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ORIGINAL ARTICLE

DNA Fingerprinting of Soybean Cultivars

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ABSTRACT
The present investigation entitled "DNA fingerprinting of soybean cultivars" was carried out with the objective to study the diversity in the soybean genotypes using ISSR markers. A set of 15 soybean genotypes were used for fingerprinting purpose. The DNA was isolated from the soybean genotypes and PCR amplification was carried out using 13 ISSR primers. Out of 13 ISSR primers used, 12 amplified and all of them showed polymorphism. A total 71 bands were generated on the amplification with these 12 primers, out of which 49 bands were polymorphic. These primers amplified 8 unique loci in the 5 soybean genotypes. These can be very useful in characterization and identification of specific soybean genotype. The size of amplification product ranged from 131-600 bp. The similarity coefficient values based on ISSR markers data ranged from 0.67 to 0.97. A consensus tree was constructed by using software programme NTSYSpc 2.02i. Divergence was observed based on cluster analysis of genotypes by using UPGMA analysis.
Key words- *Soybean, DNA Fingerprinting, ISSR Marker, Genotype.*

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INTRODUCTION

Soybean *(Glycine max)* is important pulse and oilseed crop belongs to Leguminaceae family, subfamily Papilionoideae and genus *Glycine*. It is originated in eastern Asia or china. In India, the area under soybean was 10.18 million hectares with a total production of 122.14 lakh tonnes and productivity of 1207 kg ha^{-1} during the year of 2011-2012. In Maharashtra area under soybean was 3069 hectares with production and productivity of 3969 tonnes and 1319 kg ha^{-1} respectively (Anonymous, 2014). This crop is aptly called as "Golden Bean" or "Miracle crop" of the 20th century, because of its multiple uses. In India, it is grown a large area of Madhya Pradesh, Maharashtra, Uttar Pradesh, Rajasthan, Bihar, Karnataka and Andhra Pradesh. It has revolutionized the agricultural economy with its immense potential for food, fuel and numerous industrial products. It is a major source of edible oil and high quality protein food. It contains 43.2 % crude protein and 19.5 % oil. Crude protein of soybean contains lysine (8.4 %) and other essential phospholipids (Halwankar et al*.,* 1992). Specht *et al.,* (1999) suggested that the theoretical limit of soybean production to be 8 t/ha, based on the amount of light energy available in the field.

DNA Fingerprinting

The fundamental techniques involved in genetic fingerprinting were discovered in 1984 by geneticist Alec Jeffery of the University of Leicester in Great Britain while he was studying the gene for myoglobin, a protein that stores oxygen in muscle cells. The technique has wide range of applications in the forensics.
Molecular markers have provided a powerful new tool for breeders to search for new sources of variation and to investigate genetic factors controlling quantitatively inherited traits. In soybean genetic diversity can be assessed by morphological, agronomic traits, geographic origins, isozymes, DNA markers (Nelson *et al*., 1987).
Inter Simple Sequence Repeats refers to a variant of the polymerase chain reaction that uses simple sequence repeat primers to amplify regions between their target sequences. Inter simple sequence repeats (ISSR), which involves PCR amplification of DNA using a single primer composed of a micro

satellite sequence anchored at the 3' or 5' end by 2-4 arbitrary nucleotides, is one of DNA based molecular marker which could be used to assess genetic diversity (Qian *et al.,* 2001).

MATERIAL AND METHODS

Material

Fifteen soybean genotypes were selected for the purpose of DNA fingerprinting. The seeds of genotypes were obtained from the Soybean breeder, Agricultural Research Station, K. Digraj, Dist.Sangli and from Department of Botany, College of Agriculture, Kolhapur.

Soybean genotypes used in present study

Sr.No.	Genotypes	Pedigree
1	KS-103	JS-335 X EC-241780 F1 X EC-241780 (B2)
2	KS-106	JS-335 X EC-241780 Selection from BC2
3	KS-112	JS-335 X EC-241780 Selection from BC2
4	KS-128	JS-335 X EC-241780 Selection from BC2
5	DS-228	JS-335 X PI-462312(Ankur)
6	JS-335	JS78-77 X JS71-05
7	KDS-739	JS-9305 X EC-241780(Pubescent)
8	NRC-94	Ankur x PK-1024
9	KDS-722	AMS-9933 X EC-241780
10	MAUS-71	JS 71-05 x JS 87 -38
11	Monetta	An exotic variety
12	Kalitur	Local selection khandwa, M.P
13	Birsa Soya	Mutant of Sepaya black
14	DS-9712	-
15	MACS-450	Bragg X MACS111

Methods

1) Growing of seedlings

The clean and round seeds of each genotype were planted in plastic pots in greenhouse.

