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Performance of Microsatellite Inter-Simple Sequence Repeat Marker for Molecular Diversity of *Dolichos lablab*

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ABSTRACT
Genetic variety inside and among population is the reason for survival of the population both in short and long term. Along these lines, considering the plant genetic decent variety is fundamental for any protection program. Between basic grouping rehash (ISSR) were utilized to unwind the genetic fluctuation and connections crosswise over Ten increases of Dolichos lablab. Five ISSR preliminaries yielded a sum of 48 fragments with a normal of 9.60 parts for each primer. The measure of unmistakably noticeable enhanced Inter Simple Sequence Repeat -PCR fragments extended from 257 bp - 1550 bp and the quantity of groups produced by three primers differed and is same for two primers. The normal polymorphic data content esteem got with ISSR markers was 0.394. Marker Index (3.67) and Resolving power (5.26) show that the ISSR markers were generally more proficient in uncovering the hereditary assorted variety of Dolichos lablab in light of the fact that higher the esteem, more productive the marker is. UPGMA Dendogram demonstrates the nearest hereditary assorted variety among the Genotype with every one of the preliminaries. The outcomes affirmed the value of ISSR markers to survey the hereditary assorted variety among the chose Dolichos lablab genotypes for hereditary preservation and plant change.
Keywords: *Dolichos lablab, Genetic Marker, ISSR, Primer, Diversity*

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INTRODUCTION:

Now a Days, genetic markers progressively wont to address varied queries in Agriculture. Though microsatellites area unit without doubt still the marker of selection for several studies in Molecular genetic that need markers with hyper variability, high resolution, and co-dominance [1, 2], ISSR markers with its easy application makes genetic variation studies a lot of accessible to beginners and less-funded.

For targeting multiple genomic loci, microsatellite primers uses are usually 16 −25 bp in length which are of di, tri, tetra or penta-nucleotide repeats. Anchored or unanchored primers [3-5] at 3' or 5' end extended into the flanking sequences having degenerate bases 1- 4 [6]. These primers shows polymorphism whenever there is a deletion or addition of genome and usually high level of polymorphism showed by anchored with di-nucleotide repeats either at 3' or 5' end [7-9].

Banding pattern at 3' end of anchored primers is much more clear than those anchored at 5' end [7, 8, 10]. In general, primers with (AT) repeats show lesser polymorphism because (AT) primers repeats tend to be self- annealed as compared to (GA), (AG), (CT), (AC, (TC), (CA) repeats primer. Following Mendelian inheritance, ISSR markers are usually considered as dominant markers [10-12]. However, there are cases where they act as co-dominant markers and helped to distinguish homozygotes from heterozygotes [4, 12, 13].

ISSR markers have many advantages over other markers. Like the RAPD technique, it is quite simple, quick and cheeper. Due to the longer primer length, ISSR markers have high reproducibility as compared to RAPD primers. Research on reproducibility tells that about 92 − 95% of the fragments scored could be the repeated across samples of DNA of the same cultivars and across separate PCR runs [14, 15]. Improvement of ISSR markers does not require earlier information of the genome to be investigated; subsequently, it very well may be utilized all around for plant genome examination. ISSR markers give more polymorphism [16, 14] when contrasted with isozymes, as a result of the absence of mutational limitations in the bury basic grouping rehashes as they are to a great extent part of the noncoding locales of the genome, while isozymes are from the coding areas of the genome [16].

For any harvest change program, fundamental data on the fluctuation present in the product is basic. Yield, being an intricate quality, is on the whole impacted by different segment characters, which are polygenetically acquired and exceptionally affected by ecological variety. Parceling the fluctuation into heritable and non-heritable segments with reasonable hereditary parameters, for example, genotypic and phenotypic coefficient of variety; heritability gauges and hereditary development circumstances and end results relations through phenotypic connections do assist a lot with formulating determination procedures to create appropriate genotypes. An examination concerning the nature and level of disparity empowers scientists to distinguish hereditarily different genotypes for hybridization, which would bring about instigating a wide range of inconstancy and the fuse of attractive qualities in recombinant kinds. It has additionally been seen in *Lablab* that there is wide phenotypic inconstancy however minimal hereditary decent variety [17-19].

