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MOLECULARPROFILINGANDCLUSTERANALYSISOF PIGEONPEA GENOTYPES FOR GENETIC DIVERSITY ASSESSMENT

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ABSTRACT

The present study was conducted to assess genetic diversity among 24 pigeonpea (*Cajanus cajan* (L.) Millsp.) genotypes using SSR markers. A total of 12 markers were used of which 10 showed polymorphism and were selected for diversity analysis. Genetic similarity was calculated using Jaccard's similarity coefficient, and the genotypes were grouped using the UPGMA method. The similarity coefficient ranged from 0.55 to 0.95 with an average of approximately 0.67. The dendrogram divided the genotypes into two major clusters, A and B, containing 21 and 3 genotypes, respectively. The highest genetic similarity (95%) was observed between RPS-2007-105 and RPS-2010-16-5, indicating a close genetic relationship between them. The results showed that the selected pigeonpea genotypes differed in their genetic relationships. SSR markers were found useful for studying genetic diversity and identifying potentially suitable genotypes for future breeding programmes. The information obtained from this study may help breeder's select suitable parents for developing improved pigeonpea varieties. However, the selected genotypes should also be evaluated for yield, disease resistance and adaptability before their use in breeding programmes.

KEYWORDS: Pigeonpea, genetic diversity, SSR markers, genetic similarity, UPGMA, molecular characterization.

INTRODUCTION:

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is an important pulse crop grown in tropical and subtropical regions. It is a rich source of protein and plays an important role in improving food and nutritional security, particularly in India, which is one of the major producers of pigeonpea (Varshney *et al.*, 2012; Bohra *et al.,* 2012). The crop is mainly grown under rainfed conditions and can tolerate drought and high temperatures better than many other pulse crops. However, its productivity is affected by several factors, including drought, water logging, frost, insect pests and the limited availability of high-yielding and locally adapted varieties. These constraints make it necessary to develop improved pigeonpea varieties with higher yield, better adaptability and greater tolerance to environmental stresses (Sharma *et al*., 2010; Basandrai *et al*., 2011). Genetic diversity is the variation present among individuals within a crop species. It is an important resource for plant breeding because it helps identify promising genotypes and select suitable parents for developing improved varieties. Genetically diverse parents may provide opportunities to develop new combinations of desirable traits and generate useful variation in subsequent generations. However, selection based only on visible plant characters may not always reveal the actual genetic differences among genotypes, as these characters can be influenced by environmental conditions. Therefore, reliable methods are needed to assess genetic diversity and understand the relationships among available pigeonpea genotypes. Molecular markers provide a useful approach for studying genetic variation at the DNA level. Unlike morphological characters, DNA markers are generally less affected by environmental conditions and can help distinguish genetically similar and different genotypes. Simple sequence repeat (SSR) markers are useful for molecular characterization because they can detect genetic polymorphism without requiring prior knowledge of DNA sequences. The information obtained from SSR analysis can be used to estimate genetic similarity, group genotypes and identify potentially useful genetic resources for crop improvement. Although molecular markers have been used to study genetic diversity in pigeonpea, the extent of genetic variation and relationships among the particular genotypes selected for the present investigation remains to be established. Without such information, selecting genetically diverse parents for future breeding programmes may be difficult. Therefore, the present study was undertaken to assess molecular diversity among selected pigeonpea genotypes using SSR markers, determine their genetic relationships and identify potentially

useful genotypes for future breeding programmes. The findings may help improve the understanding of genetic variation in the selected material and support the effective use of pigeonpea germplasm in crop improvement.

MATERIALSANDMETHODS:

Experimental Site and Plant Materials: The molecular diversity study was conducted at the MAS Laboratory, Department of Genetics and Plant Breeding, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India. A total of 24 pigeonpea genotypes were selected based on their phenotypic characteristics for molecular analysis.

Genomic DNA Isolation: Seeds of each genotype were germinated under natural conditions. After 20 days, approximately 300 mg of young leaf tissue was collected for DNA extraction. Genomic DNA was isolated using a modified CTAB method following Guillemant and Laurence (1992). The extracted DNA was treated with RNase, purified and dissolved in TE buffer. The DNA samples were stored at −20°C until further analysis.

DNA Quantification and Dilution: The quality and concentration of the extracted DNA were assessed using a Nano Drop 2000c spectrophotometer. The DNA samples were diluted with nuclease-free water to a final concentration of 40 ng/ µl for PCR amplification.

