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Research Article

Assessment of genetic diversity in pigeon pea (*Cajanus cajan)* using micro satellite markers

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Abstract
An effective way to use germplasm for genetic improvement is to be aware of the genetic variation among crop genotypes. The objective of the present study was to assess the genetic diversity and population structure of 30 genotypes of pigeonpea from populations that were collected from various sources. In order to show a new structure within the pigeonpea genetic pool and to give crucial information for pigeonpea breeding operations, the predetermined study's goal was to define pigeonpea genotypes using a microsatellite marker technique. The genomic DNA of 30 pigeon pea genotypes were amplified with 20 SSR primers that produced 46 amplified bands, out of which 30 band were polymorphic (65.21%) and 16 bands were monomorphic (34.82%). Primer CcM 2977 generated a maximum number of amplified bands, of which 2 bands were polymorphic. Among 20 primers, only 8 primers showed the highest polymorphism (100%) and 5 primers were monomorphic in nature. Average of 2.30 bands per primer was amplified. The dendrogram constructed from the pooled data revealed six distinct clusters of which five were solitary. Cluster analysis of pigeon pea genotypes was based on Unweighted Pair Group Method with the Arithmetic average (UPGMA) method and the 30 pigeon pea genotypes were classified into six main groups. The present study indicated that the performance of SSR markers for the evaluation of genetic diversity could be beneficial for pigeon pea breeding. They could additionally be useful in genomic mapping research, developing pigeon pea cultivars with various genetics and reaping advanced crop productivity.

Keywords: Genetic diversity, Pigeon pea, Microsatellite marker, UPGMA method

INTRODUCTION

According to the Indian Institute of Pulses Research, India's population is expected to touch 168 billion by 2030. The pulse requirement for 2030 is projected at 332 million tones anticipated required annual growth rate of 4.2% (Sarkar et al., 2018). Pigeon pea [*Cajanus cajan* (L.) Millsp.] is an important legume crop cultivated throughout the tropics and subtropical areas and cultivated in India, Malaysia, Indonesia, the Philippines, Caribbean, and Africa. Commonly known as *Arhar* in Northern India, this protein-rich pulse crop has a growing demand in Asia. Among the pulses, pigeon pea is India's second most important *kharif* grain legume. India had a growth rate of 0.8% in production between 1949- 2004 due to various stresses (Sarkar et al., 2018). Appropriate management technologies have emerged to counteract abiotic stresses like salinity, drought and waterlogging. *Cajanus cajan* productivity in its selected cropping system is enhanced by the integrated plant nutrient management system for abiotic stress soil fertility. During the green revolution, patch

pigeon pea cultivation was foster by effective procurement and enhanced productivity aid with an attractive minimum support price (Pandit et al., 2015).

The hereditary improvement of any crop generally relies upon the size of helpful hereditary contrasts inside the germplasm for the trait of interest (Carvalho and Schaal, 2001, Ojuederie et al., 2014), for hybridization and selection process, the breeder used trait association and superior parents selection. A powerful breeding program depends on parental variety to acquire variable qualities helpful in genetic improvement of a variety. Along these lines, toward the start of any breeding program, it is essential to comprehend and evaluate the extent of the genetic diversity present in the accessible germplasm.

Genetic diversity can be calculated by molecular and morphological levels (Adjebeng-Danquah et al., 2020). Morphological assessment empowers the reproducer to survey the qualities of agronomic significance for which improvement is essential. Morphological characters are simple and fast to score and are extremely helpful during the underlying appraisal of the enormous number of germplasm (Asare et al., 2011). Morphological characters have effectively been used in concentrating on hereditary contrasts in a few yields including cowpea (Mafakheri et al., 2017), mucuna (Sathyanarayana et al., 2012), sweet potato (Elameen et al., 2011), African sweet potato bean (Ojuederie et al., 2014) and cassava (Adjebeng-Danquah et al., 2016). Nonetheless, most morphological descriptors particularly quantitative characteristics, are largely impacted by the climate and genotype by climate communication. They are not viewed as dependable as markers (Adjebeng-Danquah et al., 2020).

