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GENETIC DIVERSITY ANALYSIS AMONG SOYBEAN [*Glycine max* (L.) Merr.] GENOTYPES THROUGH ISSR MOLECULAR MARKERS

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Absract

Soybean is considered as the most despised member of oilseeds and pulses in our country. The 15 ISSR primers were screened to generate 95 bands in which 74 bands were polymorphic with an average of 4.94 bands per primer. The similarity coefficient of clusters analysis was ranged from 60 to 84% for ISSR. The pooled study of ISSR generated clustering pattern which seem to be similar as ISSR clustering pattern. The ISSR primers (UBC-841, UBC-845, UBC-848 and UBC-857) produced genotype specific bands and discriminated six, four and four genotypes among all 16 soybean genotypes respectively.

Keywords: ISSR

I. Introduction

Soybean (*Glycine max* (L.) Merrill) (2n=2X=40) is also known as "Golden bean" and miracle crop of 20th century because of its multiple uses. Soybeans were cultivated as early as the 11th century in China (Hymowitz and Shurtleff, 2005). The word "soybean" was not actually used until 1804, when Dr. James Mease began to use it in the literature, called it the bean from which soy sauce was produced. The United States is the leader in soybean production followed by Brazil, China, Argentina, India and Turkey (Vural and Dageri, 2011).

The genus Glycine Wild is divided into two subgenera (16 perennial species): *G.max.* and *G.soja.* The subgenus Soja (Moench) includes the cultivated soybean *G. max* (L.) Merrill (2n=40) and wild soybean: *G. soja* Sieb. & Zucc (2n=40) in which both species are annual. *G.max* and *G.soja* form the primary gene pool for the cultivated soybean (Singh and Hymowitz, 1999). The cultivated soybean first appeared in species *Plantarum* by Linnaeus under the name *Phaseolus max* L.

The knowledge of genetic diversity in a crop species is fundamental to its improvement. However, morphological traits have many limitations, including low polymorphism, low heritability, late expression, and may be controlled by epistatic and pleiotropic gene effects. Now-a-days very powerful PCR-based techniques have also emerged which are very fast, reliable and require minimal amount of tissue for investigation. The various PCR based markers like Randomly Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSR) and Simple Sequence Repeats (SSR) are used to evaluate genetic diversity. Day by day development of such new and specific types of markers make their importance in understanding the genomic variability and the diversity between the same as well as different species of the plants (Kumar *et al.,* 2009).

The ISSRs are DNA fragments of about 100–3000 bp located between adjacent, oppositely oriented microsatellite regions. The ISSRs are amplified by PCR using microsatellite core sequences as primers with a few selective nucleotides as anchors into the non-repeat adjacent regions (16–18 bp). Genetic diversity of Asian soybean germplasm (Wang *et al*., 2006) as well as European soybean germplasm (Tavaud-Pirra *et al.,* 2009) has been studied by microsatellites.

II. Materials and method

EXPERIMENTAL MATERIALS

The seeds of soybean genotypes used for the present study were obtained by Research Scientist, Junagadh Agricultural University, Amreli. The experimental material consisted of sixteen genotypes of soybean are listed below.

List of soybean genotypes used in present study.

1	PK-805	9.	DS-86-75
2.	IC-41686	10.	PK-416
3.	JS-75-46	11.	DS-71-1-29
4.	J-79-4-11	12.	PK-820
5.	EC-77-205	13.	Himso-1548
6.	AMR-SEL-KH-06	14.	JS-80-21
7.	AGS-51	15.	JS-75-10
8.	NRC-2	16.	JS-81-607

DNA Extraction

Total genomic DNA was extracted from the leaves of 20 days old seedlings by Cetyl trimethyl ammonium bromide (CTAB) as per Doyle and Doyle (1987) method with some modifications.

List of ISSR primers used in the present study.

Sr. No	ISSR Primer	Sequence (5`→3`)	Tm	GC (%)
1	UBC 807	AGAGAGAGAGAGAGAGT	42.5	47
2	UBC 811	GAGAGAGAGAGAGAGAC	43.3	53
3	UBC 818	CACACACACACACACAG	52.1	53
4	UBC 826	ACACACACACACACACG	54.9	53
5	UBC 829	TATATATATATATATART	25.8	0
6	UBC 835	AGAGAGAGAGAGAGAGYC	42.7	50
7	UBC 841	GAGAGAGAGAGAGAGAYC	45.7	50
8	UBC 845	CTCTCTCTCTCTCTCTRC	39.4	50

9	UBC 848	CACACACACACACACARG	50.6	50
10	UBC 855	ACACACACACACACACYT	60.2	44
11	UBC 856	ACACACACACACACACYA	50.2	44
12	UBC 857	ACACACACACACACACYG	57.1	50
13	UBC 858	TGTGTGTGTGTGTGTGRT	59.4	44
14	UBC 859	TGTGTGTGTGTGTGTGRC	51.5	50
15	UBC 860	TGTGTGTGTGTGTGTGRG	56.3	50

PCR Protocol

List of different components of PCR master mix (25 µl) used for amplification reaction were given in Table 3.9.