2) Isolation of DNA

The genomic DNA was extracted using Cetyltrimethyl ammonium bromide (CTAB) protocol with some modifications given by Doyle and Doyle (1987) method.

3) PCR Analysis of DNA samples

DNA amplification by ISSR markers

List of selected ISSR Markers

Sr. No.	ISSR Primers	Sequences of Primers (5'-3')
1	ISSR 808	AGAGAGAGAGAGAGAGC
2	ISSR 812	GAGAGAGAGAGAGAGAA'
3	ISSR 817	CACACACACACACACAA
4	ISSR 825	ACACACACACACACACACT
5	ISSR 841	GAGAGAGAGAGAGAGAYC
6	ISSR 842	GAGAGAGAGAGAGAGAYG
7	ISSR 834	AGAGAGAGAGAGAGAGYT
8	ISSR 889	DBDACACACACACACAC
9	ISSR 891	GAAGAAGAAGAAGAAGAA
10	ISSR 856	ACACACACACACACACYG
11	ISSR 885	BHBGAGAGAGAGAGAGA
12	ISSR 880	GGAGAGGAGAGGAGA
13	ISSR 888	BDBCACACACACACACA

Composition of PCR reaction mixture for ISSR Primer.

PCR reaction component	Stock concentration	Volume (ul)	Final concentration
Genei *Taq* DNA Polymerase Buffer A	10X	2µl	1X
dNTP mix	2.5 mM each	2µl	0.25 mM
Primer	25 picomole	1µl	25 picomole
Genei *Taq* DNA Polymerase	3 units/ µl	0.3µl	1 unit
Sterilized distilled water	----------	13.7	--------
Template DNA	60 ng	1µl	60 ng
Total volume	20µl		

PCR Programme set in thermal Cycler for ISSR Primer

Steps	Temperature (°C)	Duration	Cycle (No.)
Initial denaturation	94	5.0 min	1
Denaturation	94	30 sec	40 Cycles
Annealing	Primer	30 sec	
Extension	72	1 min	
Final extension	72	10 min	1

5) Agarose gel electrophoresis of amplified PCR products

After PCR amplification, the PCR products (SSR analysis) were seperated by 2 % agarose gel electrophoresis.

6) Data analysis

The ISSR fragments scored after diversity database analysis and were recorded as 1 (band/peak present) and 0 (band/peak absent).

RESULTS

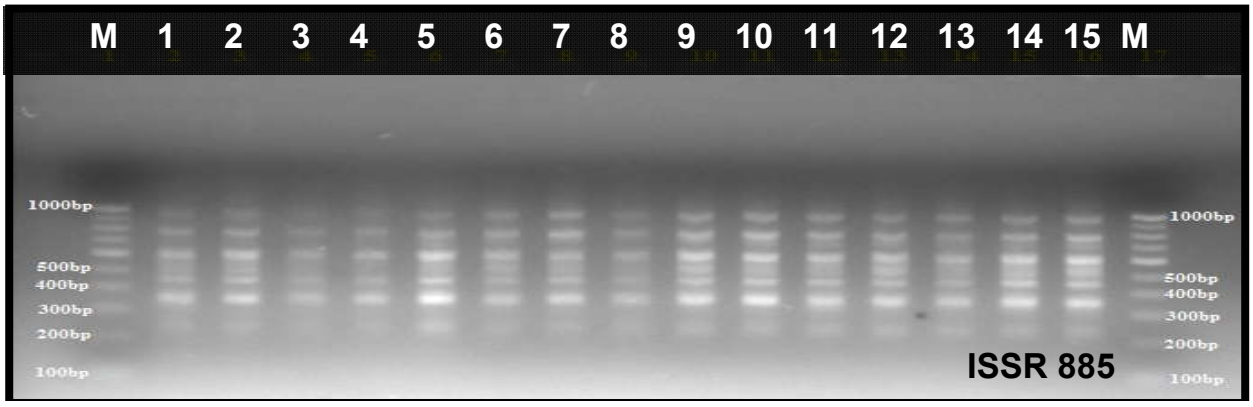
Quantification and purity analysis of genomic DNA

