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## Genetic Diversity among Pigeonpea (*Cajanus cajan* L. Millsp.) Genotypes Using Genic SSRs with Putative Function for Drought Tolerance

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### ABSTRACT

#### Keywords

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One hundred and thirty eight pigeonpea genotypes were analyzed for molecular genetic diversity using 34 SSR markers with putative function for drought tolerance. The study revealed considerable molecular genetic diversity among genotypes. Fifty two alleles were obtained with 34 SSR markers while, 1 to 3 alleles was scored with an average of ~1.6 alleles for each SSR. Three alleles were amplified by markers ASSR1, ASSR93 and ASSR97. Of these, 15 SSR markers were found to be polymorphic which identified 33 alleles among 138 genotypes. The average PIC value of these polymorphic SSRs was 0.22 with a range of 0.01 for ASSR308 to 0.38 in ASSR97. Significant positive correlation was observed between PIC values with number of alleles amplified per primer ( $r = 0.58^*$ ,  $P < 0.05$ ), and gene diversity ( $r = 0.99^{**}$ ,  $P < 0.01$ ) and between allele number and gene diversity ( $r = 0.57^*$ ,  $P < 0.05$ ). The average genetic distance for all pair wise comparisons was estimated as 0.27. The highest genetic distance of 0.69 was recorded between genotypes PAU-881 and LRG-41. Cluster analysis, done by UPGMA following Nei's similarity matrix and population structure analyses grouped 138 pigeonpea genotypes into seven sub-populations.

### Introduction

Pigeonpea (*Cajanus cajan* L. Millsp.) is predominantly a rainfed crop grown across the world. Although it is considered as a drought tolerant crop among all grain legumes and largely grown under rainfed conditions (Keller and Ludlow, 1993) across the world, productivity is highly affected by drought if it coincide with flowering and early pod development stages (Lopez *et al.*, 1997). There is large variation for days to maturity, ranging from extra early (90 days) to very long (300 days) among available pigeonpea germplasm. The intermittent periods of

drought can affect the growth and yield of specially short-duration pigeonpea sown at the start of the rainy season. As pigeonpea is cultivated under rain-fed conditions, occurrence of drought may be episodic in varying degrees in the majority of the growing season in dry land agricultural systems. An increase in temperature above 2.5°C, is known to convey negative effects on global agriculture on the whole. Adverse impact of drought on crop growth and development causes yield reduction. Despite several decades of intensive efforts in

different crop improvement programmes, the yield level reached a plateau, owing to the narrow genetic base and conventional breeding procedures. The high degree of complexity associated with the genetic enhancement through breeding procedures can be successfully overcome by the employment of biotechnological interventions (Chakravarthy and Negi, 2014).

The recent advancement in pigeonpea genomic resources resulted in the development of molecular markers, genetic maps, transcriptomic or genome sequence required for molecular breeding. Discovery of molecular markers led to genetic diversity analysis using restriction fragment length polymorphism (Sivaramakrishnan *et al.*, 2002), amplified fragment length polymorphism (Panguluri *et al.*, 2006), random amplification of polymorphic DNA (Yadav *et al.*, 2012), microsatellite markers (Singh *et al.*, 2013) and DArT (Yang *et al.*, 2006). Nevertheless, the molecular basis of most agronomic traits in pigeonpea remains unexplored due to the low level of DNA polymorphism and limited number of validated molecular markers. The presence of genetic diversity plays a vital role for a successful breeding program. Genetic diversity is essential prerequisite in breeding for drought tolerance, increased yields, wider adaptation and desirable quality. Earlier studies on genetic diversity with limited number of genotypes has been reported in pigeonpea *viz.*, 36 elite cultivated genotypes (Singh *et al.*, 2013), 45 genotypes (Datta *et al.*, 2013), 16 cultivars and 2 wild relatives (Yadav *et al.*, 2012), 15 genotypes (Shende and Raut, 2013), 49 genotypes (Rekha *et al.*, 2011), 88 accessions (Songok *et al.*, 2010), 16 genotypes (Singh *et al.*, 2008) and 14 genotypes (Chakraborty *et al.*, 2013). These studies however, focused on studying overall genetic diversity among pigeonpea germplasm using genic and genomic SSRs

not specifically SSR markers related to drought tolerance.

The objective of the present investigation was to study the level of molecular genetic diversity and population structure among pigeonpea cultivars and germplasm collection using genic SSR markers linked with putative function for drought tolerance.

