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A Comparative Analysis of Genetic Diversity in Genus *Vigna savi* Genotypes using ISSR

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Abstract: Molecular markers have great potential for use in quality control in breeding programme. The phylogenetic relationships of ten *Vigna savi* genotypes were analyzed by using inter simple sequence repeats (ISSR) markers. The number of amplified fragments ranged from 5 (UBC 819) to 17 (UBC 821) which varied in size from 125 bp to 1500 bp. Amplification of genomic DNA of ten genotypes, using ISSR markers, yielded 37 fragments, of which 20 were polymorphic while the remaining were monomorphic in nature. Of the 37 amplified bands, 20 bands (54.05%) were polymorphic with an average of 6.66 polymorphic fragments per primer. ISSR based dendrogram indicated that ten genotypes were clustered into 3 clusters (I, II and III) with 6, 3 and 1 genotypes respectively. Cluster I comprised of 2 sub clusters that is Ia and Ib, thus all 6 genotypes are grouped together in cluster I. Cluster II comprised of 3 genotypes and cluster III comprised of only one genotype, that is, *Vigna radiata* (L) Wilczek- BM 4, which is significantly genetically different from all other *Vigna radiata* cultivars used in the present investigation and suggesting utilization of this species over others for improvement programme. In comparison to other species, *Vigna radiata* (L) Wilczek- NVL 1 showed better closeness with cluster Ia and Ib cultivars .The results of the present study can be seen as a starting point for future researches on the population and evolutionary genetics of these genotypes.
Keywords: Genetic diversity, *Vigna savi*, PCR, ISSR and dendrogram.

INTRODUCTION

Inter simple sequence repeats – polymerase chain reaction (ISSR-PCR) is a simple and quick technique and useful in areas of genetic diversity, phylogenetic studies, gene tagging, genome mapping and evolutionary biology in a wide range of crop species[1]. All food legumes are valuable sources of proteins, minerals, vitamins and occupy a very important place in human nutrition. Traditionally, classification of various subgenera, species, and subspecies is based primarily on morphological attributes. However, these traits may not be significantly distinct and usually require growing plants to maturity prior to identification. Moreover, morphological characters may be unstable due to environmental influences. Over the years, the methods for detecting and assessing genetic diversity have extended from analysis of discrete morphological traits to biochemical and molecular traits. Several DNA marker systems are now commonly used in diversity studies of plants. The most commonly used marker systems are restriction fragment length polymorphism (RFLP) [2], random amplified polymorphic DNA (RAPD) [3], amplified fragment length polymorphism (AFLP) [4], ISSRs [5] and microsatellites or simple sequence repeats (SSRs) [6]. Among them to characterize DNA variation patterns within species and among closely related taxa in *Vigna* species have been RAPD [7, 8], AFLP [9, 10, 11, 12], RFLP [13], ISSR [14], SSRs [8,15] and sequence tagged microsatellite site (STMS) [16]. Molecular modeling was successfully

used to detect three dimensional arrangements of atoms in isolated compounds of curcuma longa, ginger and seeds of fenugreek mixture by using computer assisted molecular design (CAMD) [17]. ISSR fingerprinting was developed such that no sequence knowledge was required. Primers based on a repeat sequence and the resultant PCR reaction amplifies the sequence between two SSRs, yielding a multilocus marker system useful for fingerprinting, diversity analysis and genome mapping. PCR products are radiolabelled or they are separated on a polyacrylamide sequencing gel prior to autoradiographic visualization. ISSR would be a better tool than RAPD for phylogenetic studies [14, 18]. The ISSR primers produced several times more information than RAPD markers in wheat [19]. The marker index values of ISSR primers also added strength to the above results. Considering the potentials of the DNA marker based genetic diversity analysis, the present study aimed to assess and analyze the nature and the extent of genetic diversity among the genotypes of *Vigna savi* which were collected from Marathwada and Vidarbha region of Maharashtra State of India.

