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Research Article

GENETIC DIVERSITY AND PHYLOGENETIC BEHAVIOR OF 30 PIGEONPEA GENOTYPES

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Abstract: The Pigeon pea (*Cajanus cajan*), one of the major legume crops is chiefly cultivated in tropical and subtropical regions of Asia and Africa. Employing crop improvement programmes is difficult because it generally demonstrations a low degree of genetic diversity. Nevertheless, assessment of the molecular diversity is crucial in population genetic studies. Hence, genetic diversity of 30 pigeonpea genotypes was evaluated based on polymorphism data for 48 SSR markers. DNA polymorphism data, revealed moderate genetic diversity among the genotypes. The genotypes were classified into two sub clusters. The PIC value ranged between 0.02 to 0.76 for markers ASSR108 and AHSSR237 respectively with a mean of 0.39.

Keywords: *Genetic Diversity, Simple Sequence Repeat, Pigeonpea, Polymorphic Information Content*

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Introduction

Pigeonpea, is one of the most widely grown and consumed pulse crop and major source of protein for people whose major dietary intake comes from plant-based foods. Pigeonpea has a wide range of products, including the dried seed, immature seeds used as green vegetable, leaves and stems used for fodder and the dry stems used as fuel. It also improves soil fertility through nitrogen fixation as well as from the leaf fall and recycling of the nutrients [1, 2]. As it serves as a major source of protein to about 20% of the world population [3,4], it is a food and cash crops for small hold farmers. National research systems and government are making concerted efforts to promote this crop among poor. Hence focusing research on biofortification in this crop can be expected to reach the poor people and small hold farmers growing this crop. A well-framed breeding programme is essential for achieving the goal of biofortification. In any crop improvement programme, genetic diversity is an essential prerequisite for hybridization. Hence, in our study we selected 15 high zinc genotypes and 15 low zinc genotypes from the pigeonpea accessions that we had screened earlier, to study the genetic variation that existed in the different zinc types, so that they could be used in future breeding program. Genetic variation can be measured at two levels: one is phenotype - the combination of individual traits resulting from a genotype and its interacting environment; second being genotype- the particular genetic make-up of an organism [5]. Thus, this study was conducted with an objective of accessing the extent of genetic variation that existed among the pigeonpea accessions differing for zinc content using DNA based markers.

Material and Methods

Plant material

Thirty pigeonpea accessions of which 15 were high zinc types and 15 were low zinc types were sown in *kharif* season to collect the leaf sample for DNA extraction.

Collection of sample and DNA isolation

Young healthy leaves were collected and frozen in liquid nitrogen from individual lines in the field. Frozen leaves were used to grind. The fine powder obtained from each sample was used for extraction of DNA as per modified cTAB (Cetyl Trimethyl Ammonium Bromide) method of [6].

Screening with SSR markers

In the present study, 48 SSR markers comprising both genic [7] and genomic [8] were chosen for diversity studies in pigeonpea. 96 well PCR systems (Bio-Rad and Eppendorf) were used for amplification of the desired SSR sequence.

Polymerase chain reaction

SSR markers were used to amplify the SSR regions in the template DNA of pigeonpea accessions. The volume of reaction mixture per one reaction was as follows: PCRs were carried out in 10 µl volume containing1 µl of 10X reaction buffer,0.10µl of 10 mM dNTPs,1µl each of forward and reverse primers (5pmol), 1µl (60 ng/µl) of template genomic DNA and 0.04µl (0.75 U) of Taq DNA polymerase (Kappa Taq).

Fragment analysis and scoring

PCR products were resolved by electrophoresis in 3% Agarose gel containing 0.1 µg/ml ethidium bromide in 1X TBE buffer at 130 V for 4 h,. For greater resolution 3% metaphor agarose gels with 0.1µg/ml of ethidium bromide was being used. Gel documentation system (Biorad) was used to visualize and photograph the amplified bands present in the gel. The amplicons were scored for each genotype based on amplicon size with reference to a 100bp marker.

Diversity analysis

DARwin 5 software was used for diversity analysis of high seed zinc type and low seed zinc type genotypes.