## **Materials and Methods**

### **Plant material**

A total of one hundred and thirty eight pigeonpea genotypes, adapted to different climatic conditions, were received from Indian Institute of Pulses Research, Kanpur, India. Information on sources of origin of these genotypes is given in Online Resource 1. The genotypes included in the study are mostly the released varieties for different production areas in India; advanced breeding lines and germplasm accessions from Regional Research Station, National Bureau of Plant Genetic Resources, Hyderabad. All these genotypes were sown in two rows of 2.5m plot, with a row to row spacing of 90 cm and plant to plant spacing of 30 cm in augmented block design at CRIDA, Hyderabad. The recommended fertilizer doses and agronomic operations were carried out for adequate protection against pests, diseases and weeds.

### **DNA extraction and PCR**

Genomic DNA was extracted following CTAB method (Paterson *et al.*, 1993) with minor modifications from top most fully expanded leaf samples of four-week old plants for each genotype. Thirty four genic SSR markers previously reported by Dutta *et al.*, (2011) were used to amplify the DNA for genotyping. The PCR reaction contained 1.0 unit of Taq DNA polymerase, 1X Taq buffer

and 200 $\mu$ M of each dNTP. Approximately, 50ng of genomic DNA and 10 picomoles of each primer were used and the volume was made up to 20 $\mu$ l using sterile distilled water. DNA amplification was carried out in a Thermal Cycler (Applied Biosystems) with a PCR profile comprised an initial denaturation for 5 min at 94°C followed by 35 cycles with a denaturing step at 94°C for 45 seconds, a primer annealing at 60°C for 45 seconds and an extension at 72°C for 45 seconds. After the last cycle, a final extension was carried out at 72°C for 5 min. Amplified PCR products were resolved through electrophoresis at 80 volts for one hour and 30 minutes in 4% agarose gel containing 0.5 $\mu$ g/ml ethidium bromide and photographed under ultraviolet light with Vilber Loumat gel documentation system. The SSR amplification profiles were scored based on the size (bp) of the amplicons obtained among 138 genotypes using Biovision Software, USA.

### **Statistical analyses**

Gene diversity, heterozygosity and polymorphism information content (PIC) for each of the primer pair was calculated using Power Marker v.3.25 software (Liu and Muse, 2005). Genetic distances between the genotypes were also calculated (Nei, 1973). Phylogenetic tree was constructed using UPGMA (unweighted pair-group method using arithmetic average) by neighbor-joining method and dendrogram was generated by MEGA software version 5.0 (Tamura *et al.*, 2011). The STRUCTURE 2.3.4 (Pritchard *et al.*, 2000; Falush *et al.*, 2003) software was used to detect population structure and assign individuals to subpopulations following model based on clustering by Bayesian approach which identifies clusters based on a fit to Hardy–Weinberg linkage equilibrium. The population structure analysis was used to infer historical lineages that show grouping of similar genotypes. For each cluster K, five

replications were run where each run was implemented with a burn-in period of 100,000 steps followed by 100,000 Monte Carlo Markov Chain replicates derived for each K and then plotted to find the plateau of the  $\Delta K$  values (Evanno *et al.*, 2005).

### **Results and Discussion**

Research for development of drought tolerant crops is of urgent priority, as water stress is one of the main reasons for the major crop losses globally and is expected to exacerbate due to projected climate change impacts. Pigeonpea being an important source of dietary protein and a major legume in the arid and semi-arid regions, may be adversely affected due to climate change unless efforts made to develop tolerant cultivars. Identification of diverse parents is essentially required before carrying out successful crop improvement program (Tidke and Ranawade, 2017). In this section we discuss the results pertaining to molecular characterization of 138 pigeonpea genotypes adapted to diverse climatic conditions using SSR markers associated with putative function for drought tolerance.

### **Polymorphism and marker efficiency**

Pigeonpea genotypes characterized using 34 SSR markers revealed 15 SSRs as polymorphic (~44%) while 19 as monomorphic (~66%). The polymorphic SSRs was used to examine the degree of genetic variation among pigeonpea genotypes. List of polymorphic SSRs and their predicted function of genes linked with these SSRs, amplicon size was given in Table 1. A total of fifty two alleles were obtained with 34 SSR markers and number of alleles scored for each SSR loci ranged from 1 to 3 with an average of ~1.6 alleles per primer pair. Three alleles were amplified by ASSR1, ASSR93 and ASSR97. The amplification profile of

ASSR93 was given in Fig. 1. The PIC value of SSRs ranged from 0.01 (ASSR308) to 0.38 (ASSR97) with an average being 0.22 (Table 2). Among these 15 polymorphic SSR markers, 8 SSRs *viz.*, ASSR1, ASSR3, ASSR8, ASSR19, ASSR93, ASSR97, ASSR280 and ASSR648 gave PIC >0.25 with an average of 0.32 and 19 alleles with an average of 2.38 alleles/primer. The major allele frequencies among the primers tested varied between 0.59 (ASSR19) to 0.99 (ASSR213) with an average of 0.82. On the other hand, gene diversity varied from 0.01 to 0.48 with an average of 0.26.