MATERIALS AND METHODS

Plant Material

The plant material used for this study comprised of ten accession of *Vigna savi* were obtained from Department of Pulses, Punjabrao Krishi Vidyapitha, Akola (MS), India and Agricultural research station, Badnapur (MS), India (Table-1) respectively.

DNA extraction

The genomic DNA was isolated from fresh, young and disease free leaves of selected species of the genus *Vigna savi* (100 mg each) by using modified Cetyl trimethyl ammonium bromide (CTAB) method [20]. The quality and quantity of extracted DNA was checked by agarose gel electrophoresis and Eppendorf make Biophotometer which directly gives the quantity of DNA present in the sample.

ISSR analysis

ISSR primers (University of British Columbia, Canada) synthesized by Sigma Aldrich Inc., were used for the polymorphism survey. The primers were with various di-, tri- and nucleotide SSR repeats. Total nine primers were subjected to genetic diversity analysis and three primers have produced clear and unambiguous bands. Each ISSR PCR amplification was carried out in 13 µl reaction mixture containing 25 ng of genomic DNA, 2 µl of primer (3 pmol/ul), 10 mM of dNTPs (2.0 µl), 50 mM KCl, 10 mM Tris HCl (pH 8.3), 2.5 mM $MgCl_2$, 0.4 µl (3U/ul) of *Taq* DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India). PCR reactions were carried out in a Finnzyme make thermal cycler. PCR conditions were programmed for initial denaturation at 94ºC for 3 min., 40 cycles of 1 min. denaturation at 94ºC, 45 sec. annealing at 46-48ºC, temperature varied according to primer, 2 minutes extension at 72ºC, and final extension for 5 min. at 72ºC. The annealing temperature for PCR amplification was maintained based on the specificity of the primer pair used. PCR amplified products of ISSR primers were subjected to horizontal gel electrophoresis using 1.5% agarose gel in 1X TBE buffer at 60 V until loading dye reached other end of gel using Broviga™ standard submarine gel electrophoresis unit. As size marker we used a 1kb DNA ladder and the ethidium bromide stained gels were documented using Alpha Imager 1200™ (Alpha Innotech Corporation, USA).

Data analysis

For each sample, each fragment that was amplified using ISSR primers was treated as a unit rearrangement in genome. The primers which gave scorable and consistently reproducible amplicons were considered for scoring. The banding patterns obtained from ISSR markers were scored as present (1) and absent (o). The data obtained by scoring the ISSR profiles with different primers individually as well as collectively were subjected to the construction of similarity matrix using Neighbour-joining tree construction method of Nei and Li/Dice [21]. The similarity values were used for cluster analysis. The gel pictures were taken and documented to computer by using Alpha Imager gel documentation system and size of each amplicon was measured by using Alpha Imager Software with respect to standard molecular weight DNA ladder and molecular weight of each of the potential specific bands was calculated using the software program Alpha Imager. The dendrogram was plotted by using bioinformatics phylogeny Free Tree and Tree View of DNA fingerprint analysis tool.