PCR Amplification: PCR amplification was performed using SSR markers in a total reaction volume of 20µl. Each reaction contained PCR buffer, dNTPs, primers, Taq DNA polymerase, template DNA and nuclease-free water. Amplification was carried out in an Applied Biosystems thermal cycler. The PCR programme included initial denaturation at 95°C for 2 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 48–53°C for 1 minute and extension at 72°C for 90 seconds. Final extension was performed at 72°C for 15 minutes, followed by storage at 4°C.

Gel Electrophoresis and Visualization: The amplified PCR products were separated using 5% polyacrylamide gel electrophoresis. A50-bp DNA ladder was used to estimate the fragment sizes. Electrophoresis was performed at 120V and the gels were stained with ethidium bromide and visualized using a Bio-Rad Gel Doc XR+ imaging system.

Data Scoring and Statistical Analysis: Clear, reproducible bands were scored from the gel images. For binary data analysis, the presence of a band was recorded as 1 and its absence as 0 while faint or unclear bands were excluded. The binary data matrix was analyzed using NTSYS-pcversion 2.02e. Pair wise genetic similarity was calculated using Jaccard' scoefficient (Jaccard, 1908), and a dendrogram was constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to study the genetic relationships among the selected genotypes.

RESULTS:

Genetic Diversity Based on Molecular Markers in Pigeonpea Genotypes- Molecular diversity among 24 pigeonpea genotypes was studied using SSR markers to understand their genetic relationships. A total of 12 primers (Fig 1) were tested, of which 10 produced scorable amplification patterns and generated 426 bands (Details of Markers used is shown in Table-1). The bands were recorded as present (1) or absent (0), and the resulting binary data matrix was used for genetic similarity analysis. Jaccard's similarity coefficient was calculated to estimate the molecular similarity among the genotypes. The genotypes were then grouped using the unweighted pair group method with arithmetic mean (UPGMA) in NTSYS software. The similarity coefficients ranged from 0.55 to 0.95, with an average of approximately 0.67. This variation indicated that the 24 genotypes differed in their molecular relationships. Some genotypes showed a high degree of similarity, whereas others had comparatively lower similarity values. The results suggest that the selected pigeonpea material contained different levels of molecular variation. The dendrogram divided the 24 genotypes into two major clusters, designated A and B (Fig.-2). Cluster A contained 21 genotypes, while Cluster B consisted of three genotypes. The two major clusters were separated at a similarity coefficient of approximately 0.55. This separation indicated that the genotypes grouped in the two clusters had comparatively different molecular profiles.

Table1: Detail about SSR markers used diversity analysis in pigeonpea

S. No.	Marker	Primer5'to3'	No. of bases	TM (^{0}C)	Fragment Size (bp)
1	ICPM1H0 1-F ICPM1H0 1-R	CCCGAACTGCATTCAAAAAT GCGTAGGTGGAAGAAGATCG	20 20	52	200-250
2	ICPM2B0 8-F ICPM2B0 8-R	AGTTTGAAATTGCTTTTGGCT GAATTGGGAGAGACCGCATA	21 20	51	150-200
3	ICPM1E0 4-F ICPM1E0 4-R	TTTTTATGGAATATTTATGAGT TGGC AAGAGTTTCCCAACCCTGCT	26 20	52	150-200

4	CCac036-F CCac036-R	ATCGGCTTTTGTCTTGATGA AAAGCTACAAGGGATACACA TGC	20 23	52	150-300
5	CCttc033-F CCttc033-R	ATTCCCTCTCTATCTCAGACTT TT TCGTGATGGAACTCAAGATACACT	24 24	51	150-200
6	CCat011-F CCat011-R	TGCTCTAATGCTAGTTCATCC AAACACTCATGGGTTAGATTC TCC	21 24	52	100-250
7	CCttc006-F CCttc006-R	CTAGAGGAGGTTCCAAATGCA TA ATCTGTCTGGTGTTTTAGTGTGCT	23 24	52	300-550
8	ICPM1D1 0-F ICPM1D1 0-R	GGATTAACCAATTGTGAGTGA ACC TGCACTTTATAAGCATTTACC AACA	24 25	52	300-400
9	CCttc003-F CCttc003-R	ACACCACCATCGTAAAGAACA AG CCAAGCAAGACACGAGTAATCATA	23 24	52	150-250
10	CCttc008-F CCttc008-R	TCACAGAGAGGACCACACGA AG TGGACTAGACATTGCGTGAAG	22 21	52	200-250