The study revealed a total of 52 alleles using 34 SSR markers among 138 genotypes which was similar to findings made by Singh *et al.*, (2013), who reported 59 alleles using 60 SSR markers among 36 genotypes. The PIC of SSRs obtained in our study was relatively higher than those reported by Khalekar *et al.*, (2014) and Datta *et al.*, (2013). Several other workers also reported different level of genetic diversity in pigeonpea (Panguluri *et al.*, 2006; Yang *et al.*, 2006; Singh *et al.*, 2013). This variation in genetic diversity is probably attributed to diversification in morphology, use of common ancestors for the development of new cultivars (Panguluri *et al.*, 2006; Yang *et al.*, 2006). The present study also revealed significant positive correlation between PIC values with number of alleles amplified per primer ( $r = 0.58^*$ ,  $P < 0.05$ ) and gene diversity ( $r = 0.99^{**}$ ,  $P < 0.01$ ), and between allele number and gene diversity ( $r = 0.57^*$ ,  $P < 0.05$ ).

### **Genetic similarity among genotypes**

Genetic distance among the 138 pigeonpea genotypes was calculated to identify the relatedness between genotypes. The genetic distance measured through polymorphic SSRs revealed varying degree of genetic relatedness among the pigeonpea genotypes. The average genetic distance for all pair wise comparisons

was 0.27. The highest genetic distance of 0.69 was recorded between genotypes PAU-881 and LRG-41; PT-00-022 and LRG-41, followed by BWR-153 and RVK-281; RJR-292 and GT-1 which exhibited genetic distance of 0.67. Whereas, 10 genotype combinations *viz.*, CO-6 and AL-1578, GT-100 and AL-1578, ICP-84031 and AL-1578, UPAS-120 and LRG-41, AL-1816 and RVK-278, VKG-14151 and RVK-281, RVK-278 and AL-1578, PG-12 and AL-1816, Pusa-84 and CO-5, Pusa-84 and GRG-2761 had genetic distance of 0.64.

### **Cluster analysis**

The cluster analysis based on Power marker software using polymorphic SSR markers resulted in separation of the genotypes into two major clusters (Fig. 2). Cluster II was larger comprising of 97 genotypes. Further, this cluster was sub-divided into five sub-clusters with number of genotypes per cluster ranging from 6 to 35. On the other hand, cluster I is divided into two sub-clusters, consisting of 31 and 10 genotypes respectively.

### **Population structure analysis**

Population structure analysis divided 138 pigeonpea genotypes into seven different groups, assuming low levels of admixture between subpopulations (Fig. 3 and Table 3). List of pigeonpea genotypes corresponding to different groups is given in Online Resource 2. The number of genotypes ranged from 15 in group G<sub>6</sub> to 29 in group G<sub>2</sub>. Groups G<sub>1</sub> and G<sub>3</sub> comprised of 17 genotypes each whereas, groups G<sub>5</sub> and G<sub>7</sub> contained 19 genotypes each. On the other hand group G<sub>4</sub> had 22 genotypes. Among the genotypes tested, higher gene diversity was displayed within G<sub>6</sub> (0.25) followed by G<sub>4</sub> (0.23) and G<sub>1</sub> (0.22), whereas a low level (0.06) of gene diversity was displayed by G<sub>7</sub> (Table 4).