Preparation of reaction mixture for ISSR

Sr. No.	Reagent	Quantity
1	PCR buffer (10X)	2.5 µl
2	Taq polymerase (3 U.μl^{-1})	0.5µl
3	dNTPs mix (2.5 mM each)	2.0 µl
4	Primer (25 pmoles.μl^{-1})	2.0 µl
5	Template DNA (50 ng.μl^{-1})	2.0 µl
6	Millipore sterile distilled water	16.0 µl
Total		**25 µl**

Taq buffer A (10X Tris with $MgCl_2$) was added first followed by Taq DNA polymerase, dNTPs, the primer and Millipore sterilized water were added in sequence and finally template DNA. The reagents were mixed thoroughly by a short spin using micro centrifuge. The tubes were then placed on the Thermal Cycler for cyclic amplification. The conditions for amplification in the Thermal Cycler were kept as follows. (sambrook *et al*, 1985)

PCR conditions for ISSR.

Sr. No.	Steps	Temp. (^{O}C)	Duration	Cycles
1	Initial denaturation	94	5 min	40
2	Denaturation	94	1 min	
3	Annealing	52	1 min	
4	Extension	72	2 min	
5	Final extension	72	7 min	
6	Final hold	4	∞	

PCR products were subjected to electrophoresis with marker DNA of known molecular weight in 1.5% agarose gel. After electrophoresis, the gel was carefully taken out of the casting tray and photographed in a gel documentation system.

III. Results

16 soybean genotypes were subjected to ISSR analysis using 15 different primers of UBC series such as 807, 811, 818, 826, 829, 835, 841, 845, 848, 855, 856, 857, 858, 859 and 860.

Total 15 ISSR primers were amplified to generate 95 fragments. The ISSR marker UBC 859 produced maximum number of 10 bands, while UBC 857 and UBC 860 produced minimum number of 2 bands. Out of 95 bands, 74 bands were polymorphic with an average of 4.94 bands per primer and 21 bands were monomorphic. Among the 74 polymorphic bands, 70 bands were shared polymorphic within two or more genotypes, while 4 bands were unique-polymorphic (Table 4.15.).

The percent polymorphism obtained for ISSR primers were ranged from 25 (UBC 811, UBC 826, UBC 829, UBC 835, UBC 845, UBC 848, UBC 855, UBC 856, UBC 858 and UBC 860) to 100% (UBC 807, UBC 818, UBC 841, UBC 857 and UBC 859) with an average value of 74.68 % per primer. The Polymorphism Information Content (PIC) values for ISSR marker were ranged from 0.117 (UBC 857) to 0.872 (UBC 818) with an average value of 0.745 per primer and ISSR primer index (IPI) differed from 0.234 (UBC 857) to 8.706 (UBC 859) with an average value of 5.11 as presented in Table 4.15.

The performance of individual primer to amplify genomic DNA of 16 soybean genotypes is discussed as under.

Identification of Genotype Specific Marker from ISSR Primers

Among all the ISSR primers, UBC-841, UBC-845, UBC-848 and UBC-857 primers showed the amplification of unique and soybean genotype specific bands (Table 4.16.). Primer UBC-841 produced one specific band of mol. wt. of 404 bp in PK-820, UBC-845 primer produced specific band of mol. wt. 381 bp in IC-41686, UBC-848 primer produced one specific band of mol. wt. of 546 bp in AMR-SEL-KH-06 genotype and UBC-857 primer produced a specific band of mol. wt. of 558 bp in Himso-1548.

Genetic similarity

Genetic similarity was determined for each pair of 16 populations which revealed that the genetic distance was minimum 0.697 and maximum 0.938.