The population's genetic diversity can be better calculated by combining molecular and morphological characterization with minimum errors that generally evolved from environmental effects. Denwa et al. (2019) conclude that a combination of both, i.e., morphological and molecular characterization, gives more information than molecular and morphological characterization alone. Therefore, this study's major objective was embraced to learn the genetic diversity among 30 pigeon peas varieties in light of their hereditary data and select genotypes helpful in the progress of pigeon pea assortments for farmers of western Uttar Pradesh.

MATERIALS AND METHODS

Plant material

The germplasm of 30 pigeon pea genotypes was collected from Sardar Vallabh Bhai Patel University of Agriculture and Technology, Meerut, and cultivated in the field of Swami Vivekanand Subharti University, Meerut (Table 1).

Experimental site and geographical situation

The experimental site was located inside the Swami Vivekanand Subharti University Meerut. Another pot experiment was conducted at Keral Verma Subharti College of Science, Department of Biotechnology, Vivekanand Subharti University Meerut. Meerut district lies between 28^0 57' to 28^0 02' North latitude and 77^0 40' to 77^0 45' East longitude in India's Indo-Gangetic plains.

DNA extraction

Trifoliate leaves were collected from the three-week-old plant of pigeon pea genotype and kept in the Ziploc bags and sent to the Biotechnology laboratory. The leaf samples were dried at room temperature for 3 days by using silica gel and ground the samples. Ground 200mg of plant tissue to a fine paste. Paste was transferred into a microfuge tube. The incubated plant extract mixture in the water bath. After incubation, the extract mixture was spun to settle down the cell debris and transferred the supernatant into clean microfuge tubes. 2% agarose gel stained with ethidium bromide

Table 1. List of 30 pigeon pea genotypes with tolerance category

S.No.	Genotype	Tolerance category
1	Pusa 992	Moderate
2	H-2003-14	Highly Tolerant
3	ICP 5028	Tolerant
4	AH-06-5	Moderate
5	H-2001-25	Tolerant
6	AL 1758	Tolerant
7	AH-06-9	Tolerant
8	ICPL 99051	Sensitive
9	ICPA2039	Tolerant
10	JBP 110B	Tolerant
11	ICPL 332	Tolerant
12	ICPL 20128	Tolerant
13	UPAS 120	Highly Sensitive
14	H-02-59	Moderate
15	H-2000-14	Highly Tolerant
16	AH-06-7	Highly Tolerant
17	SGBS 6	Moderate
18	H-02-28	Tolerant
19	ICPL 99050	Sensitive
20	ICP 14085	Moderate
21	AL 1756	Highly Tolerant
22	AH 1760	Sensitive
23	MAL 15	Highly Sensitive
24	AL 15	Tolerant
25	AH-09-9	Sensitive
26	AL 1849	Highly Tolerant
27	AH-06-12	Tolerant
28	PAU881	Sensitive
29	ICPL 88039	Highly Sensitive
30	Manak	Highly Tolerant

was used for extraction. The samples of DNA were then diluted to 50 ng/µl before the Polymerase Chain Reaction PCR amplification. A set of 20 microsatellite marker, with 20 and 25 nucleotide sequences was used to calculate the genetic diversity of the pigeon pea accessions. After amplification of DNA, the PCR product was resolved on a 2% agarose gel electrophoresis system at 50v for 3 hr. Ethidium bromide (EtBr) was used for staining the DNA band and the image was captured by a gel doc system.The similarity coefficient of 30 pigeon pea cultivars was analyzed (Nei and Lis (1979). The Similarity matrix (Table 3) was analyzed using Jaccard's coefficient (Jaccard, 1908).