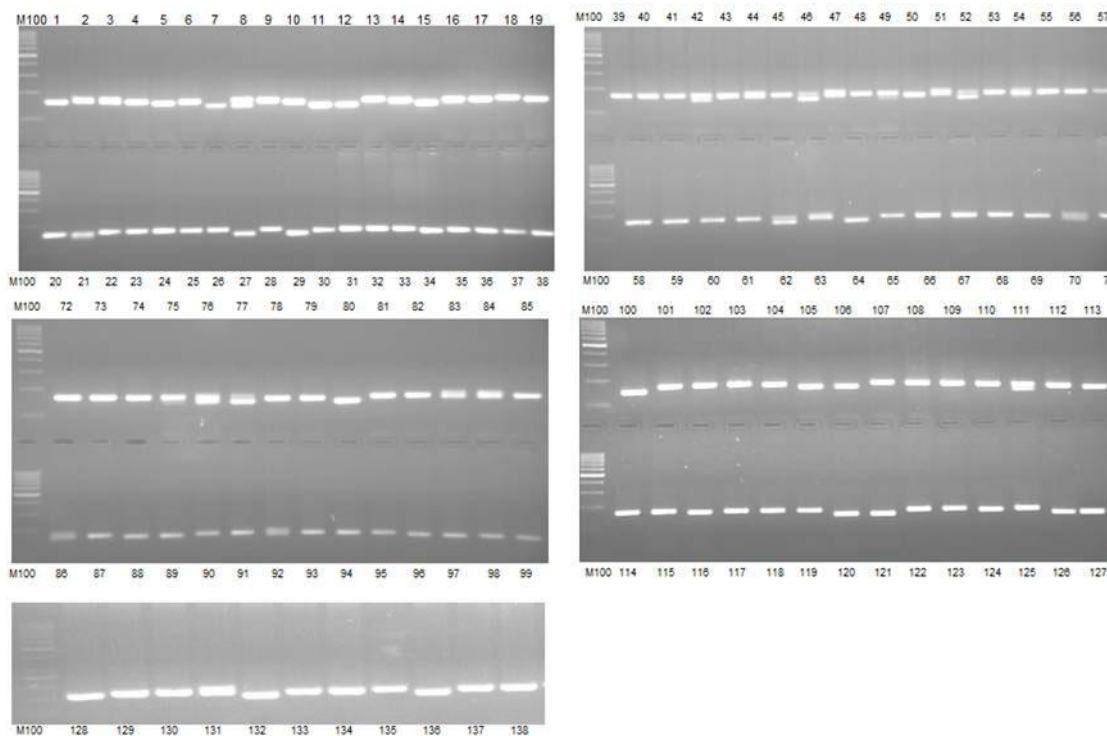
**Table.1** Details of 34 SSRs used in the present study and predicted function of their genes

| Sl. No. | SSR marker | SSR motif | Predicted function  | Amplicon Size (bp) |
|---------|------------|-----------|---|--------------------|
| 1       | ASSR-1     | (GA)10    | Putative Kinase   | 100-120            |
| 2       | ASSR-3     | (AGAAAG)5 | Cytochrome P450 Possessing cinnamate 4-hydroxylase activity | 130-150            |
| 3       | ASSR-8     | (AGA)9    | Cu/Zn-Superoxide dismutase (SOD)                            | 140-150            |
| 4       | ASSR-19    | (TGTTCA)5 | DNA binding protein (Homeodomain)                           | 150-160            |
| 5       | ASSR-23    | (CCTTCT)5 | Acetyltransferase   | 150-170            |
| 6       | ASSR-25    | (GA)10    | Ser/Thr protein kinase                                      | 180                |
| 7       | ASSR-36    | (TC)14    | Global transcription factor group                           | 160                |
| 8       | ASSR-39    | (GAA)7    | Cyclin  | 180                |
| 9       | ASSR-49    | (TC)10    | calmodulin binding protein                                  | 180                |
| 10      | ASSR-66    | (CT)12    | Hypothetical protein  | 180                |
| 11      | ASSR-70    | (GGTAGA)6 | Gamma glutamylcyclotransferase                              | 170-200            |
| 12      | ASSR-91    | (GGTTA)5  | Hypothetical protein  | 120                |
| 13      | ASSR-93    | (CATTTG)5 | Hypothetical protein  | 160-180            |
| 14      | ASSR-97    | (ATGGAC)8 | Chloroplast targeted copper chaperone                       | 150-190            |
| 15      | ASSR-121   | (TCT)8    | Ethylene responsive transcription factor                    | 180                |
| 16      | ASSR-138   | (CTT)8    | r2r3-myb transcription factor                               | 160                |
| 17      | ASSR-148   | (CAA)7    | Ethylene-responsive transcription factor                    | 110-120            |
| 18      | ASSR-163   | (TCA)8    | Heat shock protein binding                                  | 210                |
| 19      | ASSR-168   | (TCA)9    | Heat shock protein  | 150-160            |
| 20      | ASSR-213   | (AGG)7    | Mitogen-activated protein kinase 1                          | 150-160            |
| 21      | ASSR-275   | (TAAT)5   | MYB transcription factor MYB48                              | 130                |
| 22      | ASSR-279   | (ACAGGA)7 | Senescence-inducible chloroplast stay-green protein-1       | 180-190            |
| 23      | ASSR-280   | (TGGCAT)5 | Senescence-inducible chloroplast stay-green protein         | 160-170            |
| 24      | ASSR-304   | (GTT)7    | Ethylene responsive transcription factor                    | 110                |
| 25      | ASSR-308   | (TC)10    | Serine/threonine protein kinase                             | 150-160            |
| 26      | ASSR-388   | (CCA)7    | No homology   | 150                |
| 27      | ASSR-538   | (TC)9     | MYB transcription factor MYB34                              | 150                |
| 28      | ASSR-609   | (ACC)6    | Leucine Rich family protein                                 | 190                |
| 29      | ASSR-648   | (GAT)6    | Protein of early response to dehydration                    | 150-160            |
| 30      | ASSR-973   | (TTG)6    | Ethylene insensitive protein                                | 150                |
| 31      | ASSR-1092  | (CGG)6    | Serine/threonine protein kinase catalytic domain            | 160                |
| 32      | ASSR-1214  | (ACA)6    | WRKY family transcription factor                            | 170                |
| 33      | ASSR-1217  | (GGA)6    | WRKY family transcription factor                            | 190                |
| 34      | ASSR-1639  | (AAT)6    | Senescence-associated protein                               | 150                |