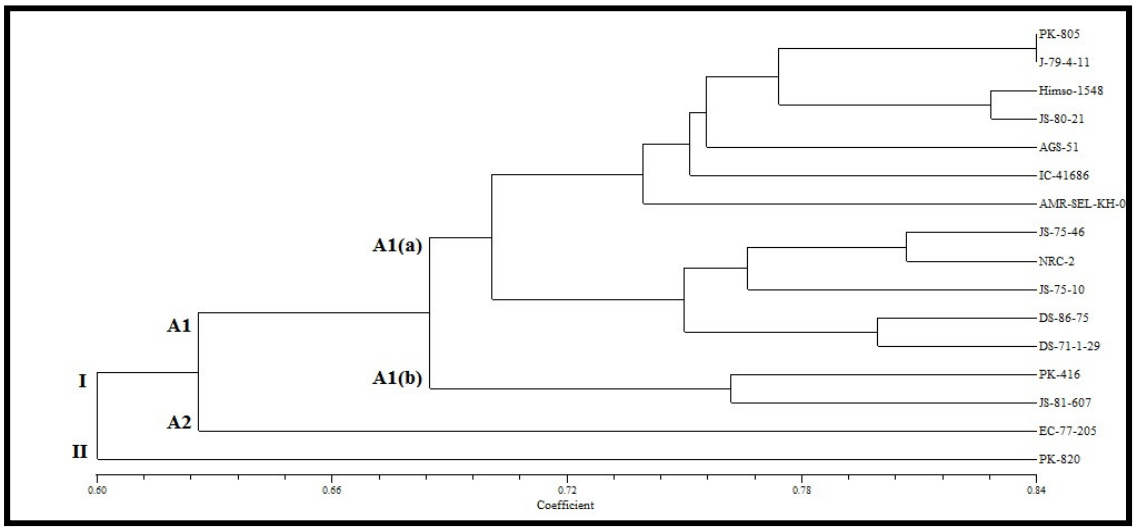

Fig.1:Dendogram depicting the genetic relationship among 16 soybean genotypes based on ISSR marker.

Cluster Analysis of ISSR

The dendogram was constructed using UPGMA based on Jaccard's similarity coefficient through NTSYSpc-2.02i software for ISSR data of 16 soybean genotypes .The genotypes were grouped into two main clusters: cluster-I and cluster-II shared 60 % similarity. The cluster-I comprised of two subclusters A1and cluster A2. Subcluster A1 further divided into A1(a) and A1(b). Subcluster A1(a) consisted of twelve genotypes PK-805, J-79-4-11, Himso-1548, JS-80-21, AGS-51, IC-41686, AMR-SEL-KH-06, JS-75-46, NRC-2, JS-75-10, DS-86-75 and DS-71-1-29 with similarity of more than 71%. However subcluster A1(b) consisted of two genotypes PK-416 and JS-81-607 with similarity of 75%. Subcluster A2 consisted of only one genotype EC-77-205. Cluster-II consisted of only one genotype PK-820.

To test the goodness of fit of the clustering of ISSR data matrix of cophenetic values were also computed using the program COPH. The cophenetic matrixes were compared to the original matrixes produced by SIMQUAL. The plots of one matrix against the other and the association statics were made and calculated by MAXCOMP. The plot and statistics of 16 soybean genotypes included in present study were shown in Figure 4.9. In the present investigation the mental test statistics Z was raw (= normalized Mantel statistic Z) and degree of goodness of fit for a cluster analysis (Matrix correlation r = 0.80) as categorized by Rohlf (1998) was found under the category of "good fit".

IV. Conclusions

The DNA based molecular marker methods such as ISSR are independent of environmental condition, which offers significant advantages of the species identification in that they are rapid, relatively cheap, eliminate the need of grow out test.

Total 15 ISSR primers were used to generate 95 fragments in which74 bands were polymorphic with an average of 4.94 bands per primer and 21 bands were monomorphic. The PIC (Polymorphism Information Content) values were varied from 0.117 (UBC 857) to 0.872 (UBC 818) with an average of 0.745 per primer while the IPI (ISSR Primer Index) differed from 0.234 (UBC 857) to 8.706 (UBC 859) with an average of 5.11. Out of 15 ISSR primers, four primers were able to produce genotype specific unique bands and identified four soybean genotypes. Out of which UBC-841 (404 bp), UBC-845 (391 bp), UBC-848 (546 bp) and UBC-857 (458 bp) primers allowed to soybean genotypes- PK-820, IC-41686, AMR-SEL-KH-06 and Himso-1548 respectively. The phylogenetic tree constructed by UPGMA method generated two main clusters and similarity coefficient was ranged from 60 to 84%. PK-805 and J-79-4-11genotypes showed maximum similarity (84%) while PK-820 had maximum variability (60%) among other genotypes.

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