RESULTS AND DISCUSSION

A small aliquot of isolated DNA was run on a 1% (w/v) Tris acetate EDTA (TAE) gel to check the quality of DNA sample and all the selected samples have shown high molecular weight intact genomic DNA band (Fig.1). The DNA quantification was carried out by using Biophotometer which ranged from 530 µg/gm to 660 µg/gm. Amplification of genomic DNA of 10 genotypes using ISSR marker analysis, yielded total amplified 304 fragments. ISSR analysis yielded 37 fragments that could be scored, of which 20 were polymorphic while the remaining were monomorphic in nature (Fig. 2 a, b and c). In ISSR analysis number of amplified fragments ranged from 05 (UBC 819) to 17 (UBC 821) (Fig.2 b and c) which varied in size from 125 bp to 1500 bp. Of the 37 amplified bands, 20 bands (54.05%) were polymorphic with an average of 6.66 polymorphic fragments per primer (Table-2). The percentage of polymorphism ranged from 20% (primer UBC 819) to 88.23% (primer UBC 821). The primers based on poly (GT) produced maximum number of bands (17 bands) while poly (GT with TA at 3') produced minimum number of bands (5 bands).The PCR amplification using ISSR primers gave rise to reproducible amplification products. The complete data was based on a total of 304 bands. A Dendogram analysis was carried out by using bioinformatics phylogeny tool Free Tree and Tree View of DNA fingerprint analysis (Fig.3). Distance similarity matrix was calculated by using Neighbour-joining tree construction method of Nei and Li/Dice which ranged from 0.01587 to 0.29825. Earlier the comparative analysis of genetic diversity was also attempted by using ISSR and RAPD markers in *Vigna* genotypes, *Jatropa curcus* and *blackgram* also [14, 22, 23].

The ten *Vigna savi* genotypes were clustered into 3 viz., cluster I, II and III with 6, 3 and 1 genotypes respectively (Fig.3). Cluster I comprised of 2 sub-clusters that is I-a and I-b. Subcluster I-a consisted of 3 genotypes namely *V. radiata* (L) Wilczek BM 2002-01,
V. aconitifolia (Jacq.) Marechal, *V. umbellata* (Thunb.) Ohwi and Ohashi while I-b comprised of 3 genotypes namely *V. radiata* (L) Wilczek BPMR 145, *V. radiata* (L) Wilczek AKM 8802, *V. mungo* (L) Hepper -Tau 1. Thus, all 6 genotypes are grouped together in cluster I. Cluster II comprised of 3 genotypes namely *V. radiata* (L) Wilczek NVL 1, *V. mungo* (L) Hepper - BDU 1 and *V. unguiculata* (L.) Walp. Cluster III consisted of only one genotype viz., *V. radiata* (L) Wilczek BM 4 which was significantly different from all other genotypes used in the present investigation.

CONCLUSION

Cross breeding between genetically different individuals is a recommended, rather than involving individual belonging to related genetic group [24]. The evaluation of genetic diversity and construction of linkage maps has been considered desirable for the efficient use of genetic variations in the breeding programme [25]. ISSR analysis reported in the present work could be useful to select parents to be crossed for generating appropriate populations intended for both genome mapping and breeding purposes [22]. In comparison to other species, *V. radiata* (L) Wilczek BM 4 showed most genetic variability with cluster I and II, suggesting utilization of this species over others namely *V. radiata* (L) Wilczek AKM 8802, *V. radiata* (L) Wilczek BPMR 145, *V. radiata* (L) Wilczek NVL 1 and *V. radiata* (L) Wilczek BM 2002-01 for breeding programme and in transferring the characters into *Vigna radiata* cultivars. Comparatively, *V.mungo (L) Hepper* - BDU 1 showed more genetic variability as compared to *V. mungo* (L) Hepper -Tau 1.

The genotype *V. radiata* (L) Wilczek NVL 1 showed more closeness with sub cluster I-a and I-b as compared to other genotypes of *Vigna savi*. The genotype of *V. umbellata* (Thunb.) Ohwi and Ohashi showed most genetical similarity with *V. aconitifolia* (Jacq.) Marechal and *V. radiata* (L) Wilczek BM 2002-01. The genotype of *V. unguiculata* (L.) Walp. showed less genetical similarity with rest of the genotypes of *Vigna savi*. The *V. radiata* (L) Wilczek BM 4 and *V.mungo (L) Hepper* - BDU 1 showed more genetic variability, thus, may ultimately be useful for breeding programme and in transferring the characters into *Vigna radiata* cultivars.

Table -1: *Vigna savi* genotypes used for genetic diversity study through ISSR markers.