**Table.2** Number of alleles, gene diversity and PIC of polymorphic microsatellite markers

| Sl. No. | Marker  | Number of Alleles | Amplicon Size (bp) | Gene Diversity | PIC  | Major allele frequency |
|---------|---------|-------------------|--------------------|----------------|------|------------------------|
| 1       | ASSR1   | 3                 | 100-120            | 0.39           | 0.33 | 0.75                   |
| 2       | ASSR3   | 2                 | 130-150            | 0.31           | 0.26 | 0.81                   |
| 3       | ASSR8   | 2                 | 140-150            | 0.29           | 0.25 | 0.83                   |
| 4       | ASSR19  | 2                 | 150-160            | 0.48           | 0.37 | 0.59                   |
| 5       | ASSR23  | 2                 | 150-170            | 0.28           | 0.24 | 0.83                   |
| 6       | ASSR70  | 2                 | 170-200            | 0.20           | 0.18 | 0.89                   |
| 7       | ASSR93  | 3                 | 160-180            | 0.45           | 0.38 | 0.69                   |
| 8       | ASSR97  | 3                 | 150-190            | 0.48           | 0.38 | 0.62                   |
| 9       | ASSR148 | 2                 | 110-120            | 0.22           | 0.20 | 0.87                   |
| 10      | ASSR168 | 2                 | 150-160            | 0.12           | 0.11 | 0.94                   |
| 11      | ASSR213 | 2                 | 150-160            | 0.01           | 0.01 | 0.99                   |
| 12      | ASSR279 | 2                 | 180-190            | 0.04           | 0.04 | 0.98                   |
| 13      | ASSR280 | 2                 | 160-170            | 0.38           | 0.31 | 0.75                   |
| 14      | ASSR308 | 2                 | 150-160            | 0.01           | 0.01 | 0.99                   |
| 15      | ASSR648 | 2                 | 150-160            | 0.31           | 0.26 | 0.81                   |
|         | Average | 2.20              |                    | 0.26           | 0.22 | 0.82                   |