An all around soaked hereditary guide is a need for a rearing system in view of marker helped choice. To encourage the reproducing exercises, considers were started in connection to marker and populace advancement with the point of giving instruments to the investigation of Indian *Dolichos bean* hereditary and phenotypic changeability.

MATERIAL AND METHODS

DNA Extraction and PCR Reactions

Genomic DNA was extracted from fresh leaf tissues for each gene type using the modified CTAB method. DNA integrity was identified through Gel electrophoresis (0.8%) and quantification was made by recording its absorbance at 260 nm and 280 nm using a UV-VIS Spectrophotometer.

PCR Analysis

In a study a total of 5 primers (Table 1) were selected for analysing 10 Genotype of *Dolichos***.** SSR-PCR were performed in a 25 µl reaction volume containing 1xPCR buffer, 1.5 mM $MgCl_2$, 0.4 mM of each primer, 0.4 µM of mixed dNTPs, 0.5U Taq DNA Polymerase and 50-100 ng/µl template genomic DNA. The amplification was performed in a Bio-Rad Thermo cycler at an Initial Denaturation at 94^{o} C for 1 minute followed by 40 cycles of Denaturation at 94^{o} C for 1 minute drop to 55°C at a 22% ramp rate, 2 min primer annealing at 55°C, rise to 72°C at a 55% ramp rate, 0.5 min primer extension at 72°C and rise to 94°C at a 57% ramp rate and a final Extension at 72°C for 5 minutes. Finally, amplified products were resolved on 2.5% Agarose gel at 50V for 120 min and gel was visualized under UV light and the image was captured.

Data analysis and Polymorphic information Content:

To score banding information, a photo of the gel was clicked by advanced camera, in Gel documentation unit. Groups of ISSR were appeared based on their molecular weight (length of increased polynucleotide). The separation gone by pieces which are opened up from the well were meant molecular sizes in connection to molecular weight marker. Band nearness was scored as '1' and band nonappearance as '0'. Groups which are faintly observed were not scored but rather a noteworthy groups identical to a faintly band were estimated for scoring. Keeping in mind the end goal to confirm the groups nearness and to decide the reproducibility every one of the ground works was recreated thrice. The information lattice of markers was then changed over into hereditary comparability network utilizing Jaccard coefficient (Jaccard 1908). The hereditary relatedness among the 32 people of three populaces was broke down utilizing unweighted combine bunch strategy with number juggling normal (UPGMA) in light of pairwise Nei's [29] hereditary separation.

To estimate the genetic profile of *D. lablab* genotypes, the markers performance were measured using 4 parameters (1) PIC, (2) MRP, (3) RP and (4) MI.
PIC of a band was evaluated as follow:PIC = 2fi (1-fi), where fi is the frequency of the band present, and 1 - fi is the frequency of band absent. The resolving power (RP) of each primer was calculated as: RP = ΣIb, where Ib shows the informative fragments. The Ib can be represented on a scale of 0 –1 by the following formula: Ib = 1-[2 x (0.5-p)], where p is the proportion of genotypes having band. Further, we calculated mean resolving power for each primer as: MRP = 1/n ΣBIi, where *n* is the No. of Polymorphic band for that primer (Prevost and Wilkinson, 1999). The MI was evaluated as [19]: MI = EMR x PIC, where, EMR = fraction of polymorphic loci x the number of polymorphic loci for an individual assay.