RESULTS AND DISCUSSION

Genetic diversity is pre-needful for any crop development programme to be successful. Molecular markers are significant materials for evaluating genetic diversity and act as flexible equipment to look at variability in distinct plant species. Microsatellite markers or SSRs are presently available to understand the range and

Table 2. Analysis of SSR banding patterns for Pigeon pea genotype

S. No.	Primer	Annealing temperature (^{0}C)	Total Band	Polymorphic Band	Monomorphic Band	PIC Value	Polymorphism %
1	PKS30	59.50	3	3	0	0.210	100%
2	CCac006	59.00	1	0	1	0.210	0%
3	CCtc009	63.00	3	3	0	0.270	100%
4	CCttc018	58.00	3	3	0	0.290	100%
5	CCac029	60.00	2	1	1	0.110	50%
6	CCB4	64.00	3	1	2	0.370	33.33%
7	CCtta015	58.00	3	2	1	0.130	66.66%
8	CCttat001	58.00	2	1	1	0.180	50%
9	CCB7	60.00	2	1	1	0.200	50.66%
10	CCB10	61.00	3	3	0	0.370	100%
11	CcM 2977	59.50	4	2	2	0.654	50%
12	CcM0039	59.00	3	3	0	0.519	100%
13	CcM1381	63.00	2	2	0	0.033	100%
14	CcM0252	58.00	3	2	1	0.183	66.66%
15	CcM0268	60.00	2	2	0	0.180	100%
16	CcM1538	64.00	3	0	3	0.485	0%
17	CcM0008	58.00	1	0	1	0.130	0%
18	CcM0093	58.00	1	0	1	0.180	0%
19	CcM0306	60.00	1	0	1	0.110	0%
20	CcM0353	61.00	1	1	0	0.130	100%
TOTAL			46	30	16	–	

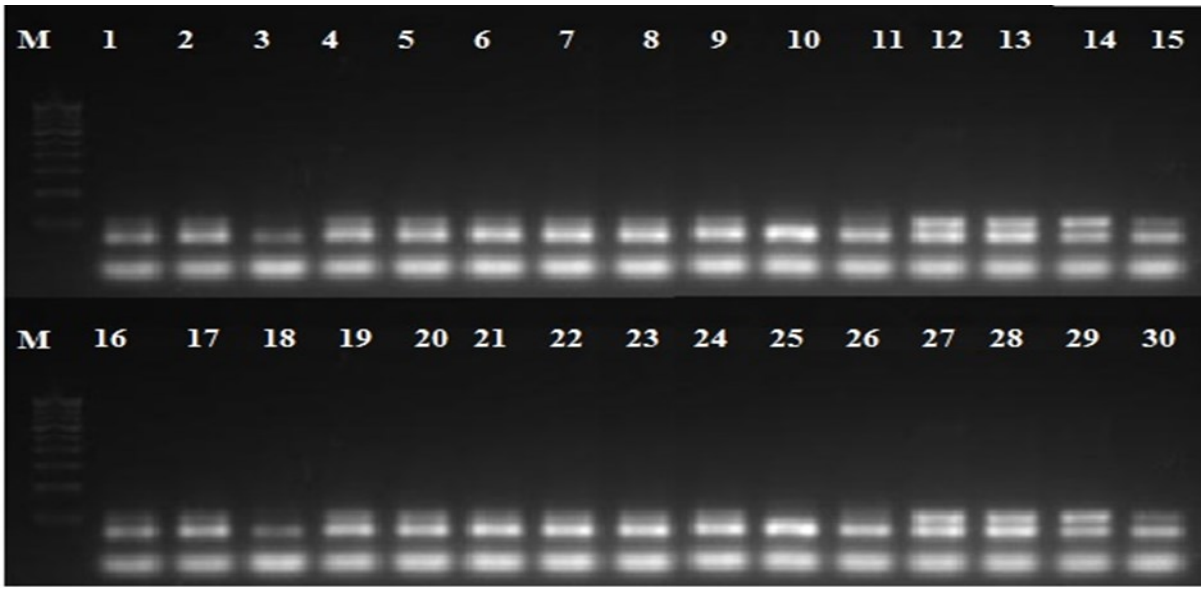

Fig.1. *PCR amplification profile of 30 Pigeon pea genotypes (Pusa992, H200314, ICP5028, AH065, H200125, AL1758, AH069, ICPL99051, ICPA2039, JBP110B, ICPL332, ICPL20128, UPAS120, H0259, H200014, AH067, SGBS6, H0228, ICPL99050, ICP14085, AL1756, AL1760, AL15, MAL15, AH099, AL1849, AH 0612, PAU881, ICPL88039 and Manak) with CcM0252 SSR*

