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Genetic evaluation of M_3 population of pigeon pea (*Cajanus Cajan* (L.) Millispaugh) through molecular markers

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Abstract
Farmers are hesitant to plant pigeon pea crop due to a lack of irrigation infrastructure during the *rabi* season because the crop lasts more than 180 days. As a result, there is a need for pigeon pea genotypes with early maturity (150 days). Present investigation meant to evaluate genetic diversity and variability of M_3 generation of pigeon pea through ISSR markers where laboratory work performed at Plant biotechnology Centre, College of Agriculture, Dapoli. Phenotypically 50 mutants were selected for growing further generation on the basis of yield and yield contributing characters. Variability of 50 selected mutants was confirmed through molecular markers which are not affected by environment. DNA isolation was done by using CTAB method. 20 ISSR primers were showed polymorphism. UBC 885 recorded maximum polymorphism percent *i.e.*, it shows more variation. Primer UBC 886 exhibited minimum polymorphism percentage. The dendrogram demonstrated that mutants. T1(21)7-8 and T1(67)11-5 had relatively more genetic distances over the control (Konkan Tur-1), *i.e.*, 0.505 bp and 0.470 bp, respectively, while T1(77)9-1(0.095bp) and T2(36)15-2(0.058 bp) had a few genotypic distances with respect to control.

Keywords: ISSR markers, genetic diversity, genetic variability, Polymorphism percentage, polymorphic information content

Introduction
Legumes have been acknowledged for their nutritional importance with high protein content, along with energy values and important vitamin and mineral content. Amongst legumes, Pigeon pea (*Cajanus cajan* L.) is mainly grown and consumed in India. It is also known as red gram, arhar, tur which belongs to the family of Fabaceae.

India is the native of pigeon pea because of its natural genetic variability available in the local germplasm and the presence of its wild relatives in the country. In Konkan region, area of Tur is very meagre due to unavailability of short duration crop. Most farmers produce this crop on rice bunds. During *kharif* season it required more than 180 days. As a result, there is a need for pigeon pea genotypes with early maturity. There is a scarcity of early duration cultivars suitable for Konkan conditions during the *kharif* season among the available germplasm. Similarly, long-lasting local genotypes are vulnerable to pod borer infestation, which will need to be addressed through different breeding approaches.

Mutation breeding is a viable option for creation of genetic variability. Similarly, through mutation breeding we can rectify the one or two defects present in widely adapted genotypes. Konkan Tur 1 is a variety widely adopted genotype for Konkan region of Maharashtra. The area under this crop is limited due to its longer duration (> 180 days) and susceptibility to pod borer. The mutants having less than 180 days duration and tolerant to pod borer has a great chance to obtain from mutants under study. Keeping the foregoing in mind, this project was approved with the general goal of examining genetic diversity patterns across 50 pigeon pea mutants derived from Konkan Tur 1 using ISSR markers in order to create genomic information that will be useful in accelerating molecular breeding in pigeon pea in future.

The DNA fingerprinting of varieties will aid in the development of a variety-specific fingerprint that reflects the stable genetic descriptor as well as existing morphological markers. Given the potential of DNA markers, the current study was carried out with the following goals in mind:

1. To study variability of M_3 population by molecular markers of Pigeon pea (*Cajanus cajan* (L.) Millsp.) generated through gamma irradiation
2. Diversity study of M_3 population of Pigeon pea (*Cajanus cajan* (L.) Millsp.)

2. Material and Methods

The current study, "Genetic evaluation of M3 population of Pigeon pea (*Cajanus cajan* (L.) Mill.) through molecular markers," was conducted at "Plant Biotechnology Centre", Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli-415712, Maharashtra state, India during the *kharif* period of 2021-2022.

2.1 Plant material and DNA extraction

Fifty morphologically distinct plants were chosen from a pigeon pea population cultivated in M3 generation during *kharif* 2021-22. DNA was extracted from young fresh leaves of 50 pigeon pea mutants and control.