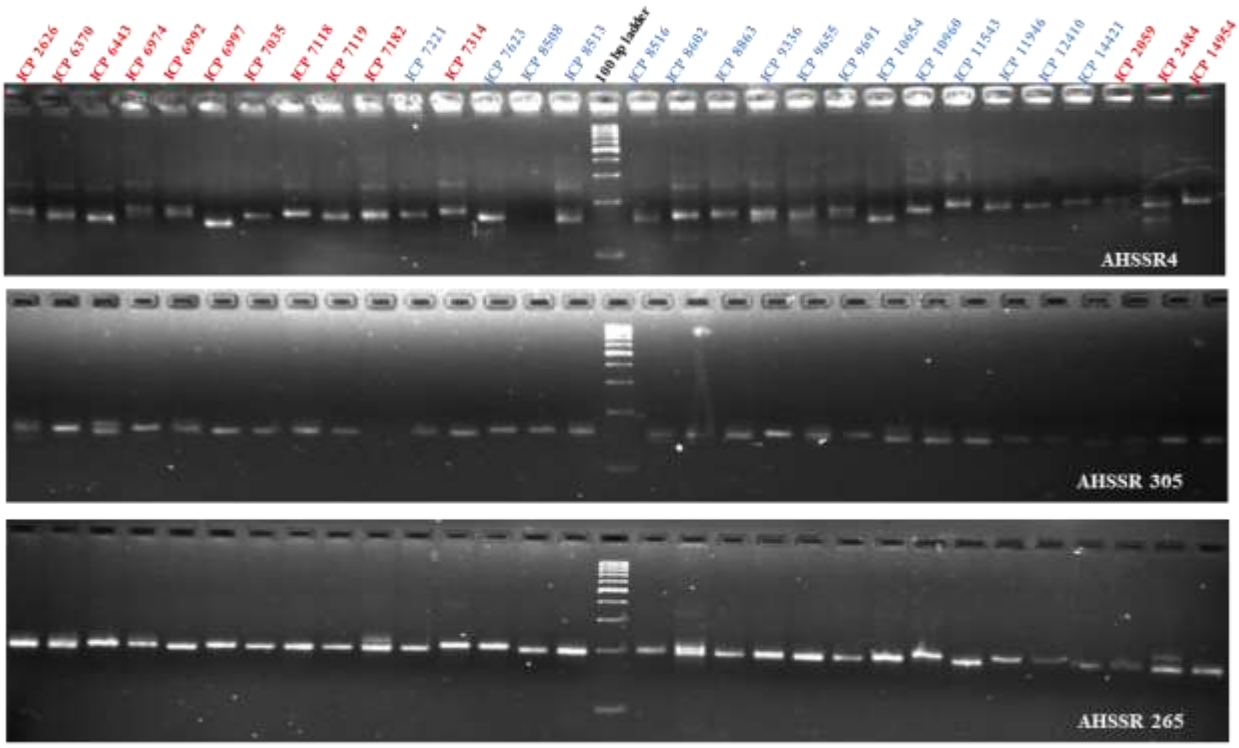

Fig-1 Representative agarose gel showing PCR amplicons of genomic SSR markers for 30 genotypes of pigeonpea
Note: Accession names mentioned in red are the high Zn types and those mentioned in blue are the low Zn types.
The top panel is the PCR amplicons for AHSSR4, middle one is for AHSSR305 and bottom panel is for AHSSR265

