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# Correlation between phenotypic and genotypic distances among selected chickpea cultivars and breeding lines

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**ABSTRACT**

Phenotypic and genetic diversity of thirty diverse short duration chickpea cultivars and breeding lines were assessed using twelve agro-morphological characters and STMS primers. Phenotypic diversity was assessed by Mahalanobis's $D^2$-statistics in two contrasting environments ($E_1$ and $E_2$) using grain yield and other agronomic characters. Mahalanobis's $D^2$ analysis revealed that 30 genotypes were grouped into eight clusters at $E_1$ and 5 clusters at $E_2$. The distance values for all the 435 pairs of genotypes for different traits at $E_1$ ranged from 2.0 between ICCV2 and BGD9920 to 19.1 between ICCV4958 and Karikadale and at $E_2$ it ranged from 0.7 between BGD9706 and JG11 to 47.9 between BGD132 and BG1103. Crossing between these genetically diverse genotypes may result in promising derivatives for grain yield and other agronomically important traits. The genetic diversity studies using STMS markers revealed that among the primers used PIC was highest for the STMS primer CAM0443 and CAM0886, which indicated that these primers might be an effective and useful tool to determine the genetic differences among the genotypes and to study the phylogenetic relationship. The STMS marker profiles resulted in seven clusters at nearly 52 per cent similarity revealing that the presence of genetic diversity at molecular level was high among the selected genotypes. The highest similarity index was observed between BGD72 and Annigeri-1 whereas the lowest similarity index between BGD9920 and ICC92944. The correlation between morphological and STMS dissimilarity matrices was positive 0.079 and non-significant ($p< 0.001$, random permutations) for both locations suggesting that the two systems gave different estimates of genetic relations among the genotypes.

**Key words :** *Chickpea, Short duration genotypes, Morphological diversity, Phenotypic & genotypic distances, STMS.*

## INTRODUCTION

Chickpea (*Cicer arietinum* L.), one of the major food legumes in the world, is an important component of rainfed cropping systems in the Indian subcontinent and West Asia and North Africa (WANA) region where terminal drought and heat stress are the major abiotic constraints to its productivity (Saxena, 1990). Therefore, crop duration plays an important role in determining its adaptation and productivity (Kumar and Abbo, 2001). The shortening of crop duration along with fast initial growth has also been suggested as one of the ways of enhancing yield and yield stability in such stress environments (Subbarao *et al.,* 1995). The short duration types have better adaptation to such environments characterized by terminal drought and heat stress. It is observed that the short duration (75-80 days duration) chickpea genotypes available at present are generally poor in their biomass and grain yield potential and hence farmers may not accept them unless they are genetically improved to increase their yield potential similar or superior to locally adopted cultivars. Therefore, a study was undertaken to assess the genetic diversity based on agronomic traits among selected short duration chickpea cultivars and breeding lines of diverse origins in two contrasting environments.

The diversity analysis and grouping based on the morphological/agronomic traits and DNA markers could indicate those cultivars with the greatest genetic differences before a breeding programme is initiated & different genotypes crossed to obtain a high number of transgressive segregant forms.

Although STMS polymorphisms have been analyzed in elite chickpea germplasm, there is a distinct need to utilize these markers in molecular characterization of landraces, short duration chickpea cultivars and breeding lines for their genetic yield potential. Recent experiments on STMS in the pulse family revealed that some of the STMS identified in one species are also capable of revealing polymorphism in other pulse species, it means once developed in chickpea they can also be used in other chickpea related species (Choumane *et al.,* 2000).

The present study was undertaken to measure the genetic diversity in the short duration chickpea cultivars & breeding lines which represent the whole range of variation in the cultivated chickpea by using both morphological & DNA markers in order to identify the most diverse parents for their utilization in hybridization aiming at yield improvement. The objectives of the study were, to assess the level of correlation between agronomic traits & based on STMS markers among the selected short duration chickpea cultivars & breeding lines of diverse origins.

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## MATERIALS AND METHODS

### Plant materials

**Phenotypic analysis :** Thirty diverse short duration cultivars and breeding lines selected from IARI Reg. Res. Centre, Dharwad were included for the study (**Table 1**). They comprised of short- duration cultivars adapted to southern India and central India, short-duration breeding lines selected for high yield, landraces adapted to short-season environment, short to extra-short duration genetic stocks, genetic stocks for drought, heat and pod borer tolerance; resistant to wilt/root rot, cultivars with wide adaptability & short-duration derivatives of *Cicer arietinum* x *C. reticulatum* cross. These thirty genotypes were evaluated at two different locations during *rabi* 2012-13 in a R.B. Design with two replications at IARI Reg. Res. Centre, Dharwad ($E_1$) and three replications at the IARI New Delhi ($E_2$). The data were recorded on twelve economically important agronomic traits on each of the five randomly selected plants from each plot. The mean data thus obtained were subjected to Mahalanobis's $D^2$ – statistics analysis (Mahalanobis, 1936) as suggested by Rao (1952). All the chickpea genotypes were grouped into respective clusters on the basis of distance values by following Tocher's method.