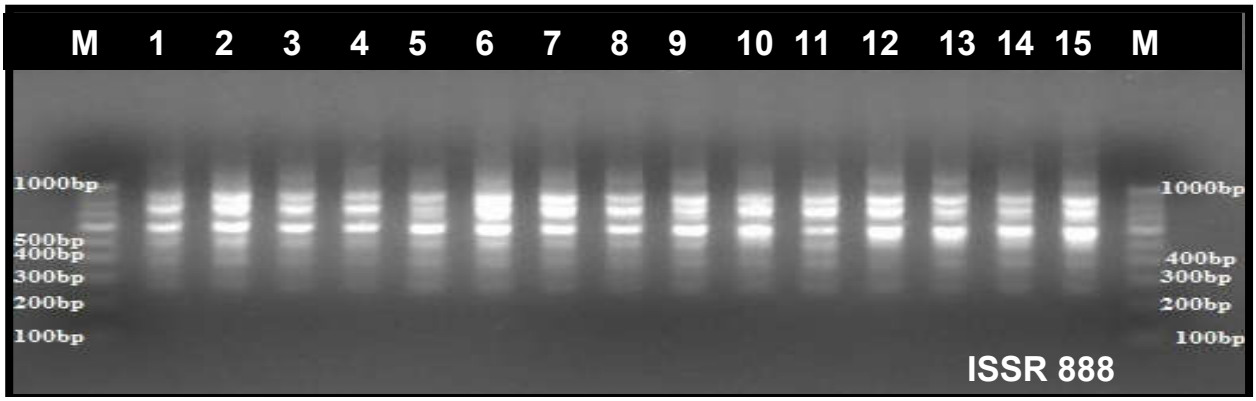
The amount and purity of DNA was quantified by measuring the Optical Density (O.D) both at 260 and 280 nm wavelength on UV-Spectrophotometer. The DNA was diluted for individual sample working concentration of 60 ng/µl.

ISSR analysis of genomic DNA.

The genomic DNA isolated from 15 soybean genotypes were subjected to PCR amplification using 13 ISSR primers. It was observed that out of these 13 ISSR primers, 12 of them amplified and showed polymorphism.

From the ISSR analysis of 15 genotypes, it was observed that a total of 71 bands were generated by amplification with 12 polymorphic primers. Out of which 49 bands were polymorphic with an average of 69.01% polymorphism. Each primer thus produced on an average 4.08 polymorphic bands.