Table 3. Similarity matrix by Jaccard's coefficient showing the relationship among 30 pigeon pea genotypes based on SSR markers

Genotypes																													
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1.00																													
0.86	1.00																												
0.84	0.91	1.00																											
0.85	0.89	0.87	1.00																										
0.80	0.87	0.89	0.81	1.00																									
0.82	0.87	0.87	0.87	0.85	1.00																								
0.82	0.90	0.86	0.86	0.88	0.90	1.00																							
0.79	0.77	0.81	0.78	0.80	0.85	0.80	1.00																						
0.83	0.89	0.87	0.85	0.87	0.85	0.92	0.78	1.00																					
0.79	0.82	0.84	0.84	0.82	0.90	0.87	0.80	0.84	1.00																				
0.81	0.88	0.88	0.86	0.84	0.90	0.87	0.82	0.86	0.89	1.00																			
0.81	0.88	0.86	0.88	0.80	0.92	0.89	0.84	0.84	0.87	0.93	1.00																		
0.78	0.82	0.80	0.80	0.76	0.80	0.82	0.81	0.80	0.79	0.81	0.81	1.00																	
0.85	0.87	0.83	0.83	0.79	0.81	0.84	0.78	0.83	0.78	0.82	0.80	0.85	1.00																
0.83	0.88	0.92	0.84	0.92	0.88	0.89	0.84	0.86	0.83	0.89	0.85	0.81	0.86	1.00															
0.86	0.89	0.89	0.85	0.87	0.89	0.88	0.81	0.87	0.84	0.90	0.86	0.78	0.87	0.92	1.00														
0.82	0.84	0.82	0.82	0.80	0.84	0.83	0.77	0.82	0.79	0.85	0.81	0.78	0.84	0.85	0.90	1.00													
0.82	0.88	0.86	0.84	0.88	0.88	0.91	0.78	0.88	0.85	0.85	0.85	0.81	0.84	0.87	0.88	0.87	1.00												
0.76	0.82	0.80	0.78	0.80	0.78	0.82	0.77	0.83	0.79	0.83	0.83	0.76	0.77	0.81	0.82	0.78	0.86	1.00											
0.85	0.85	0.85	0.83	0.83	0.85	0.88	0.78	0.87	0.86	0.84	0.82	0.79	0.86	0.84	0.89	0.86	0.88	0.81	1.00										
0.84	0.91	0.89	0.85	0.89	0.89	0.90	0.79	0.87	0.84	0.88	0.86	0.80	0.83	0.90	0.91	0.84	0.88	0.82	0.85	1.00									
0.84	0.82	0.82	0.78	0.82	0.84	0.79	0.77	0.80	0.81	0.83	0.79	0.73	0.78	0.85	0.86	0.83	0.83	0.78	0.78	0.84	1.00								
0.76	0.88	0.82	0.80	0.80	0.82	0.81	0.75	0.81	0.81	0.83	0.81	0.72	0.81	0.83	0.82	0.80	0.81	0.76	0.76	0.82	0.80	1.00							
0.85	0.91	0.87	0.85	0.81	0.85	0.86	0.77	0.85	0.80	0.86	0.86	0.83	0.89	0.86	0.87	0.84	0.88	0.81	0.83	0.87	0.84	0.85	1.00						
0.83	0.84	0.88	0.82	0.86	0.86	0.83	0.82	0.82	0.83	0.91	0.87	0.79	0.84	0.89	0.90	0.83	0.85	0.79	0.82	0.88	0.87	0.79	0.88	1.00					
0.85	0.90	0.90	0.84	0.86	0.86	0.87	0.78	0.84	0.83	0.89	0.87	0.79	0.82	0.89	0.88	0.85	0.87	0.81	0.84	0.90	0.83	0.83	0.88	0.87	1.00				
0.74	0.78	0.74	0.74	0.70	0.76	0.75	0.71	0.76	0.73	0.77	0.77	0.65	0.75	0.73	0.76	0.78	0.75	0.74	0.73	0.72	0.75	0.89	0.80	0.75	0.77	1.00			
0.83	0.84	0.81	0.80	0.75	0.82	0.81	0.74	0.80	0.77	0.84	0.85	0.73	0.80	0.80	0.81	0.77	0.77	0.75	0.78	0.79	0.75	0.83	0.86	0.80	0.81	0.85	1.00		
0.87	0.90	0.92	0.86	0.90	0.90	0.89	0.82	0.86	0.85	0.89	0.87	0.81	0.86	0.93	0.94	0.87	0.91	0.81	0.88	0.94	0.87	0.81	0.88	0.93	0.89	0.73	0.80	1.00	
0.85	0.85	0.83	0.82	0.81	0.87	0.84	0.78	0.82	0.84	0.84	0.84	0.73	0.78	0.84	0.85	0.83	0.82	0.75	0.82	0.85	0.83	0.81	0.82	0.80	0.92	0.79	0.84	0.84	1.0