Cluster B was further divided into two sub-clusters, B1 and B2, at approximately 0.76 similarity. Sub-cluster B1 contained ICP7373, whereas sub-cluster B2 contained ICP7376 and ICP7382. The latter two genotypes were separated at approximately 0.846 similarity. Their grouping indicated a relatively close molecular relationship between ICP7376 and ICP7382, while ICP7373 occupied a separate subgroup within Cluster B. Cluster A, which contained the majority of the genotypes, was further divided into sub-clusters A1 and A2 at approximately 0.675 similarity. Sub-cluster A1 consisted of 12 genotypes, whereas A2 contained nine genotypes. This division showed that the genotypes in Cluster A also differed in their molecular relationships and could be further separated into smaller groups. Sub-cluster A1 was divided into two groups, A1(a) and A1(b). BDN-2 was placed in A1(b), while the remaining 11 genotypes were included in A1(a). Further divisions within A1(a) separated the genotypes into smaller groups at similarity levels ranging from approximately 0.68 to 0.90. These groups included ICPL 87119, ICP6996, ICP7004, ICL-87, RPS-2008-4, RPS-2008-5 and ICP7406. The grouping pattern indicated that these genotypes had different degrees of molecular similarity within the larger cluster. Sub-cluster A2 also showed further divisions into smaller groups. UPAS-120 was placed in a distinct subgroup, indicating a different molecular relationship from the other genotypes grouped in this part of the dendrogram. RPS-2007-105 and RPS-2010-16-5 showed the highest reported similarity coefficient of approximately 0.95. This result indicated that these two genotypes had highly similar banding patterns for the markers used in the study. Other genotypes, including ICP-7391, ICP7393, ICP7349, RPS-2010-14-1, ICP7002 and ICP6999, were grouped into smaller sub-clusters at different similarity levels. These groupings further demonstrated the variation in molecular relationships among the selected genotypes. The overall clustering pattern revealed that the 24 pigeonpea genotypes were distributed into groups according to their molecular similarity. The presence of two major clusters and several sub-clusters indicated differences in the genetic relationships within the selected material. The results provide preliminary information for identifying closely related and comparatively distinct genotypes for further evaluation and use in pigeonpea breeding programmes.

DISCUSSION:

Genetic diversity plays an important role in plant breeding because it provides opportunities to select parents with different genetic backgrounds. The selection of suitable parents is essential for developing improved varieties with desirable characteristics. Molecular markers help breeders understand genetic relationships at the DNA level and provide information that may not always be apparent from the external appearance of plants. Unlike many morphological traits, DNA marker profiles are generally less affected by environmental conditions. Molecular analysis can therefore complement field evaluation when characterizing germplasm. In the present study, molecular diversity among 24 pigeonpea genotypes was assessed using SSR markers. Ten of the 12 primers tested produced scorable amplification patterns, generating a total of 426 bands. The similarity coefficients ranged from 0.55 to 0.95, with an average of approximately 0.67. This range indicated that the genotypes differed in their molecular relationships, with some showing high similarity and others showing comparatively greater differences. The clustering pattern further supported the presence of variation among the selected materials. Earlier studies have demonstrated the importance of molecular markers in plant genetic diversity analysis. Vos *et al.* (1995) introduced amplified fragment length polymorphism (AFLP) as a technique for DNA finger printing, while Karp *et al.* (1996) discussed the use of molecular tools in plant genetics source conservation and characterization.

These approaches established an important basis for studying genetic variation and relationships among plant materials. Research specifically conducted on pigeonpea has also shown the value of molecular characterization. Nadimpalli *et al.* (1993) investigated genetic relationships in pigeonpea and related material using restriction fragment length polymorphism (RFLP) markers. Their study demonstrated the usefulness of DNA-based approaches for examining relationships among genetic resources.