The DNA was isolated using the procedure described by Edwards *et al*., 1991 [3], with minor changes to the buffer composition and concentration. To avoid contamination, the young newly flushing leaves were gathered and sterilised with 70% ethanol. Quality and quantity of isolated DNA was checked by 0.8% agarose gel electrophoresis. The DNA yield obtained was in the range of 1.0µg to 3.0 µg

2.2 ISSR Amplification

A set of 20 ISSR primers made entirely of defined, short tandem repeat sequences with anchor and representing various microsatellites (di and tri-repeats) were utilised as general primers in PCR amplification of inter simple sequence repeat areas using the Adawy *et al*. approach (2004) [1]. (Table 1).

Table 1: Annealing temperatures of 20 ISSR amplified markers used in the study

Sr. No.	Primer	Primer sequence (5' – 3')	Standardized Annealing temperature (^{0}C)
1	UBC 811	GAG AGA GAG AGA GAG AC	43.4
2	UBC 812	GAG AGA GAG AGA GAG AA	52.0
3	UBC 813	CTC TCT CTC TCT CTC TT	47.0
4	UBC 814	CTC TCT CTC TCT CTC TA	47.0
5	UBC 815	CTC TCT CTC TCT CTC TG	45.0
6	UBC 824	TCT CTC TCT CTC TCT CG	56.7
7	UBC 825	ACA CAC ACA CAC ACA CC	50.4
8	UBC 834	AGA GAG AGA GAG AGA GYT	49.8
9	UBC 841	GAG AGA GAG AGA GAG AYC	45.4
10	UBC 843	CTC TCT CTC TCT CTC TRA	50.0
11	UBC 844	CTC TCT CTC TCT CTC TRC	52.0
12	UBC 845	CTC TCT CTC TCT CTC TRG	50.0
13	UBC 857	ACA CAC ACA CAC ACA CYG	51.7
14	UBC 876	GAT AGA TAG ACA GACA	40.0
15	UBC 878	GGA TGG ATG GAT GGAT	50.0
16	UBC 879	CTT CAC TTC ACT TCA	50.0
17	UBC 881	GGG TGG GGT GGG GTG	50.0
18	UBC 885	BHB GAG AGA GAG AGA GA	40.7
19	UBC 886	VDV CTC TCT CTC TCT CT	51.4
20	UBC 895	AGA GTT GGT AGC TCT TGA TC	55.0

Because pipetting small volumes is laborious and often inaccurate, a master mixture was created in which ingredients common to all reactions were blended in one tube and the volume for one reaction was multiplied by the total number of samples. Following that, the necessary amount of master mixture was dispensed to each tube, and template DNA was separately added to each tube. (Table 2)

Table 2: Master mixture for polymerase chain reaction (PCR)

Components	Stock concentration	Vol. for one reaction/10 µl
Taq buffer	10X	1.25µl
$MgCl_2$	25 mM	0.25µl
dNTP mix	10 mM	0.5µl
Primer	25picomole/µl	1.0µl
TaqDNA polymerase	3 U/µl	0.25µl
Template DNA	30-50 ng	1.0µl
Sterile Distilled water	-	5.75 µl
Total		10 µl

The PCR amplification was carried out for 35 cycles in a thermal cycler. The reaction had initial denaturation step at 94˚C for 5 min, followed by 35 cycles of 94˚C for 30 sec, annealing temperature for 1 min, 72˚C for 1 min. The final extension step was at 72˚C for 7 min. Amplified products were separated on 1.8% agarose gels having 0.5 µg·ml−1 of the ethidium bromide at 50V for 2hrs. The gels were observed under a UV light source in a gel documentation system. The gel images were captured by documentation systems (Uvi-Tech, Fire reader, Cambridge, England) and recorded on a computer for future examination.