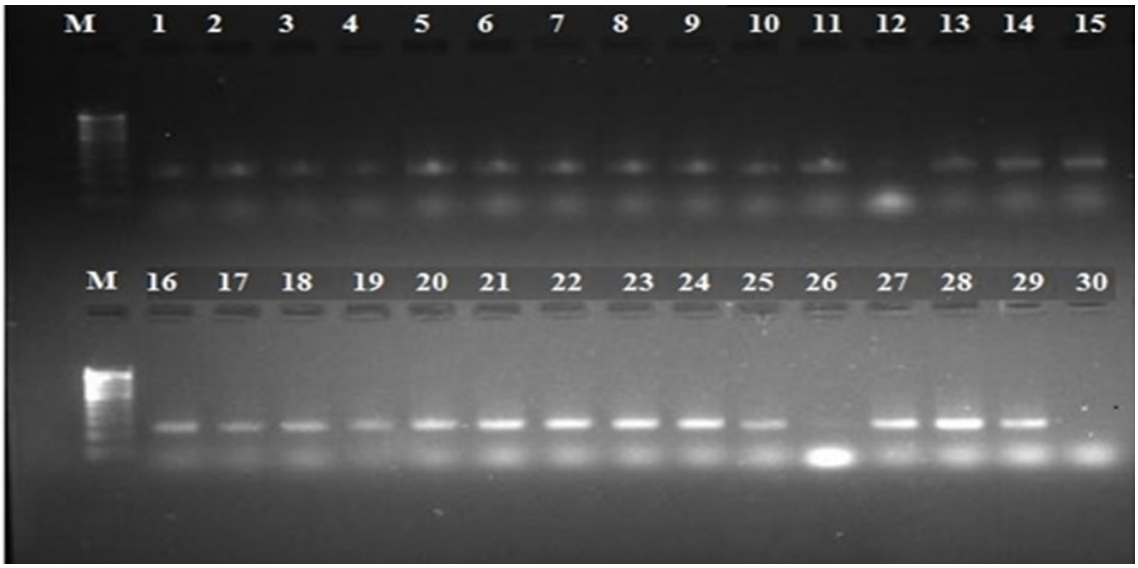

Fig. 2. *PCR amplification profile of 30 Pigeon pea genotypes (Pusa992, H200314, ICP5028, AH065, H200125, AL1758, AH069, ICPL99051, ICPA2039, JBP110B, ICPL332, ICPL20128, UPAS120, H0259, H200014, AH067, SGBS6, H0228, ICPL99050, ICP14085, AL1756, AL1760, AL15, MAL15, AH099, AL1849, AH 0612, PAU881, ICPL88039 and Manak) with CcM0268 SSR marker*

variety at the molecular level (Palombi and Damiano, 2002, Kinhoegbe 2022, Kimaro 2020).