RESULT AND DISCUSSION

The utilized of ISSR marker is suggested for mutagenesis consider and concurred with Zahra et al. (2012) that ISSR uncovered high polymorphism and valuable for concentrate hereditary decent variety, introgression investigation and distinguishing proof of germplasm. Microsatellite preliminaries increasing dinucleotide, trinucleotide, tetranucleotide, or pentanucleotide rehash themes of 16bp –to 25 bp long are generally used to focus on different genomic loci in Inter Simple Sequence Repeat investigation [20, 21]. As a rule, dinucleotide continues having groundworks tied down either at the 3' or 5' end uncover high polymorphism [22]. Carvalho *et al.* [23] detailed that dinucleotide preliminaries were more appropriate for intensifying ISSRs in bread and durum wheat. In any case, every one of the preliminaries dissected in the present investigation demonstrated a high level of polymorphism (99.52%) among the chose 10 Genotypes of *Dolichos lablab*. In general, a high level of polymorphism was additionally detailed in a hereditary decent variety think about [24-27].
The intensification results of the ten genotypes yielded a sum of 48 fragments with a normal of 9.60 fragments for every primer in the present examination. The measure of plainly perceivable intensified ISSR-PCR sections went from 257 bp to 1550 bp and the quantity of groups created by three groundwork changed and same for two primers. The UBC 836 primer delivered the most elevated number of polymorphic fragments (13), while the least number of polymorphic fragments (03) was gotten with the UBC 8 primer. For instance, the PCR banding example of the UBC 880 and UBC 836 ISSR primer is appeared in Figure 2. The parallel information lattice created by the opened up pieces of the Ten *Dolichos lablab* genotype in the ISSR-PCR examinations was utilized for the calculations of hereditary characters for each pairwise correlation of the genotypes (Table 3, 4, 5, 6 and 7) for the investigation of ISSR information. The evaluated Similarity framework ran from 0 to 0.79 (Primer 836), 0.13 to 0.53 (Primer 840), 0.09 to 0.55 (Primer 880), 0 to 1 (Primer 881), 0.10 to 0.60 (Primer 888). These qualities can be utilized in a rearing project to such an extent that the genotypes with the least hereditary could be chosen as guardians to enhance the *Dolichos lablab* assortments. The UPGMA calculation was utilized for gathering all promotions in light of their genetic distances. Dendrograms speaking to most plausible genetic connection between increases relating to ISSR are introduced in Fig. 1.
The level of polymorphism is 100% for every one of the primers. To decide PIC estimations of every primer, the mean of PIC esteems is broke down for all loci. High PIC estimation of 0.443 (UBC 881 01) and low PIC estimation of 0.337 (UBC 836), with a normal estimation of PIC per primer 0.394 were gotten. The viable multiplex proportion relies upon the part of polymorphic sections. In this examination, the most astounding Matrix Index (MI) 4.43 was seen with the primer UBC 880 and the least Matrix Index (MI) 1.33 was seen with the primer UBC 881 with a normal MI of 3.67 for each primer. The resolving power (RP) is a parameter that demonstrates the prejudicial capability of the primers picked. The most astounding RP esteem was seen with the primer UBC 881 (6.60) and the least with the primer UBC 836 (4.30) with a normal RP of 5.26 for every primer where as most astounding Mean settling Power (MRP) was seen with the primer UBC 881 (2.20) and most reduced with the primer UBC 836 (0.33) (Table 2). The Phylogenetic tree prepared from Inter Simple Sequence Repeat profiles showed two major clusters with all the five primers used (Fig. 2).

