Primers	Sequences (5′-3′)	Number of bands (a)	Number of polymorphic bands	% Polymorphism (b/a × 100)	Discrimination index (D)
OPC-5	GATGACCGCC	8	7	87.5	0.964
OPC-10	TGTCTGGGTG	–	–	–	–
OPC-12	TGTCATCCCC	2	0	0	0.000
OPC-13	AAGCCTCGTC	–		–	–
OPC-14	TGCGTGCTTG	6	5	83.3	0.892
OPC-15	GACGGATCAG	8	7	87.5	0.964
OPC-16	GACACTCCAG	–	–	–	–
OPC-17	TTCCCCCCAG	3	2	66.6	0.464
OPC-18	TGAGTGGGTG	6	2	33.3	0.733
OPC-19	GTTGCCAGCC	10	10	100.0	0.964
OPC-20	ACTTCGCCAC	7	6	85.7	0.250
OPD-4	TCTGGTGAGG	4	4	100.0	0.857
OPD-10	GGTCTACACC	–	–	–	–
OPD-13	GGGGTGACGA	6	4	66.6	0.785
OPD-14	CTTCCCCAAG	8	7	87.5	0.892
OPD-17	TTTCCCACGG	6	5	83.3	0.964
OPD-18	GAGAGCCAAC	3	0	0	0.000
OPD-19	CTGGGGACTT	–	–	–	–
OPD-20	ACCCGGTCAC	6	3	50.0	0.750
Total		83	62	74.69 (Aver.)	

- = No amplification.

Material and methods-

Plant material and DNA extraction

To study the genetic variability in soybean, eight varieties (Table 1) were procured from Agriculture Research Station, Kota (Rajasthan, India). The complete details of varieties are available on http://agropedia.iitk.ac.in/content/soybean-varieties-releasednotified-india. DNA was extracted from 3 to 4 weeks old seedlings using the method of Sharma et al. [16]. The quantitation of DNA sample was done using a UV visible spectrophotometer (UNICAM).

DNA extraction and RAPD

PCR amplifications were performed using RAPD primers of OPC and OPD series with the following cycling program: initial extended step of denaturation at 94 °C for 5 min followed by 40 cycles of denaturation at 94 °C for 1 min, primer annealing at 37 °C for 1 min and elongation at 72 °C for 2 min followed by a final step of extension at 72 °C for 4 min. PCR amplification was carried out in a total volume of 25 μL, consisting of 50 ng template DNA, 2.5 μL 10 × PCR buffer, 200 μM each of dNTPs, 10 pmols/reaction random primers, 1 U of *Taq* polymerase (Banglore genei). The PCR reactions were carried out in DNA thermal cycler (Model-CGI-96, Corbett Research, Australia) and reiterated twice for each primer to ensure the repeatability of RAPD results. The amplicons with a DNA ladder (λ DNA EcoRI/HndIII) were electrophoresed on 1.2%. Agarose gel (Himedia), prepared in 1 × TBE buffer containing 0.5 μg/ml of the ethidium bromides. The gel was photographed under UV transillluminator for band documentation. For RAPD, without considering the faint bands, the presence of each band was scored as 1 and its absence as 0.

SDS-PAGE

For SDS-PAGE analysis, seed powder was treated with hexane and acetone to remove fats and oils [17]. Protein was extracted with 0.1 M Tris-borate (pH 8.5) from the seed powder. The concentration of extracted Protein was estimated using Biuret method and finally equal amount of protein was loaded on 12% acrylamide resolving gel for resolution of polypeptides. The gel was electrophoresed at 200v followed by stained with Commassie Brilliant blue R-250 for 2–3 h and then destained in a solution of methanol and water (1:1) and 10% acetic acid. For SDS-PAGE the presence of each band was also scored as 1 and its absence as 0. The bands were designated on the basis of Protein Molecular Weight Marker (PMW).

Data analysis

Pair-wise association coefficients were calculated using Jaccard's similarity coefficient. Cluster analysis based on genetic distances was carried out using un-weighed pair-group method with arithmetic averaging (UPGMA) [18] to generate a dendrogram showing relationships among varieties using NTSYSpc 2.02 [19]. The degree of polymorphism was also quantified using Simpson's index of diversity [20]. The discriminatory power (Dj) of RAPD primers and SDS-PAGE profile was analyzed as per Tessier et al. [21].

Results

RAPD analysis

Fourteen informative primers (summarized in Table 1) of the 19 tested generated 83 unambiguous polymorphic amplification products in the range of 225–3630 bp. The total number of bands scored per primer ranged from 2 (OPC 12) to 10 (OPC 19) with an average of 5.92 bands per primer. The number of polymorphic bands lying between 0 and 7 with polymorphism range from 0% (OPC 12 and OPD 19) to 100% (OPC 19 and OPD 4) (Table 1) In total, 74.69% of bands were polymorphic. The patterns obtained with primer OPC 15 for varieties suggested that this primer has the ability to produce fingerprints specific to particular variety. Maximum variety specific bands were generated by OPC 15 distinguishing 4 varieties. Variety Punjab-1 had maximum (7) variety specific bands generated by 4 primers. The value of D ranged from 0 to 0.964 for single primer based RAPD. All the primers having 0.964 D value had polymorphism level of bands above 80% *viz*. OPC 5 (87.5), OPC 15 (87.5), OPC 19 (100), OPD 17 (83.3). A combination of two of these primers would distinguish all the varieties. However, primer OPC 12 and OPD 18 failed to reveal any polymorphism.

Similarly, of the 17 primer combinations, 10 pairs consisted of primers both generating bands singly whereas 7 of these pairs included one non amplifying primer with another one generating amplicons. Out

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