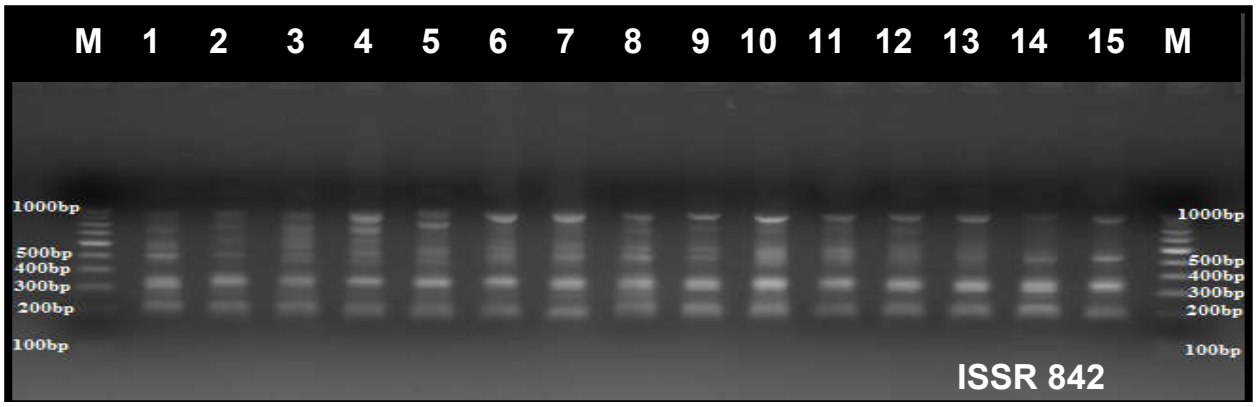
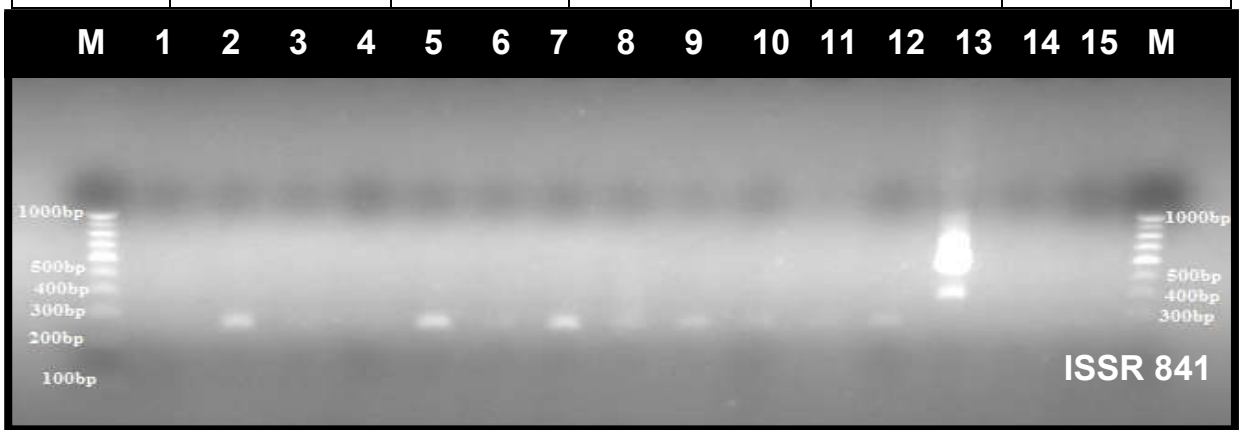
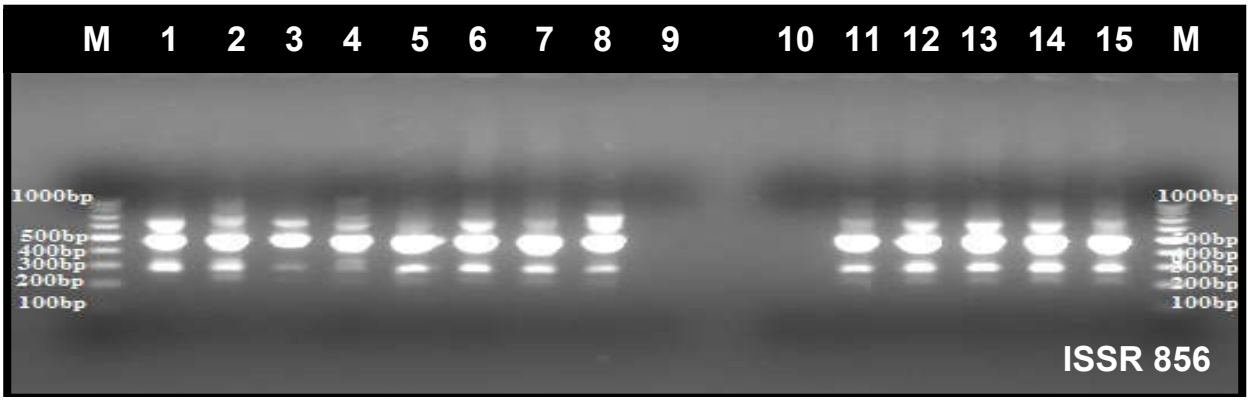
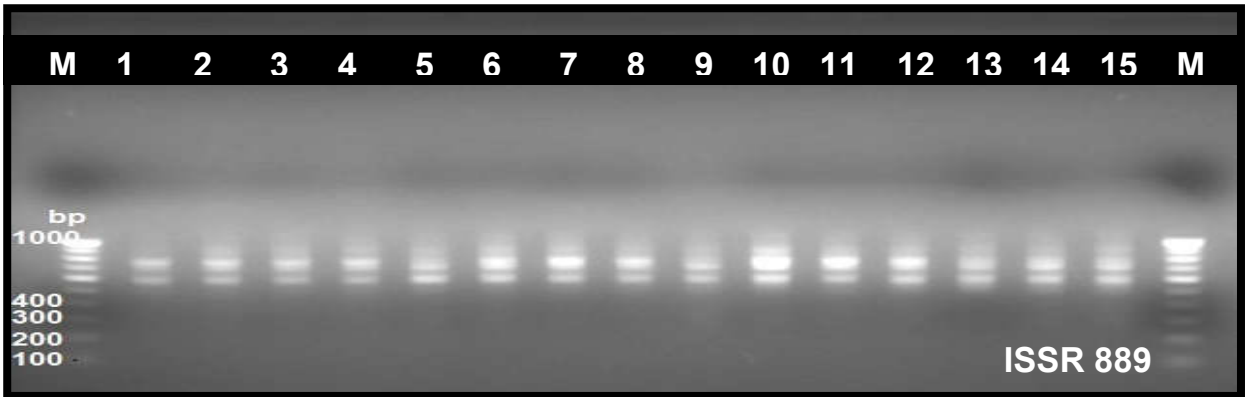


Plate : ISSR primer based DNA polymorphisms of soybean genotypes
Lane M = Marker 100 bp

Lane No.	Genotype	Lane No.	Genotype	Lane No.	Genotype
1	KS-103	6	JS-335	11	Monetta
2	KS-106	7	KDS-739	12	Kalitur
3	KS-112	8	NRC-94	13	Birsa soya
4	KS-128	9	KDS-722	14	DS-9712
5	DS-228	10	MAUS-71	15	MACS-45