Microsatellites are highly popular genetic markers because of their co-dominant inheritance, high abundance, the enormous extent of allelic diversity, and the ease of assessing SSR size variation by PCR with pairs of flanking primers. Numerous authors said that the genetic map was a comparison of the genetic map generated via means of exclusive molecular markers in various research (Russell et al., 1997) and that the best mild settlement among genetic distance estimates made the use of SSR. Recently, different kinds of gene -based molecular markers such as SSR, ISSR, SLP, EST-SSR, SRAP, TRAP, SNP, STMS are accessible, which detect polymorphism at the gene level (Kumar et al.2015, Kumar et al.2017, Kumar et al., 2018, Addae Frimpomaah et al., 2021). The present study employed that microsatellite, short tandem repeats, or simple sequence repeats are monotonous repetitions of very short nucleotide motifs, which occur as interspersed repetitive elements in all eukaryotic genomes.

The outcome of the present study suggests that quite a huge wide variety SSR primers may be used to differentiate genetic versions amongst pigeon pea genotypes. These markers offer essential facts approximately the genetic version of the Pigeon pea (Addae Frimpomaah, 2021). The polymorphism amongst genotypes may be detected through the usage of random primers. The discrepancy in the banding prototype of the amplification products occurs due to the variation in the size of DNA sequences flanked through the primers. The present study utilized 30 Pigeon pea genotypes for SSR analysis with 20 random primers. Some primers produced a high degree of polymorphism (Fig. 1 and (Fig. 2), but some primers did not produce polymorphism. Among 20 primers, only 8 primers showed the highest polymorphism (100%) and 5 primers were monomorphic in nature. On average 2.30 bands per primer were amplified. The dendogram constructed from the pooled data revealed seven distinct clusters of which five were solitary (Fig. 3). In the fifth cluster, the highest genetic similarity (0.95) was found between AH-067 and ICPL88039, while the lowest genetic similarity (0.79) was found between PAU-881 and AH 0612 & MAL-15. The diversity ranged from 5 to 21%. The two main clusters were separated at 15.00% diversity. The first cluster was separated at 21% diversity, the second at 20.4% diversity, the third at 19.2% diversity and the fourth at 17% diversity. 79% diversity was observed between ICPL99050 with PAU-881 and AH 0612. Similarly, Addae-Frimpomaah et al. (2021) reported the genetic diversity in pigeon pea varieties as high as 94 per cent with marker CCttaa015, while the least diversity was observed in marker CCB10 i.e., 0.73. Three Dimensional structures of 30 pigeon pea cultivars were examined and drawn based on molecular data (Fig. 4). The clustering pattern did not follow any definite character base, geographic area of adoption or ploidy level. In the future, many cultivars should be analyzed with more primers. The knowledge of genetic diversity among pigeon pea cultivars can be applied in future breeding programs to improve pigeon pea crops with respect to yield and different quality traits to meet the increasing demand of various plant breeding industries and consumers.

The PIC values derived from allelic diversity and frequency among the genotypes were not uniform for all the SSR loci tested. The PIC value for 20 primers var-