**Fig.1** PCR amplification pattern of 138 pigeonpea genotypes using ASSR93 primers. M100=100bp DNA size marker



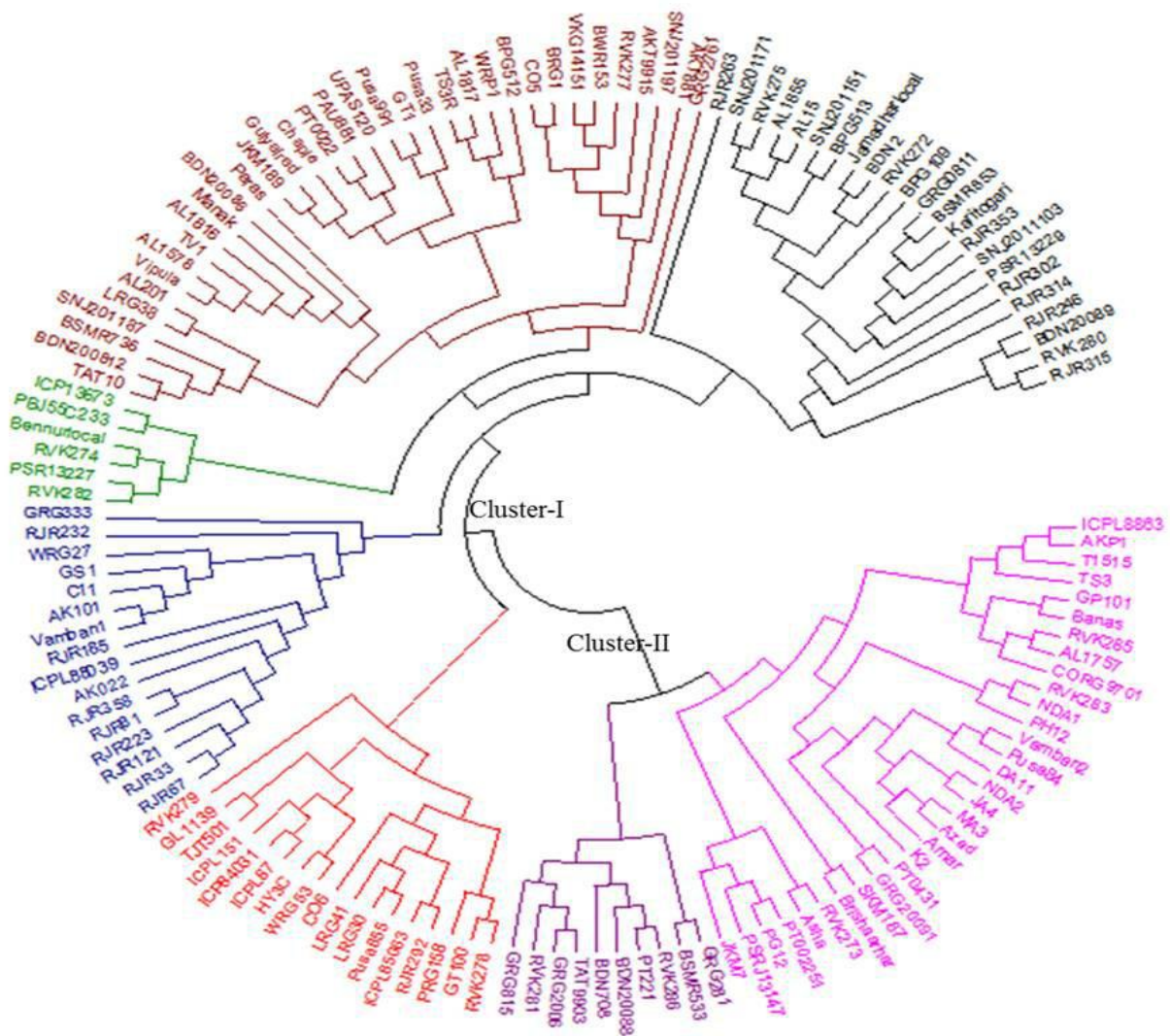
**Table.3** list of pigeonpea genotypes corresponding to a group in population structure analysis G<sub>1</sub>: 54-63; G<sub>2</sub>: 105-44; G<sub>3</sub>: 88-116; G<sub>4</sub>: 129-4; G<sub>5</sub>: 22-114; G<sub>6</sub>: 134-123; G<sub>7</sub>: 33-118