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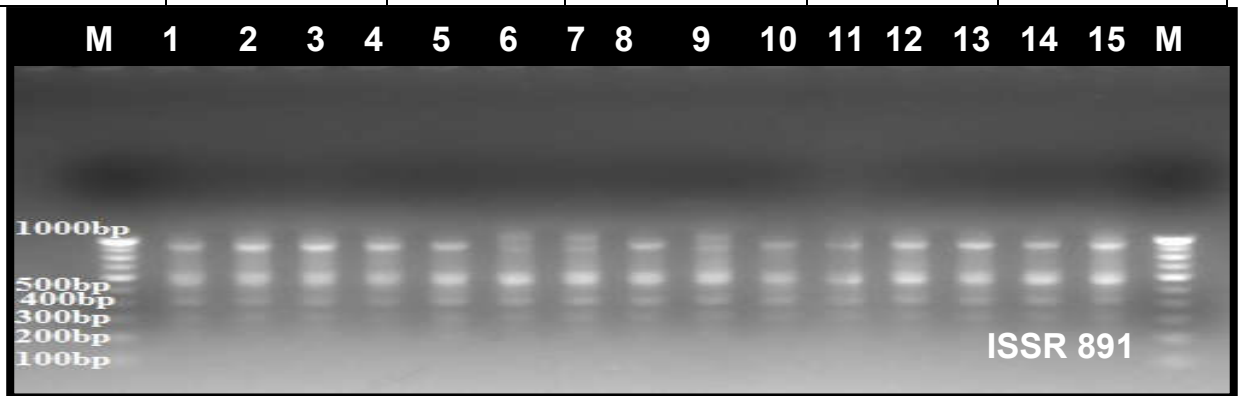
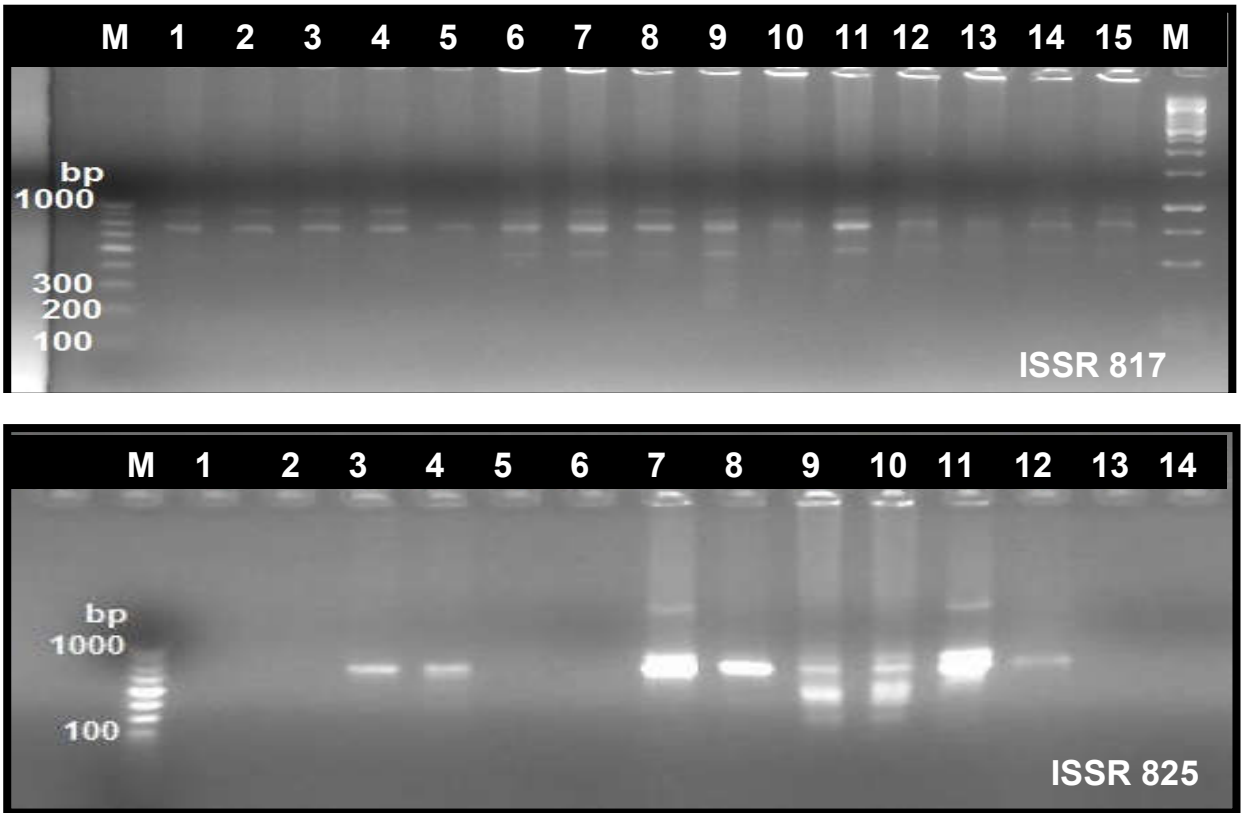
5	DS-228	10	MAUS-71	15	MACS-450