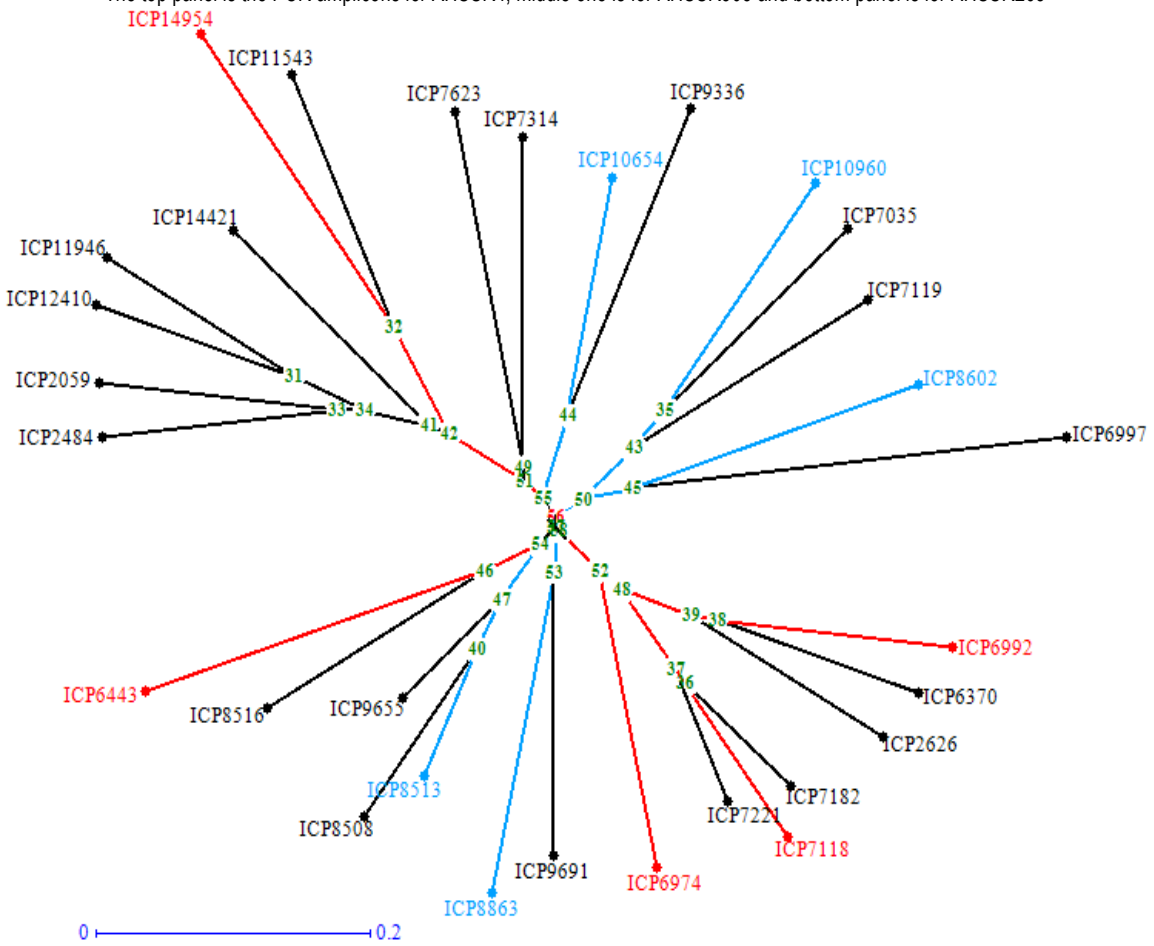

Fig-2 Distance based structure of UPGMA (Unweighted pair group method arithmetic average) phenogram of 30 germplasm accessions based on genotypic data using DARwin5
Note: Red colour depicts the high Zn content genotypes, Blue colour depicts the low Zn content genotypes

Distance based structure of UPGMA (Unweighted Pair Group Method Arithemetic average) was drawn for 30 pigeonpea genotypes based on the genotypic data of 48 SSR markers.

Table-1 *Polymorphic information content (PIC) value of the SSR markers used for diversity analysis*

S	Marker	PIC	S	Marker	PIC
1	ASSR 1	0.3	25	AHSSR 23	0.5
2	ASSR 108	0.02	26	AHSSR 233	0.42
3	ASSR 109	0.03	27	AHSSR 235	0.18
4	ASSR 11	0.03	28	AHSSR 236	0.4
5	ASSR 120	0.03	29	AHSSR 237	0.76
6	ASSR 148	0.35	30	AHSSR 239	0.25
7	ASSR 17	0.03	31	AHSSR 249	0.43
8	ASSR 19	0.15	32	AHSSR 263	0.42
9	ASSR 20	0.13	33	AHSSR 265	0.26
10	ASSR 23	0.34	34	AHSSR 269	0.47
11	ASSR 379	0.06	35	AHSSR 271	0.49
12	ASSR 66	0.04	36	AHSSR 29	0.42
13	ASSR 77	0.03	37	AHSSR 301	0.45
14	ASSR 9	0.03	38	AHSSR 302	0.4
15	AHSSR 114	0.54	39	AHSSR 303	0.58
16	AHSSR 126	0.46	40	AHSSR 305	0.32
17	AHSSR 128	0.35	41	AHSSR 42	0.16
18	AHSSR 160	0.55	42	AHSSR 45	0.47
19	AHSSR 163	0.57	43	AHSSR 59	0.3
20	AHSSR 164	0.23	44	AHSSR 89	0.36
21	AHSSR 199	0.75	45	AHSSR 90	0.58
22	AHSSR 20	0.34	46	AHSSR 92	0.52
23	AHSSR 21	0.4	47	AHSSR 95	0.45
24	AHSSR 228	0.29	48	AHSSR 99	0.52