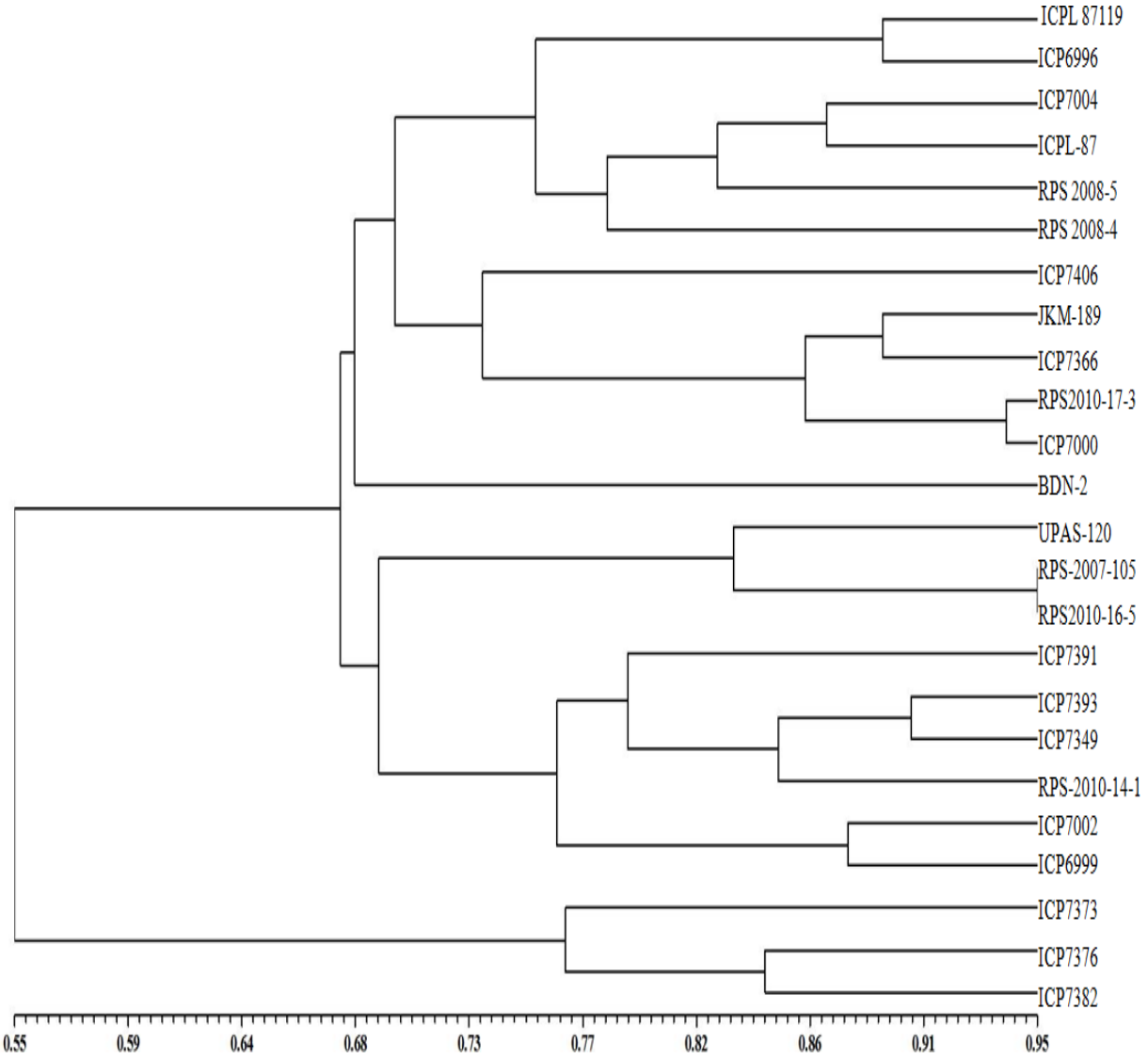
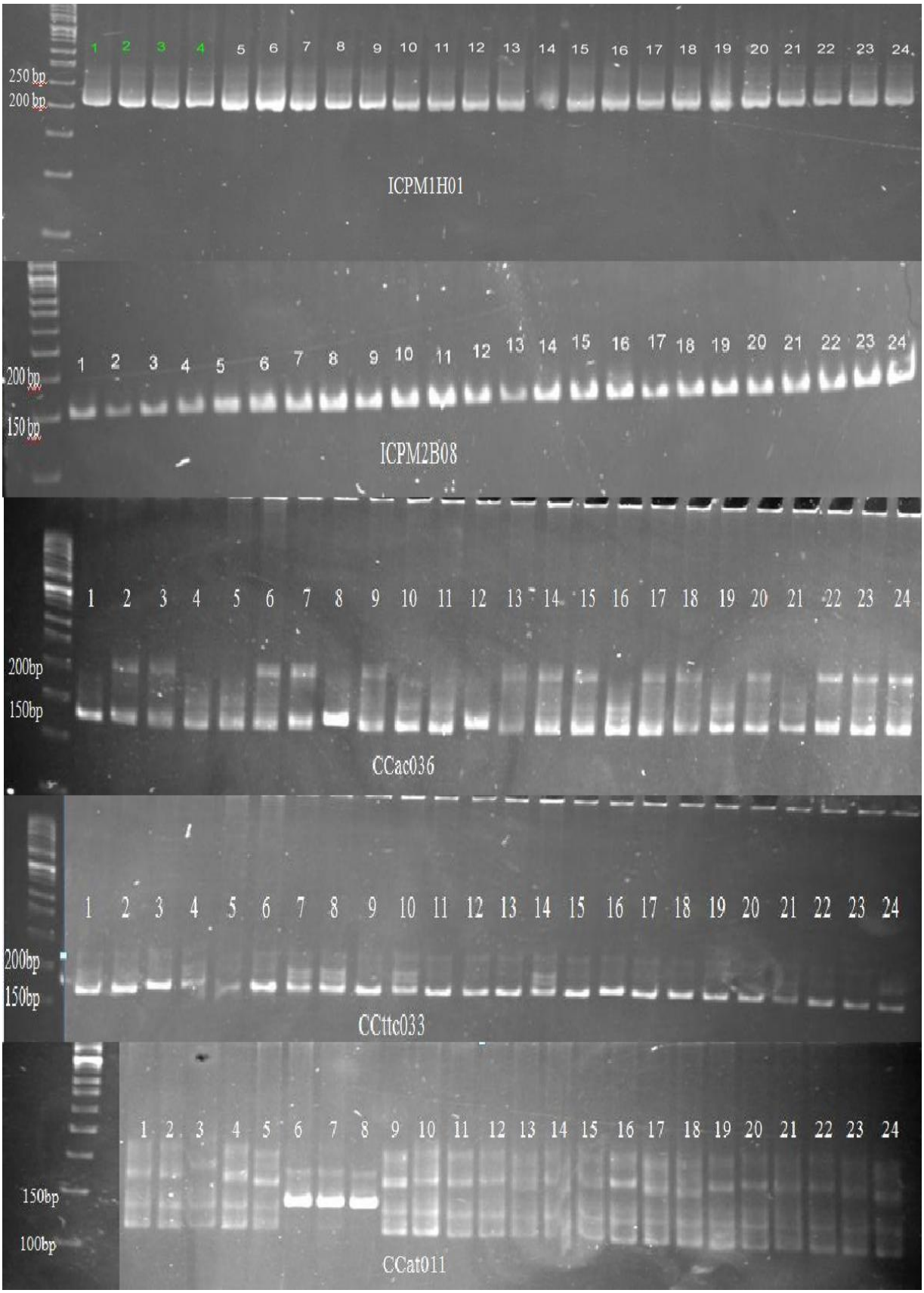