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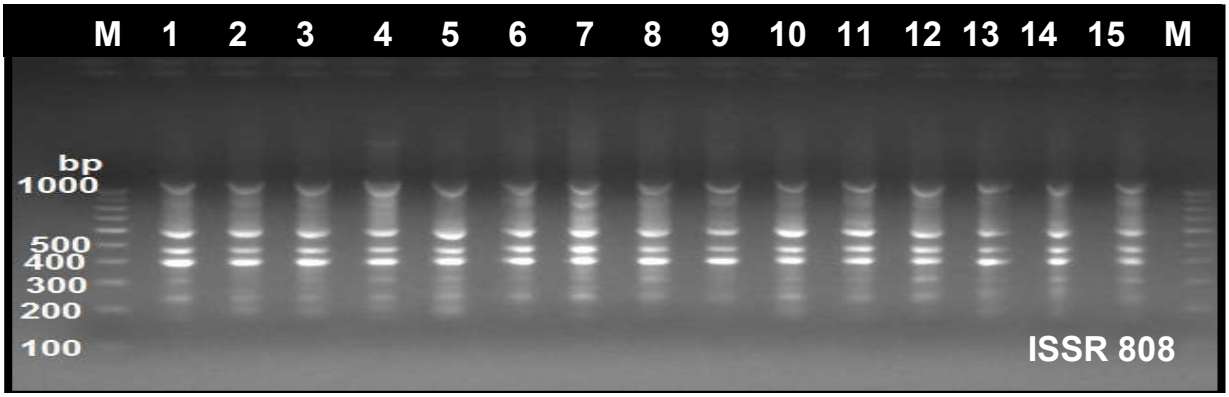

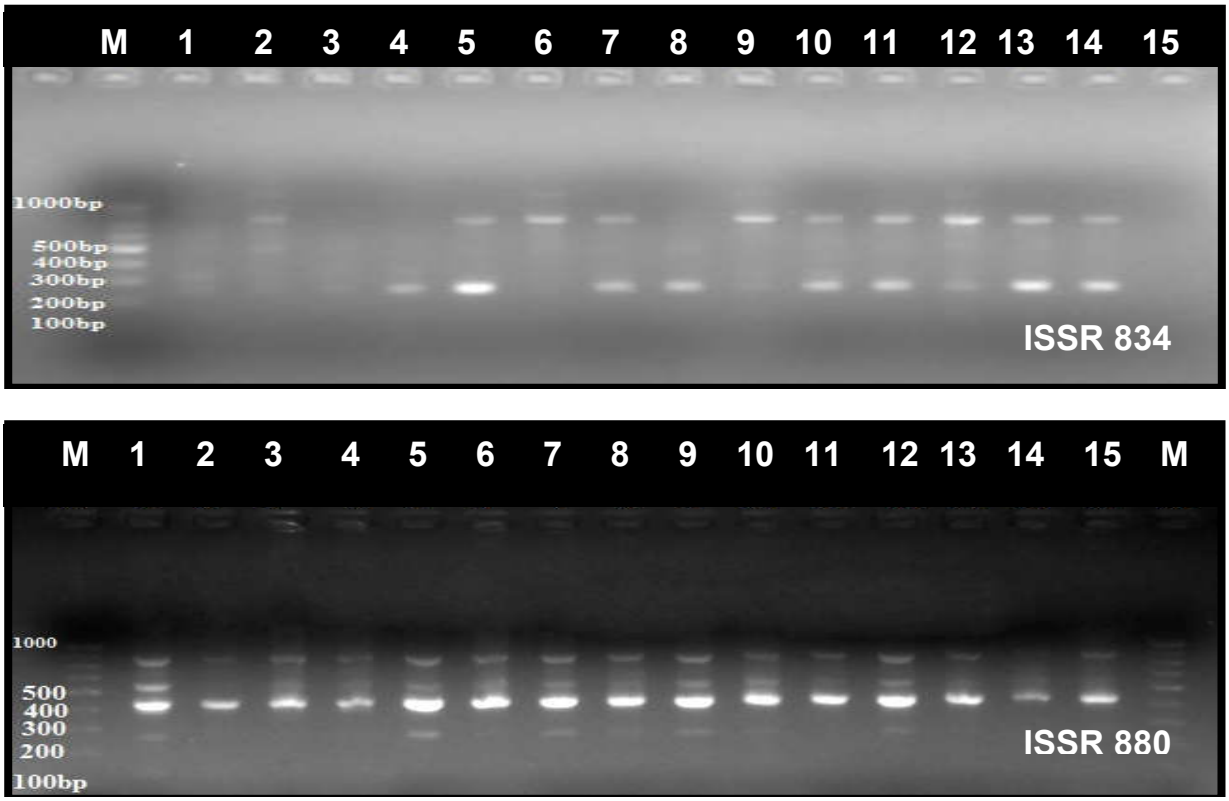

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Analysis of soybean genotypes with ISSR primers