Table 1: List of ISSR primer sequences, Annealing temperature, and GC content of ISSR Primers

No.	Primer Name	Sequence (5`□ 3`)	Tm (°C)	GC- Content
1	UBC836	AGAGAGAGAGAGAGAGYA	46(°C)	44%
2	UBC840	GAGAGAGAGAGAGAGAYT	46(°C)	44%
3	UBC880	GGAGAGGAGGAGAGGAGA	53(°C)	61%
4	UBC881	GGGTGGGGTGGGGTG	53(°C)	80%
5	UBC888	BDBCACACACACACACA	42(°C)	41%

Table 2: Marker parameters calculated for each ISSR primer used with *D. lablab*

Name of Primer	Total no. of Fragments	No. of Polymorphic Fragments	% of Polymorphic band	PIC	RP	MRP	MI
UBC 836	13	13	100	0.337	4.30	0.33	4.05
UBC 840	11	11	100	0.403	5.30	0.48	4.20
UBC 880	10	10	100	0.420	5.20	0.52	4.43
UBC 888	11	11	100	0.369	4.90	0.44	4.38
UBC 881	3	3	100	0.443	6.60	2.20	1.33

Table 3: Similarity matrix Data of Primer UBC 836

	730	843	733	847	746	776	739	757	702	799
730	0	0	0.29	0.21	0.14	0.36	0	0.07	0.29	0.5
843		0	0.29	0.21	0.14	0.36	0	0.07	0.29	0.5
733			0	0.36	0.29	0.21	0.29	0.21	0.43	0.64
847				0	0.36	0.57	0.21	0.29	0.5	0.43
746					0	0.36	0.14	0.21	0.43	0.5
776						0	0.36	0.29	0.21	0.71
739							0	0.07	0.29	0.5
757								0	0.21	0.57
702									0	0.79
799										0

Table 4: Similarity matrix Data of Primer UBC 840

	730	843	733	847	746	776	739	757	702	799
730	0	0.33	0.47	0.33	0.40	0.47	0.20	0.20	0.27	0.47
843		0	0.27	0.40	0.47	0.40	0.13	0.27	0.20	0.40
733			0	0.53	0.47	0.53	0.40	0.27	0.33	0.40
847				0	0.33	0.13	0.27	0.27	0.47	0.53
746					0	0.20	0.33	0.20	0.27	0.47
776						0	0.27	0.27	0.47	0.53
739							0	0.13	0.20	0.40
757								0	0.20	0.27
702									0	0.47
799										0

Table 5: Similarity matrix Data of Primer UBC 880

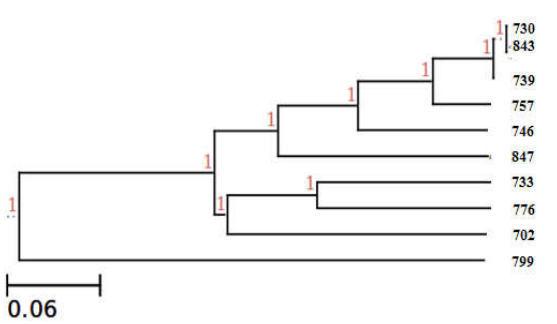
	730	843	733	847	746	776	739	757	702	799
730	0	0.55	0.27	0.55	0.27	0.27	0.36	0.55	0.27	0.46
843		0	0.64	0.36	0.46	0.46	0.36	0.36	0.27	0.46
733			0	0.27	0.36	0.36	0.46	0.27	0.36	0.18
847				0	0.46	0.46	0.36	0.36	0.46	0.09
746					0	0.18	0.27	0.46	0.18	0.36
776						0	0.27	0.46	0.18	0.36
739							0	0.36	0.27	0.46
757								0	0.46	0.09
702									0	0.36
799										0