Fig-2: Dendrogram constructed using UPGMA based on Jaccard's coefficient of 24 pigeonpea genotypes (SSR Markers)

Similarly, Ratnaparkhe *et al.* (1995) used random amplified polymorphic DNA (RAPD) markers to study pigeonpea and its wild relatives. Their work highlighted the ability of molecular markers to distinguish genetic materials and examine differences in genetic variation. Yang *et al.* (2006) reported lower genetic diversity in cultivated pigeonpea than in its wild relatives using diversity arrays technology (DArT). This finding is important for pigeonpea improvement because the identification and use of diverse genetic resources can help broaden the genetic base of cultivated material. In the present study, the differences observed among the 24 genotypes may help identify material for further evaluation, although the diversity within this selected set should not be taken as a measure of the diversity of the entire pigeonpea gene pool. Microsatellite markers have also contributed substantially to pigeonpea research. Odeny *et al.* (2007) demonstrated the usefulness of microsatellite markers for characterizing pigeonpea germplasm, while Odeny *et al.* (2009) developed additional microsatellite markers for the crop. These studies showed that molecular markers can support genotype identification, germplasm characterization and the study of genetic relationships. Other researchers have investigated pigeonpea genetic variation using different marker systems, including RAPD, AFLP, microsatellite and single nucleotide polymorphism (SNP) markers (Sivarama krishnan *et al*., 2002; Yan *et al*., 2007; Choudhury *et al*., 2008; Saxena *et al*., 2010, 2014; De Sousa *et al*., 2011; Datta *et al*., 2013; Walunjkar *et al.,* 2013; Yadav *et al.*, 2014). Collectively, these studies emphasize the role of molecular approaches in understanding and utilizing pigeonpea genetic resources. The separation of the 24 genotypes into two major clusters in the present study provides a useful overview of their molecular relationships. Cluster A contained 21 genotypes, whereas Cluster B contained three.