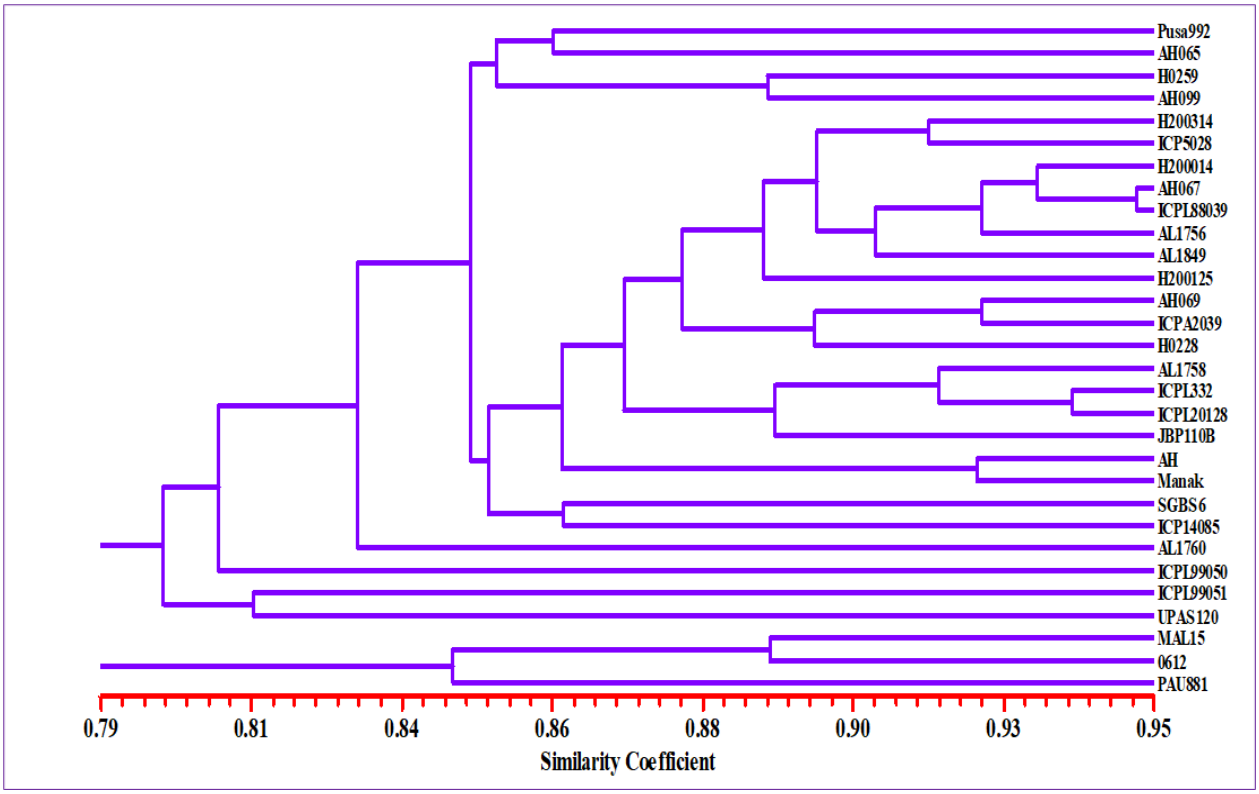

Fig. 3. *Dendogram showing the clustering pattern of 30 pigeon peas using present and absence of a band in the amplification*