| G <sub>1</sub> |           | G <sub>2</sub> |                | G <sub>3</sub> |           | G <sub>4</sub> |            | G <sub>5</sub> |          | G <sub>6</sub> |           | G <sub>7</sub> |              |
|----------------|-----------|----------------|----------------|----------------|-----------|----------------|------------|----------------|----------|----------------|-----------|----------------|--------------|
| 54             | ICP84031  | 105            | RVK272         | 88             | Pusa855   | 129            | TS3R       | 22             | BDN708   | 134            | Vipula    | 33             | C11          |
| 55             | ICPL151   | 17             | BDN2           | 66             | LRG30     | 138            | WRP1       | 85             | PT221    | 130            | TV1       | 136            | WRG27        |
| 57             | ICPL87    | 61             | Jamadhar local | 60             | JA4       | 25             | BPG512     | 117            | RVK286   | 35             | CO5       | 132            | Vamban1      |
| 87             | Pusa84    | 26             | BPG513         | 68             | LRG41     | 34             | Chaple     | 42             | GRG2006  | 9              | AL1816    | 48             | GS1          |
| 50             | GT100     | 120            | SNJ201151      | 56             | ICPL85063 | 51             | Gulyal red | 40             | GP101    | 19             | BDN20086  | 93             | RJR232       |
| 83             | PT002251  | 101            | RJR353         | 96             | RJR292    | 62             | JKM189     | 126            | TAT9903  | 122            | SNJ201187 | 2              | AK101        |
| 81             | PSRJ13147 | 31             | BSMR853        | 72             | NDA2      | 131            | UPAS120    | 11             | AL1855   | 73             | Paras     | 91             | RJR185       |
| 38             | DA11      | 41             | GRG0811        | 13             | Amar      | 82             | PT0022     | 6              | AL15     | 70             | Manak     | 1              | AK022        |
| 52             | HY3C      | 65             | Karitogari     | 109            | RVK277    | 74             | PAU881     | 59             | ICPL8863 | 89             | Pusa991   | 58             | ICPL88039    |
| 115            | RVK283    | 24             | BPG109         | 36             | CO6       | 10             | AL1817     | 3              | AKP1     | 27             | BRG1      | 90             | RJR121       |
| 137            | WRG53     | 97             | RJR302         | 37             | CORG9701  | 75             | PBJ55C233  | 124            | T1515    | 49             | GT1       | 92             | RJR223       |
| 133            | Vamban2   | 119            | SNJ2011103     | 39             | GL1139    | 18             | BDN200812  | 128            | TS3      | 69             | MA3       | 104            | RJR81        |
| 64             | K2        | 98             | RJR314         | 127            | TJT501    | 135            | VKG14151   | 16             | Banas    | 113            | RVK281    | 102            | RJR358       |
| 110            | RVK278    | 80             | PSR13229       | 47             | GRG815    | 30             | BSMR736    | 20             | BDN20088 | 5              | AKT9915   | 46             | GRG333       |
| 77             | PH12      | 79             | PSR13227       | 8              | AL1757    | 12             | AL201      | 84             | PT0431   | 123            | SNJ201197 | 67             | LRG38        |
| 76             | PG12      | 108            | RVK275         | 15             | Azad      | 125            | TAT10      | 45             | GRG281   |                |           | 103            | RJR67        |
| 63             | JKM7      | 121            | SNJ201171      | 116            | RVK285    | 86             | Pusa33     | 43             | GRG20091 |                |           | 100            | RJR33        |
|                |           | 94             | RJR246         |                |           | 7              | AL1578     | 29             | BSMR533  |                |           | 28             | Brisha arhar |
|                |           | 21             | BDN20089       |                |           | 71             | NDA1       | 114            | RVK282   |                |           | 118            | SKM187       |
|                |           | 112            | RVK280         |                |           | 32             | BWR153     |                |          |                |           |                |              |
|                |           | 99             | RJR315         |                |           | 53             | ICP13673   |                |          |                |           |                |              |
|                |           | 95             | RJR263         |                |           | 4              | AKT881     |                |          |                |           |                |              |
|                |           | 107            | RVK274         |                |           |                |            |                |          |                |           |                |              |
|                |           | 23             | Bennur local   |                |           |                |            |                |          |                |           |                |              |
|                |           | 111            | RVK279         |                |           |                |            |                |          |                |           |                |              |
|                |           | 78             | PRG158         |                |           |                |            |                |          |                |           |                |              |
|                |           | 14             | Asha           |                |           |                |            |                |          |                |           |                |              |
|                |           | 106            | RVK273         |                |           |                |            |                |          |                |           |                |              |
|                |           | 44             | GRG2761        |                |           |                |            |                |          |                |           |                |              |

**Table.4** Summary statistics for the whole group of pigeonpea genotypes and subpopulations detected by structure analysis based on 15 polymorphic SSR markers