2.3 Data Analysis for ISSR

ISSR markers in the 50 mutants and control were assessed for the presence (1) or absence (0) of bands for each primer. The resulting binary data was used to assess polymorphism levels by dividing the number of polymorphic bands by the total number of scored bands. Jaccard's similarity coefficients were determined for each pairwise comparison of germplasm and a similarity co-efficient matrix was created. This matrix was used to create a dendrogram using the Unweighted Pair Group Method for Arithmetic Average Analysis (UPGMA). MVSP-A Multivariate Statistical Package-5785 was used for the similarity co-efficient analysis and dendrogram creation (Version 3.1)

The distance matrix and dendrogram were created from pooled data using the unweighted pair group method of arithmetic means (UPGMA), a computer software for distance estimation.

Other parameters computed were,

2.4 Per cent polymorphism

$$\text{Per cent polymorphism} = \frac{\text{Total number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

2.5 Polymorphism Information Content

Polymorphism Information Content (PIC) values were calculated as per formula developed by Powell *et al*., (1996) [7].

$$PIC = 1-\sum P_{ij}^2$$

Where,

P_{ij} is the frequency of i^{th} and j^{th} locus, summed across the entire locus over all lines.

PIC values range from 0 (monomorphic) to 1 (very highly discriminative, with many

alleles each in equal and low frequency) were estimated for each profile generated across 50 pigeon pea mutants along with control.

3. Result and Discussion

In the present project 50 phenotypically different mutants from the M_3 population as well as the base variety Konkan Tur-1 we used for molecular analysis to analyse their inheritance pattern with 20 ISSR primers.

3.1 Per cent polymorphism and number of alleles

The twenty primers synthesise 271 alleles. They produced 2960 DNA fragments, with 1737 of them being polymorphic with an average of 86.85 bands per primer. They had a polymorphism of 61.31%. The same results have been published by Yadav *et al.* (2014) [5] and Rani *et al.* (2015) [4]. In this investigation, the ISSR marker did not generate any amplification in a specific genotype of pigeon pea. This might be due to a mutation in the primer binding site or the absence of the locus entirely.

3.2 Range of amplification and Genetic relationship among mutants

Amplification range shown in this study from 101 bp to 2950 bp with number of bands produced by ISSR primer ranged from 6 to 24. The mutant T1(21)7-8 shows the highest genetic distance over Konkan Tur-1, *i.e.*, 0.505, while T2(36)15-2 has the lowest genetic distance with respect to Konkan Tur-1, which is 0.058. The amplified ISSR fragments varied from 200 bp to 4 kb and the number of bands generated by each ISSR primer ranged from 5 to 12. An investigation conducted by Yadav *et al.* (2014) [5] was found to be genetic distance between pigeon pea cultivars in the range of 0.1210 to 0.4776. Result obtained by Datta *et al.* (2011) [2] is the number of loci per primer ranged from 4 to 17, with an average of 7.9. The fragment sizes ranged from 0.2 kb to 2 kb.

3.3 Polymorphic information content

The ISSR loci's PIC values varied from 0.69 to 0.93, with an average of 0.86. According to previous report of Rani *et al.* (2015) [4] PIC value observed for ISSR analysis was 0.76. The average PIC value was 0.70, with a range of 0.13-2.49 in ISSR analysis for genetic differentiation between chickpea and pigeon pea (Datta *et al.* 2011) [2]. The PIC values for the SSR loci ranged from 0.24 to 0.86 with an average value of 0.50 in pigeon pea cultivar (Yadav *et al.* 2017) [6].