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
250 bp
200 bp
ICPM1H01
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
200 bp
150 bp
ICPM2B08
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
200bp
150bp
CCac036
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
200bp
150bp
CCttc033
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
150bp
100bp
CCat011

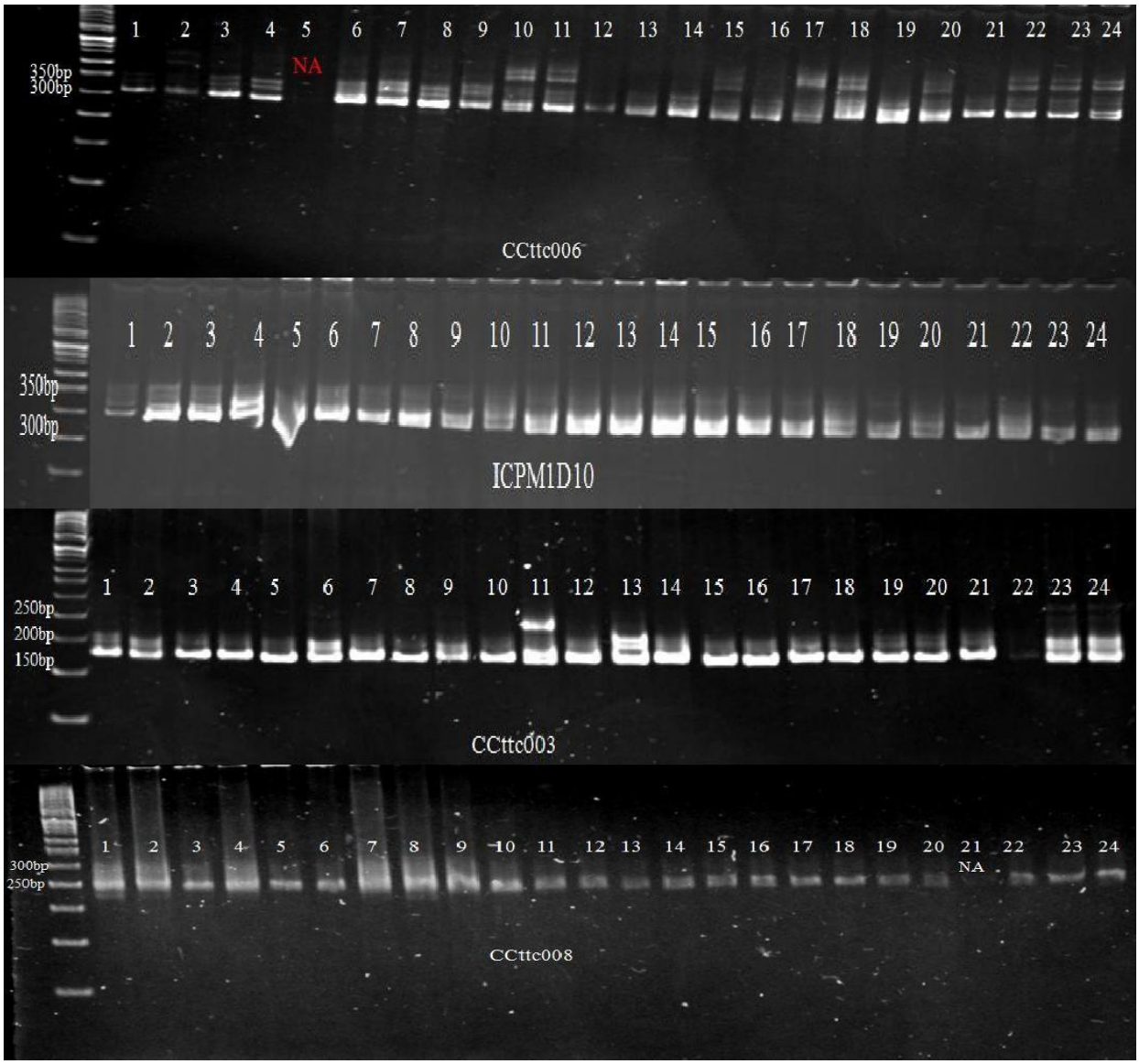


Fig 1. SSR Profile generated by ICPM1H01, ICPM2B08, ICPM1E04 (not amplified), CCac036, CCttc033, CCat011, CCttc006, ICPM1D10, CCttc003 and CCttc008 (10) primer of 24 pigeonpea germplasm including varieties.