Sr.No.	Selected species of *Vigna savi*	Code
1.	*Vigna mungo* (L.) Hepper -Tau 1	S 1
2.	*Vigna umbellata* (Thunb.) Ohwi and Ohashi	S 2
3.	*Vigna radiata* (L.) Wilczek AKM 8802	S 3
4.	*Vigna radiata* (L.) Wilczek- BPMR 145	S 4
5.	*Vigna mungo* (L.) Hepper - BDU 1	S 5
6.	*Vigna unguiculata* (L.) Walp.	S 6
7.	*Vigna radiata* (L.) Wilczek- NVL 1	S 7
8.	*Vigna radiata* (L.) Wilczek- BM 4	S 8
9.	*Vigna radiata* (L.) Wilczek- BM 2002-01	S 9
10.	*Vigna aconitifolia* (Jacq.) Marechal	S 10

Table-2: ISSR primers with properties of amplified products in *Vigna* genotypes

Sr.No.	UBC[1] Primer	Sequence (5' – 3')	TNB	NPB	P%
1	813	CTCTCTCTCTCTCTCTT	15	04	26.6
2	819	GTGTGTGTGTGTGTGTA	05	01	20.0
3	821	GTGTGTGTGTGTGTGTT	17	15	82.3
	Total		37	20	-
	Average		12.3	6.6	54.05

TNB = Total number of bands
NPB = Number of polymorphic bands
P% = Polymorphism percentage
([1] – University of British, Columbia)

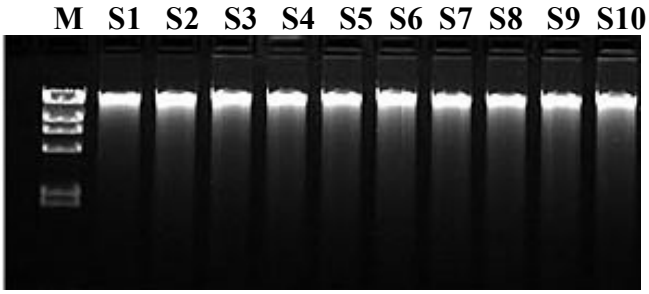

Figure 1: Isolated genomic DNA on 1% Agarose gel (M = DNA ladder)

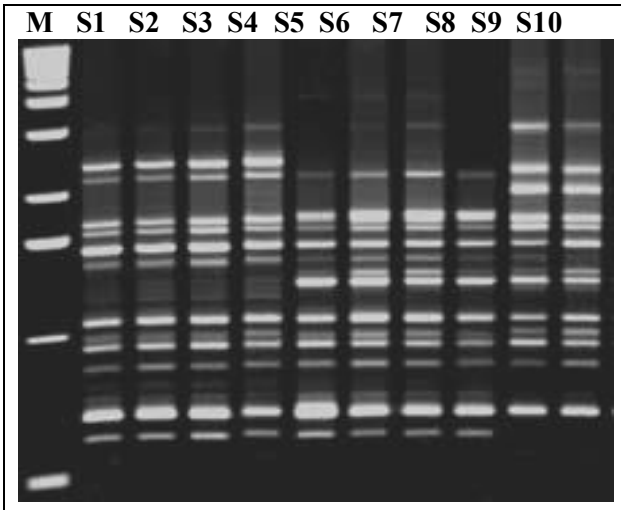

Fig.-2 (a)

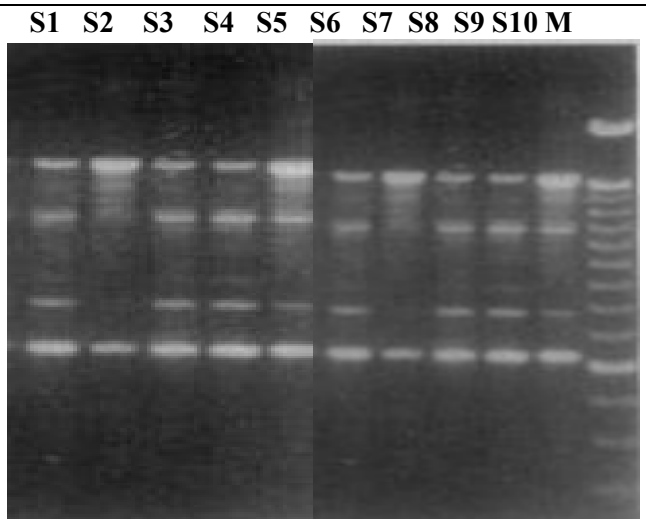

Fig.-2 (b)