Table 6: Similarity matrix Data of Primer UBC 881

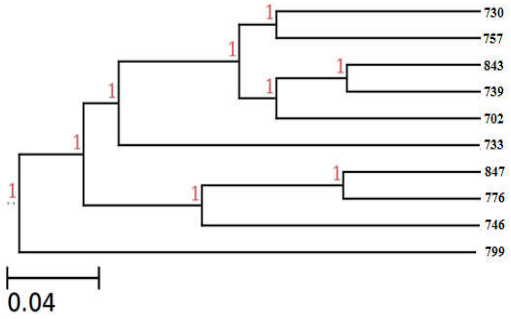
	730	843	733	847	746	776	739	757	702	799
730	0	0.33	0.66	0.66	0	0.33	0.66	0	0	0.66
843		0	0.33	0.33	0.33	0.66	0.33	0.33	0.33	0.33
733			0	0	0.66	1	0	0.66	0.66	0
847				0	0.66	1	0	0.66	0.66	0
746					0	0.33	0.66	0	0	0.66
776						0	1	0.33	0.33	1
739							0	0.66	0.66	1
757								0	0	0.66
702									0	0.66
799										0

Table 7: Similarity matrix Data of Primer UBC 888

	730	843	733	847	746	776	739	757	702	799
730	0	0.2	0.2	0.3	0.2	0.3	0.2	0.4	0	0.3
843		0	0.4	0.5	0.4	0.5	0.4	0.2	0.2	0.1
733			0	0.1	0.4	0.1	0.4	0.4	0.2	0.5
847				0	0.5	0.2	0.5	0.5	0.3	0.6
746					0	0.5	0.2	0.6	0.2	0.3
776						0	0.5	0.5	0.3	0.6
739							0	0.6	0.2	0.3
757								0	0.4	0.3
702									0	0.3
799										0



836



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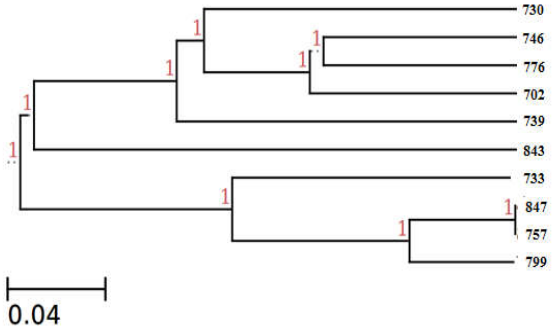

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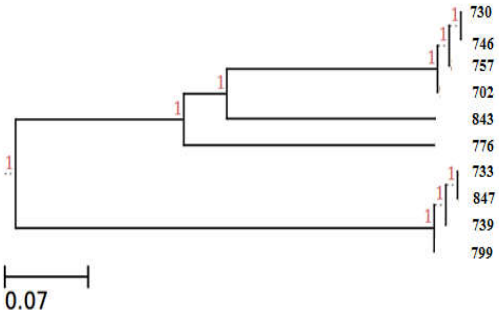

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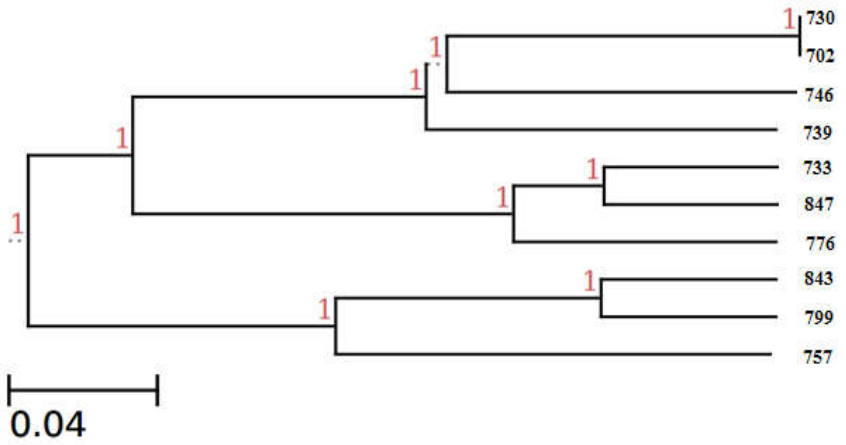

Figure 1: Dendrogram of *D. lablab* based on genetic distance obtained from ISSR markers using the UPGMA method.