Their separation at approximately 0.55 similarity suggested that the two groups were relatively different according to the marker data. However, cluster size alone does not indicate the amount of genetic diversity within a cluster. The relationships among individual genotypes and their distribution within the smaller sub-clusters also need to be considered. The grouping of ICP7376 and ICP7382 in the same sub-cluster, with a reported similarity of approximately 0.846, indicated a close molecular relationship between them. ICP7373 was placed separately within Cluster B. Similarly, the division of Cluster A into A1 and A2 showed that molecular differences were also present among the genotypes in the larger cluster. Further divisions within these sub-clusters helped distinguish groups of genotypes with different similarity levels. The highest reported similarity coefficient of approximately 0.95 between RPS-2007-105 and RPS-2010-16-5 indicated that these genotypes had highly similar molecular profiles for the markers used. Such information can help breeders understand relationships among breeding materials and avoid selecting parents solely on the basis of their names or visible characteristics. Never the less, high molecular similarity does not necessarily mean that two genotypes are identical in all their genes or agronomic characteristics. The result reflects similarity based on the markers examined in this study. The similarity coefficients observed in the present investigation should be interpreted in relation to the marker system and the genotypes evaluated. Earlier pigeonpea studies used different molecular techniques, including RFLP, RAPD, AFLP, microsatellite and SNP markers. These marker systems differ in the way they detect genetic variation. In addition, the number of genotypes, their genetic backgrounds and the methods used to calculate similarity can influence the results.

Therefore, numerical comparisons between the present study and earlier reports should be made only when the methods and data are sufficiently comparable. From a breeding perspective, the observed diversity may be useful for identifying potential parents for crossing programmes. Genotypes that show comparatively lower similarity may be considered for further evaluation when the objective is to combine different genetic backgrounds. However, molecular dissimilarity alone cannot predict the performance of hybrids or segregating populations. A genetically distant parent may not necessarily possess the desired yield, maturity or disease-resistance traits. Therefore, the selection of parents should also be based on field performance, combining ability where appropriate, and the breeding objectives of the programme. For pigeonpea improvement, important traits to consider include grain yield, maturity duration, plant height, resistance to major diseases and insect pests, and adaptation to the target production environment. Combining these traits with molecular diversity information can improve the basis for parent selection. Further evaluation of the selected genotypes under field conditions, along with additional molecular characterization where necessary, may help identify promising material for future breeding programmes. The present study therefore provides useful preliminary information on the molecular relationships among 24 pigeonpea genotypes. The results demonstrate that marker-based analysis can help distinguish genotypes and organize germplasm according to their similarity. The combined use of molecular data and agronomic evaluation will be important for making effective use of these genetic resources and supporting the development of improved pigeonpea varieties.

CONCLUSION:

The present study was conducted to assess molecular diversity among 24 pigeonpea genotypes using SSR markers. Out of the 12 primers tested, 10 produced scorable amplification patterns and generated 426 bands. The similarity coefficients ranged from 0.55 to 0.95, with an average of approximately 0.67. Cluster analysis grouped the genotypes into two major clusters, A and B, containing 21 and 3 genotypes, respectively. The observed clustering pattern indicated differences in the genetic relationships among the selected genotypes. The highest similarity (95%) was observed between RPS-2007-105 and RPS-2010-16-5, suggesting a close molecular relationship between them. The variation observed among the genotypes provides useful information for understanding their genetic backgrounds and identifying potential parents for future breeding programmes. However, molecular diversity alone is not sufficient to select superior parents. Important traits such as grain yield, maturity, disease resistance and adaptability should also be considered. Overall, the study highlights the usefulness of molecular markers for characterizing pigeonpea germplasm and understanding genetic relationships among genotypes. Combining molecular information with field evaluation may help breeders identify suitable parents, utilize available genetic resources more effectively and develop improved pigeonpea varieties with desirable agronomic traits.

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