Sr. No.	ISSR Analysis	Observations
1.	Total number of primars used	13
2.	Number of primers amplifying DNA	12
3.	Number of primers not amplifying DNA	01
4.	Total number of polymorphic primers	12
5.	Total numbers of bands	71
6.	Total number of polymorphic bands	49
7.	Total number of monomorphic bands	22
8.	Total number of unique bands	08
9.	Per cent polymorphism	69.01
10.	Average number of bands/primers	5.91
11.	Average polymorphic bands produced per marker	4.08

Among the ISSR primers, ISSR-842 produced maximum number of 9 bands followed by ISSR-888 and ISSR-808 (8 bands) and ISSR-885 (7 bands). However, least number of bands was amplified by ISSR-856, 889, 817 and 834 primers (4 bands). The highest (100%) polymorphism was shown by ISSR 888, ISSR 841, ISSR 817, ISSR 825, while ISSR 885 primer showed minimum i.e., 14.28% polymorphism as shown below.

Amplification details of individual ISSR primer pairs used in soybean genotypes studies.

Sr. No	ISSR Primer	No. of bands amplified	Poly-morphic bands	Mono-morphic bands	Unique bands*	% Poly-morphic bands	Size range (bp)	PIC
1	885	07	01	06	00	14.28	235-980	0.845
2	888	08	08	00	00	100	300-866	0.844
3	842	09	08	01	00	88.88	219-966	0.856
4	841	05	05	00	04	100	282-916	0.444
5	856	04	01	03	00	25	240-600	0.725
6	889	04	03	01	01	75	566-750	0.604
7	891	06	05	01	01	83.33	251-980	0.801
8	817	04	04	00	00	100	457-850	0.736
9	825	06	06	00	02	100	376-700	0.744
10	808	08	03	05	00	37.50	131-1000	0.839
11	834	04	03	01	00	75	284-850	0.694
12	880	06	01	05	00	16.66	350-950	0.823

*Unique bands are also counted under polymorphic bands.

The PIC values and were calculated to find out the efficiency of primers in distinguishing individual genotypes. The polymorphism information Content (PIC) values of ISSR primers ranged from 0.444 in ISSR primer 841 to 0.856 in ISSR primer 842. ISSR primers ISSR 888, ISSR 841, ISSR 817 and ISSR 825 showed maximum per cent polymorphism and ISSR primer 856 showed maximum PIC values. Further it was revealed that minimum per cent polymorphism showed by ISSR 885.

Of the 12 polymorphic ISSRs, 4 markers showed unique amplification in soybean genotypes. Out of 15 genotypes, 4 markers amplified specific unique bands in 5 genotypes. Details of these markers are given below. These primers amplified unique bands that can be very useful in characterization and identification of specific soybean genotypes.

Table 14: Unique ISSR bands amplified in 5 soybean genotypes

Sr. No.	soybean genotypes	Primer revealing unique ISSR (Size of base pairs of amplified fragment)
1.	KS -103	ISSR 891 (455 bp)
2.	KDS-739	ISSR 825 (687 bp)
3.	MAUS-71	ISSR 825 (425 bp)
4.	Birsa soya	ISSR 841 (407 bp), ISSR 841 (588 bp), ISSR 841 (700 bp), ISSR 841 (916 bp)
5.	MACS-450	ISSR 889 (640 bp)

Genetic diversity analysis by ISSR primers.