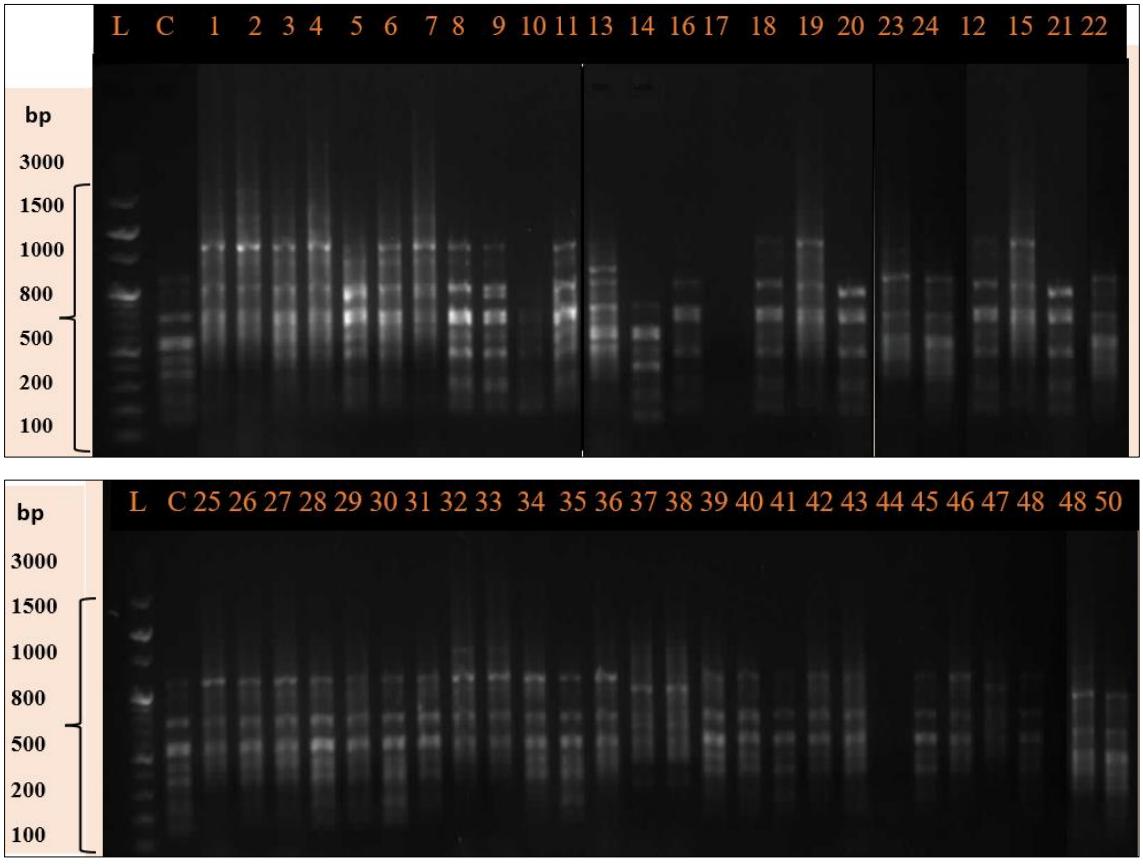

Plate 1: Amplification of range of 834

3.4 Cluster analysis

All of the primers used for data analysis have a considerable amount of polymorphism and reliable repeatable banding characteristics. The phylogenetic tree constructed by UPGMA exhibited considerably more clear analysis and demonstrated genetic diversity across pigeon pea cultivars to some extent. The 50 mutants and one control, Konkan Tur-1, were grouped into two clusters. First cluster contain only one genotype T2(36)15-2 while another cluster includes 49 mutants and base variety Konkan Tur-1. Second cluster divided into sub clusters II A and II B. II A cluster divided into II A (a) having 3 mutants and II A (b) containing 16 mutants. II B cluster again divided into II B (a) includes maximum number of mutants (28) and II B (b) had 3 mutants including control (Konkan Tur-1). (Table 3)

Datta *et al.* (2011) [2] discovered the same results using SSR markers on 8 genotypes of chickpea and pigeon pea. The test genotypes were divided into two groups by the dendrogram. The first cluster was divided into two subclusters: BG 256, ICC 4958, and JG 62 were clustered together, while WR 315 was separated. The second cluster divided into two subclusters, with Bahar, Type 7, and ICP 8863 clustering together and ICPL 87119 clustering apart.

Table 3: Clustering pattern of 50 mutants of Pigeon pea.