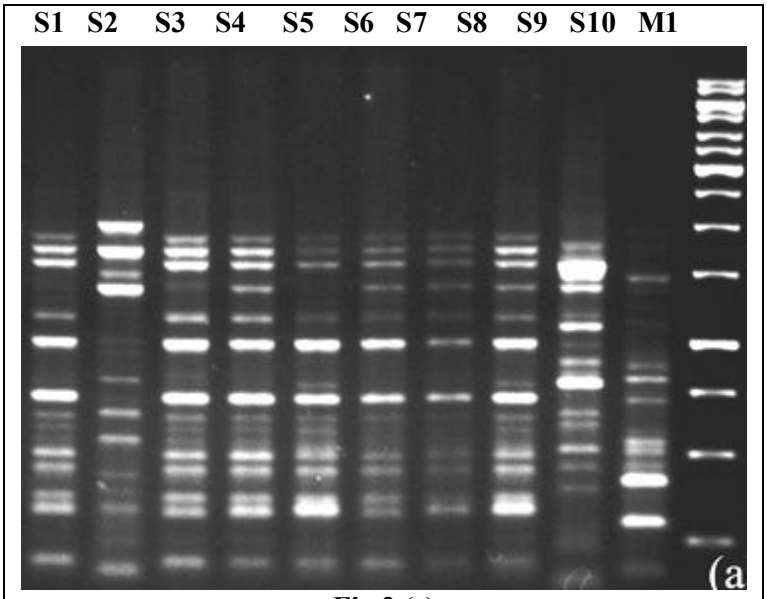

Fig.2 (c)

Figure 2: (a) ISSR profiles of 10 *Vigna savi* genotypes with primer UBC 813 (b) ISSR profiles with primer UBC 819 (c) ISSR profiles with primer UBC 821, M = 100 bp DNA ladder and M1 = 2 KB DNA ladder.

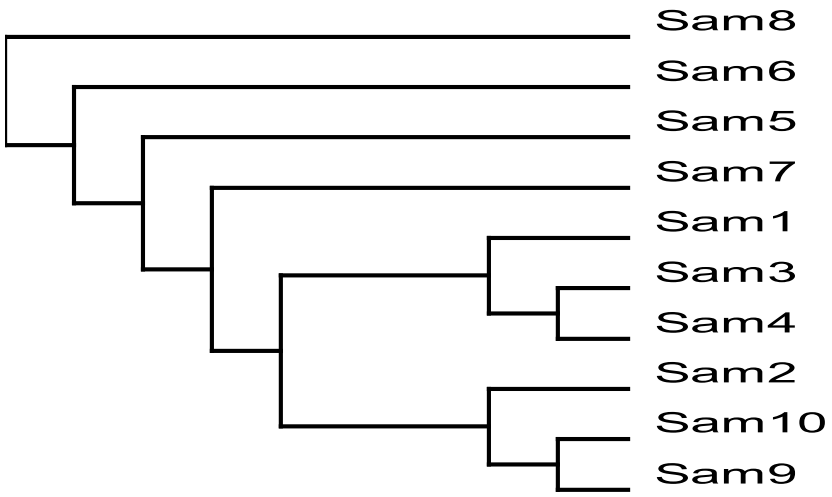

Figure 3: Dendrogram based on bioinformatics phylogeny tool Free Tree and Tree View of DNA fingerprint analysis.

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