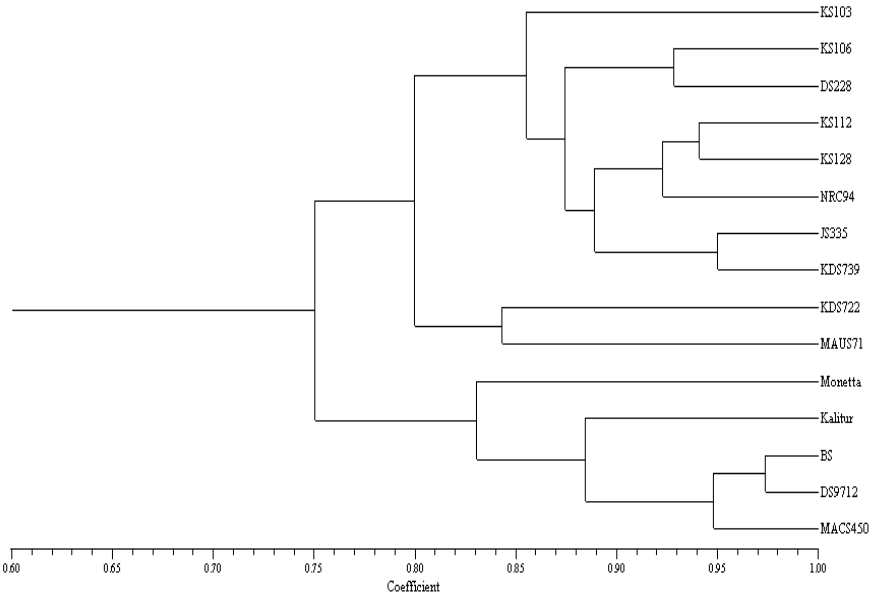
The Dice similarity coefficient values among soybean on ISSR analysis are presented below. The pair wise similarity values ranged from 0.67 to 0.97. Maximum similarity value of 0.97 was noticed in between Birsa soya and DS-9712. Minimum similarity value of 0.67 was observed in between KS-106 and Birsa soya.

To visualize the genetic relationship among 15 soybean genotypes, a dendrogram was constructed based on the UPGMA method from similarity matrix using NTSYSpc 2.02i Programme was presented. Based on cluster analysis using ISSR markers, genotypes were grouped into three clusters (A, B and C). First cluster A comprised of eight genotypes *viz.* KS-103, KS-106, KS-112, KS-128, DS-228, JS-335, NRC-94 and KDS-739. Second cluster B comprised of two genotypes *viz.* KDS-722 and MAUS-71.

Third cluster C consist of five genotypes *viz.* Monetta, Kalitur, Birsa soya, DS-9712, MACS-450.

The Dice similarity coefficient value based on ISSR markers data

	KS103	KS106	KS112	KS128	DS228	JS335	KDS739	NRC94	KDS722	MAUS71	Monetta	Kalitur	BS	DS9712	MACS450
KS103	1.0000														
KS106	0.8861	1.0000													
KS112	0.8608	0.9048	1.0000												
KS128	0.9231	0.9157	0.9412	1.0000											
DS228	0.8608	0.9286	0.8810	0.8434	1.0000										
JS335	0.8052	0.8537	0.8780	0.8395	0.8780	1.0000									
KDS739	0.8052	0.8780	0.9176	0.8571	0.9024	0.9500	1.0000								
NRC94	0.8462	0.8434	0.9412	0.9048	0.8434	0.9136	0.9286	1.0000							
KDS722	0.7467	0.7750	0.7952	0.8049	0.8250	0.8462	0.8537	0.8293	1.0000						
MAUS71	0.7429	0.7467	0.8000	0.7595	0.8267	0.8219	0.8101	0.8101	0.8434	1.0000					
Monetta	0.7561	0.7816	0.7556	0.7865	0.7586	0.8000	0.7640	0.7865	0.8140	0.7711	1.0000				
Kalitur	0.7160	0.7209	0.7273	0.7126	0.7442	0.7619	0.7126	0.7356	0.7619	0.7654	0.8696	1.0000			
BS	0.6842	0.6667	0.7143	0.6747	0.7143	0.7561	0.7561	0.7470	0.7342	0.8108	0.8046	0.8372	1.0000		
DS9712	0.7105	0.7342	0.7342	0.6923	0.7848	0.7792	0.7792	0.7692	0.7568	0.8406	0.8293	0.9136	0.9737	1.0000	
MACS450	0.7013	0.7250	0.7250	0.6835	0.7750	0.7949	0.7692	0.7595	0.7733	0.8286	0.8193	0.9024	0.9351	0.9610	1.0000



Consensus tree showing clustering of fifteen soybean genotypes using ISSR markers.

CONCLUSIONS

Molecular analysis of soybean in relation to assessment of diversity using ISSR analysis revealed that ISSR markers have a higher discrimination capacity. Among ISSR primers four primers showed 100% polymorphism represents capability of these primers to amplify the less conserved regions of the DNA. The ISSR analysis depicted that soybean genotypes used in the present investigation were divergent. In ISSR markers 4 primers were found to be informative out of 12 ISSR primers. The Dice similarity coefficient values for ISSR primers indicated large diversity among soybean genotypes. The identical

clustering pattern was observed by ISSR primers. Unique bands produced by ISSR primers may be the variety specific.

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