Results and Discussion

Thirty contrasting genotypes were selected for accessing molecular diversity using 48 SSR markers. PCR profile of AHSSR4, AHSSR305 and AHSSR265 among 30 pigeonpea genotypes is given in [Fig-1]. These 48 AHSSR markers amplified a total of 192 SSR alleles which were scored from 48 AHSSR markers across 30 accessions. The frequency of alleles amplified ranged from 2 (ASSR1, ASSR108, AHSSR128, ASSR129, ASSR109 and ASSR23) to 8 (AHSSR237) with a mean of 3.86. [Table-1] gives the information of the 48 SSR markers used for diversity studies along with their respective PIC values. Heterozygosity was observed to some extent as expected in an often-cross pollinated crop with an average of 0.13 and the maximum was observed (0.53) for AHSSR21. Polymorphic information content (PIC) was also estimated which is a measure of the probability of two randomly chosen alleles from population are distinctly different. The PIC value ranged between 0.02 (ASSR108) to 0.76 (AHSSR237) with a mean of 0.39. Around 50 % of the markers were highly informative with PIC values more than 0.50. While 30% markers were reasonably informative (0.5>PIC>0.25) and rest of the markers had low PIC values of <0.25. Assessment of the molecular diversity is crucial in population genetic studies. Diversity analysis of 30 genotypes using UPGMA algorithm, grouped them into a single major cluster. The top five high zinc types and the bottom 5 low zinc types are represented in red and blue colour respectively [Fig-2]. However, there were two sub clusters based on the bootstrap values (range < 80) the accessions had to be considered as single clade. The subcluster-I comprised the maximum of 16 accessions. Among the 30 accessions, ICP7182 and ICP7221 (0.2) were the closest while ICP9336 and ICP14954 (0.86) showed the maximum genetic distance based on UPGMA (DARwin V 5.0). A higher genetic distance of 0.73 was observed between the high zinc and low zinc lines ICP6443 and ICP10960 respectively. Significant genetic variability is one of the primary prerequisites for improving Zn acquisition. Such genotypic variations can be exploited in breeding programmes to produce genotypes with higher Zn efficiency. These 30 genotypes contrasting for seed zinc content were characterized for molecular diversity with 48 SSR markers. Being an often-cross pollinated spp., pigeonpea is reported to have a significant level of residual heterozygosity. Thus, 48 co-dominant SSR markers that were used to decipher the molecular diversity observed a mean heterozygosity of 0.13 among the 30 contrasting accessions including the parental lines. The SSR markers used were quite capable of detecting polymorphism with a mean PIC value of 0.39 ranging between 0.02 to 0.76. The microsatellite markers revealed moderate genetic diversity among genotypes of pigeonpea, which were clustered into two groups in a single clade. Some of the high Zn genotypes were clustered along with few low Zn genotypes and *vice versa*. Therefore, a more elaborate linkage mapping needs to be carried out to identify the causal genomic region controlling seed Zn content. Low variability was observed in genetic diversity studies for 77 genotypes of pigeonpea. It was speculated that low variability in these collections may be due to a narrow genetic base of the original germplasm collection or pre-selection of these genotypes based on some agronomic characteristics [9]. Several other workers also reported a quite narrow genetic diversity [10-12] in pigeonpea. Narrow genetic base has also been reported due to use of only few genotypes with high degree of relatedness in crossing programmes for the development of new cultivars [11,13]. It was observed that 16 (34 %) pigeonpea cultivars released in India involved only one or two genotypes as one of the ancestors in their pedigree [13]. To increase genetic diversity of pigeonpea breeding material, new diversity from wild relatives will be extremely useful, although there is substantial variation in existing collections. In addition, use of genome wide markers could aid in accessing genetic diversity largely. Besides the narrow genetic diversity, presence of heterozygosity is yet another factor that potentially slows the progress in crop improvement in pigeonpea. Heterozygocity profile in pigeonpea was as high as 0.53. Although pigeonpea is considered an autogamous species, in the presence of pollinators, the cross-pollination can occur, ranging from 3 % to 26 % [14].

Conclusion

Moderate genetic diversity was observed among the 30 pigeonpea genotypes using 48 SSR markers. Further, the resolution of the diversity observed can be increased by screening with greater number of markers. Retrotransposons markers are gaining a lot of importance in uncovering polymorphism due to their large proportion and dispersed localization within the genome, Hence, using retroelement sequences can be employed as other alternative for diversity studies.

Application of research: Based on the diversity studies the most distinct genotypes can be identified and can be utilized in hybridization programme to develop mapping populations for molecular breeding studies. Population structure can also be studies for large number of accessions.

Research Category: Genetic diversity studies

Abbreviations:
SSR: Simple sequence repeats
PIC: Polymorphic information content
UPGMA: Unweighted Pair Group Method Arithemetic average

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Study area / Sample Collection: UAS, GKVK, Bangalore

Cultivar / Variety / Breed name: Pigeonpea accessions from ICRISAT

Conflict of Interest: None declared

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Ethical Committee Approval Number: Nil

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