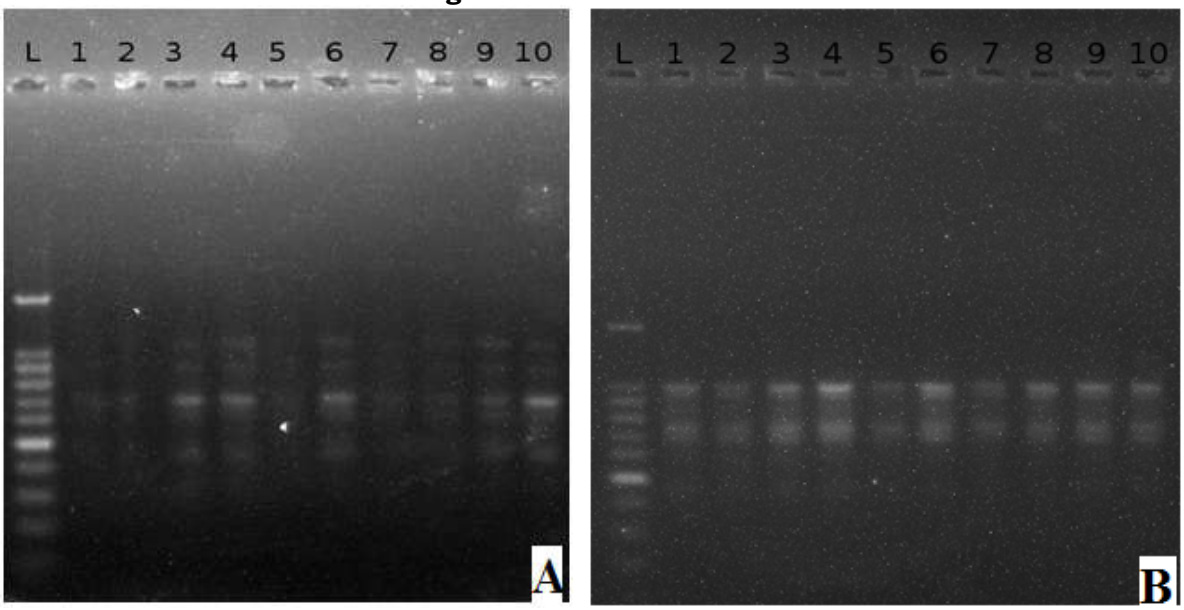

Fig 2: ISSR marker profiles of the amplified loci among the *Dolichos lablab* genotypes using primer UBC 880 (A) & UBC 836 (B). Each lane contains a different *Dolichos lablab* variety (1–10).

CONCLUSION

Investigation of ISSR items got from PCR enhancement is principal significance to fingerprinting as these data can be used and acknowledgment a standard in building national open databases. When all is said in done, these markers distinguish an adequate level of polymorphism with reproducible fingerprinting profiles to assess the hereditary decent variety among an assortment of plants including agricultural and field species. It is even announced that ISSR primers could identify more polymorphism than cpDNA, mtDNA, RAPD, and isozyme markers in firmly related plants. Inter Simple Sequence Repeat markers include enhancement of DNA parts between two indistinguishable recurrent areas. In the study, an high state of polymorphism was acquired among the chosen *D. lablab* genotypes utilizing ISSR markers. The high state of polymorphism among the *D. lablab* genotypes proposed that trained *D. lablab* germplasm was not restricted. The nearness of the polymorphic marker information is a critical apparatus for various rearing procedures. For instance, since the couple of genotypes have the most minimal genetic likeness in the present investigation they could be chosen as guardians to enhance the local *lablab* assortments. The present examination showed the benefits of Inter Simple Sequence Repeat primers to describe the genetic decent variety among ten developed genotypes of the class *lablab* with interesting, particular, and reproducible banding designs. These information

can additionally serve to reinforce the pertinence of rose rearing projects, likewise contrasting and morphological information for the assessment of *lablab* genotypes.

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