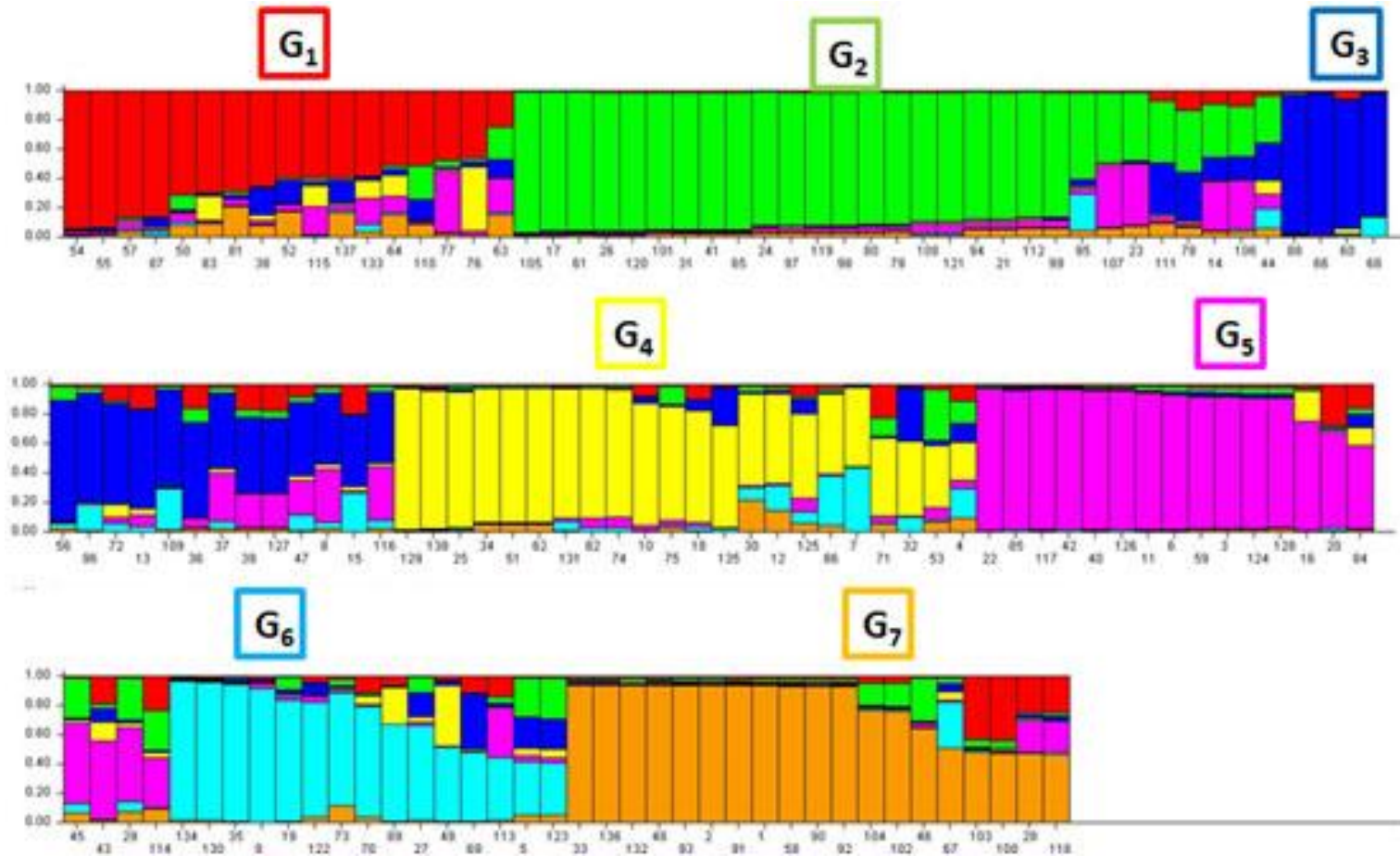
| Statistics              | Overall | G1   | G2   | G3   | G4   | G5   | G6   | G7   |
|-------------------------|---------|------|------|------|------|------|------|------|
| Sample size             | 138     | 17   | 29   | 17   | 22   | 19   | 15   | 19   |
| Total number of alleles | 33      | 25   | 24   | 25   | 27   | 22   | 28   | 19   |
| Mean number of alleles  | 2.20    | 1.67 | 1.60 | 1.67 | 1.80 | 1.47 | 1.87 | 1.27 |
| Major allele frequency  | 0.82    | 0.84 | 0.93 | 0.87 | 0.84 | 0.91 | 0.82 | 0.96 |
| Gene diversity          | 0.26    | 0.22 | 0.10 | 0.19 | 0.23 | 0.13 | 0.25 | 0.06 |

**Fig.2** UPGMA tree using Nei similarity coefficient





**Fig.3** Population structure analysis. The y-axis is the subgroup membership, and the x-axis is the accessions. G (G<sub>1</sub> to G<sub>7</sub>) stands for a subpopulation



Population structure analysis using SSR data revealed seven subpopulations, with varying degrees of admixture among subpopulations (Fig. 3). Structure analysis indicated the patterns of allele sharing among different pigeonpea genotypes from diverse agro-climatic regions and large scale sharing of alleles among the genotypes. In addition, UPGMA tree using neighbor joining also grouped the genotypes into seven subpopulations. The clusters found in structure analysis were almost consistent with the cluster analysis following UPGMA method using Nei similarity coefficient. Most of the genotypes were classified into the corresponding sub-population and branch was similar with a few exceptions.

Molecular analysis using drought linked genic SSR provided a good insight of genetic diversity and population structure among pigeonpea materials used in the present investigation. These findings will be useful in selection of diverse genotypes for development of new cultivars with adaptation to a broad range of environments. Further, the genotypes producing specific amplicons with SSR markers can be used for cultivar identification.

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