Cluster	Sub cluster	Sub-sub cluster	No. of genotypes	Genotypes
I	-	-	1	T2(36)15-2
II	II A	II A (a)	3	T2(47)11-1, T1(77)3-3, T2(11)3-4
		II A (b)	16	T1(20)5-5, T1(17)10-2, T1(77)9-1, T2(1)13-8, T1(67)11-6, T1(67)2-2, T1(67)2-3, T1(82)7-8, T5(96)11-6, T1(82)7-4, T1(67)15-2, T1(57)4-8, T2(10)8-1, T6(26)9-3, T2(29)1-1, T3(19)8-8.
	II B	II B (a)	28	T6(14)8-1, T9(37)10-4, T9(37)10-2, T2(23)5-4, T5(106)1-2, T5(101)1-7, T1(67)11-4, T3(20)2-6, T1(73)12-1, T1(51)9-4, T1(77)9-6, T1(77)9-3, T2(11)3-3, T5(96)11-1, T5(79)3-8, T5(96)11-2, T1(77)9-5, T3(5)7-5, T7(2)2-2, T5(56)4-5, T1(85)2-3, T1(47)2-1, T1(30)9-2, T1(72)5-1, T1(77)3-4, T1(68)1-2, T1(77)1-3, T1(73)13-4
		II B (b)	3	T1(67)11-5, T1(21)7-8, Konkan Tur-1

4. Conclusion

The study emphasized that ISSR markers are suitable for evaluation of genetic variability and diversity among different mutant lines of pigeon pea. The ISSR analysis shows substantial polymorphism in pigeon pea. The results indicated the efficiency of ISSR markers in investigating genetic diversity at molecular level, which is important for detecting distinctness of mutants in relation to base variety Konkan Tur 1. Such information may be useful for selection of desirable mutants and its utilisation for further breeding programme. Based on the similarity coefficient and cluster analysis, the genotypes T1(73)13-4 and T2(36)15-2 were determined to be relatively different than Konkan Tur-1 and can be employed in breeding programmes for their desired characteristics. The genetic similarities discovered during the research can also be utilised to choose parents for mapping populations and for breeding purposes.

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6. References

1. Adawy SS, Hussein EHA, Saker MM, EI-Itriby HA. Intra- and Inter- varietal variation of Upper Egypt date palm cultivars (*Phoenix dactylifera* L.): 1 As revealed by RAPD and ISSR markers. Int. Conf. Gent. Eng. 2004;11:165-179.
2. Datta J, Lal N. Genetic differentiation in *Cicer arietinum* L. and *Cajanus cajan* L. Millspaugh using SSR and ISSR marker systems. Advanced Biotech. 2011;11(05):39-44.
3. Edwards K, Johnstone C, Thompson C. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucl. Aci. Res. 1991;19(6):1349.
4. Rani P, Sirohi A, Parihar A, Gupta A. Assessing genetic variation of pigeonpea [*Cajanus Cajan* (L.)] genotype using RAPD and ISSR Markers Systems. The Bioscan. 2015;10(2):957-962.
5. Yadav K, Yadav SK, Yadav A, Pandey VP, Dwivedi UN. Comparative analysis of genetic diversity among cultivated pigeon pea (*Cajanus cajan* (L) Millsp.) and its wild relatives (*C. albicans* and *C. lineatus*) using randomly amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) fingerprinting. Am. J. Plant Sci. 2014;5(11):1665-1678.
6. Yadav M, Kumar YY, Pushpendra K, Kumar SR, Renu Y, Pawan K, *et al*. Molecular diversity analysis as an improvement tool for pigeon pea [*Cajanus cajan* (L.)]. Res. J. Biotechnol. 2017;12(9):75-86.
7. Powell WW, Koput KW, Smith-Doerr L. Interorganizational collaboration and the locus of innovation: Networks of learning in biotechnology. Administrative science quarterly. 1996 Mar 1:116-45.