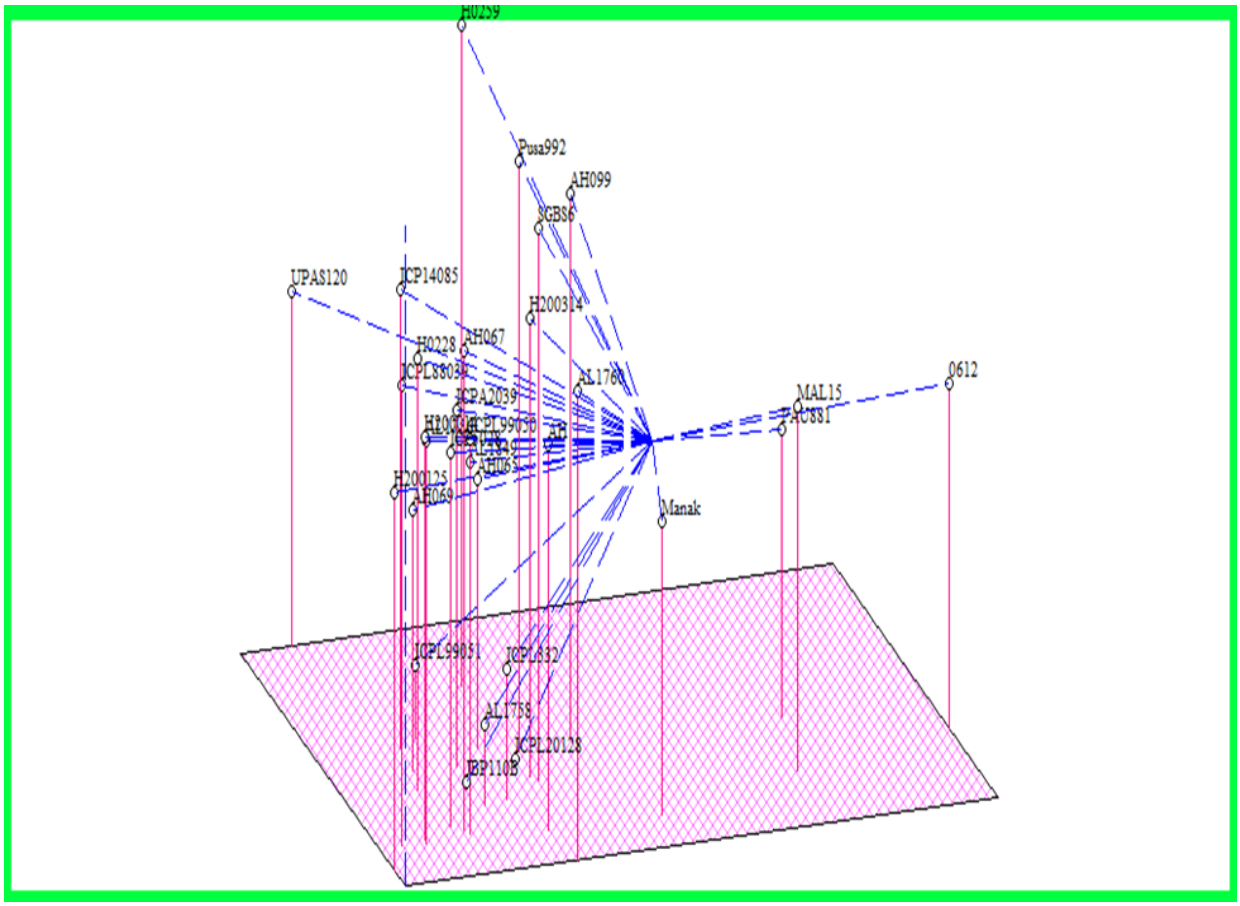

Fig. 4. *Three-dimensional plot of 30 Pigeon pea clutivars obtained using principal component analysis of SSR data*

ied from 0.033 (CcM1381) to 0.654 (CcM 2977) with a mean of 0.247.Lower PIC values may result from closely related genotypes and higher PIC values might result from diverse genotypes (Table 2). Some other primers in pigeon pea had low PIC values earlier reported by Addae-Frimpomaah *et al.* (2021). Among the primers used in the present study, CCB10 was highly informative since it recorded a high PIC value (0.37). The markers showed an average PIC value of 0.25, indicating that SSR markers used in this study were not highly informative because only PIC values higher than 0.5 indicate high polymorphism.

Conclusion

The present study used microsatellite markers to determine the genetic range among 30 Pigeon pea varieties. The use of molecular markers inclusive of SSR was validated for a significant genetic range and genetic dating among Pigeon pea cultivars. The facts acquired from this examination showed that the performance of SSR marker was excessive for the dedication and estimation of genetic similarity amongst extraordinary Pigeon pea genotypes. It was discovered that the extreme stage of range appeared in 30 Pigeon pea cultivars. The most genetic similarity occurred in AH-067 and ICPL88039, while minimal genetic similarities were observed in PAU-881 and AH 0612 & MAL-15. The finding of this examination unravels the performance of SSR markers for the evaluation of genetic diversity. Microsatellite markers might be a beneficial resource for Pigeon pea breeding. These SSR markers might also be helpful in genomic mapping research, improving Pigeon pea cultivars with various genetic histories and gaining progressed crop productivity.

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Conflict of interest

The authors declare that they have no conflict of interest.

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