**Molecular analysis :** The genotyping of all the 30 lines using STMS markers was carried out at Chickpea Molecular Breeding Laboratory, Genetics Division, IARI, New Delhi.

**STMS primers screening :** Thirty three STMS primers were initially screened for their repeatable amplification with thirty genotypes. Among them twenty one primers were selected for further analysis based on their ability to detect polymorphic amplified products across the genotypes (**Table 2**). To ensure reproducibility the primers generating weak products were discarded.

**DNA isolation and quantification :** DNA was extracted from thirty chickpea genotypes using CTAB method. DNA of about 100 mg of fresh young leaf tissue was collected , immediately frozen in liquid nitrogen and stored at −80◦C.

**PCR amplification of DNA and electrophoresis :** STMS markers were synthesized as per the sequences of Winter *et al.* (1999) from Bioneer, Daejeon, South Korea. BioRad My Cycler thermal cycler, Richmond, USA was used to carry out amplifications in 10 µL volume reaction mixture. This mixture contained 1 µL of 20 ng plant genomic DNA, 1.6 µL of 10 Tris buffer (15mM $MgCl_2$ and gelatine), 1 µL of 10 mM dNTP mix, 1.0 µL each of forward and reverse primer and 0.3 µL of 3 U µL−1 Taq polymerase. PCR was performed with following conditions 150 s at 90°C followed by 18 cycles of denaturation at 94°C for 20 s, annealing for 50 s at 50°C (touch down of 0.5°C for every repeat cycle) and 1 min elongation at 72°C for 50 s. Further 20 cycles of denaturation at 94°C for 20 s, annealing for 50 s at 55°C and 50s elongation at 72°C were given and final extension at 72°C for 7 min were performed. The resolution of PCR products was done on six per cent polyacrylamide gels. Band patterns for each of the microsatellites markers were recorded for each genotype by assigning a letter to each band. Alleles were numbered as 'a1', 'a2' etc., sequentially from the largest to the smallest sized band.

**Table 1.** Genotypes used in the study and their important characteristics

| Code | Genotype | Seed type | Important characters |
|---|---|---|---|
| G1 | BGD 128 | *Kabuli* | High yielding variety adapted to central India, medium duration |
| G2 | ICC 4958 | *Desi* | Drought tolerant, deep and dense root system, short duration |
| G3 | ICCV42 | *Desi* | High yielding, wilt resistant, high nodulation, short duration |
| G4 | KAK 2 | *Kabuli* | High yielding variety, large seed size |
| G5 | JG 11 | *Desi* | High yielding variety, high harvest index, short duration, wilt resistant |
| G6 | ANNIGERI | *Desi* | Local adaptation, wide adaptability, high yielding variety |
| G7 | PhG 95311 | *Kabuli* | High yielding variety, large seed size, wilt resistant |
| G8 | ICCV 2 | *Kabuli* | Extra-short duration, wilt resistant |
| G9 | BGD 72 | *Desi* | High yielding variety, wide adaptability |
| G10 | BG 1105 | *Kabuli* | High yielding variety, large seed size |
| G11 | BG 256 | *Desi* | High yielding, wide adaptability |
| G12 | ICC 92944 | *Desi* | Heat tolerant, short duration |
| G13 | BG 1103 | *Desi* | High yielding variety, Cicerreticulatum derivative |
| G14 | BG 5023 | *Kabuli* | High yielding variety, extra-large seed size |
| G15 | BG 1088 | *Kabuli* | High yielding variety, large seed size |
| G16 | BG 1108 | *Kabuli* | High yielding variety, large seed size |
| G17 | BG 2024 | *Kabuli* | High yielding variety, large seed size |
| G18 | BGD 103 | *Desi* | High yielding variety, large seed size, short duration |
| G19 | ICCV 10 | *Desi* | High yielding variety, drought tolerant, resistant to wilt & root rot |
| G20 | ICCV 5 | *Kabuli* | High yielding variety, wilt resistant, more secondary branches |
| G21 | Kari kadale | *Desi* | Land race, black seeds, more secondary branches, short duration |
| G22 | BGD 9617 | *Desi* | Super-early genotype |
| G23 | BGD 9812 | *Desi* | Super-early genotype |
| G24 | BGD 132 | *Kabuli* | Extra-short duration genotype |
| G25 | BG 1054 | *Kabuli* | Extra-short duration genotype, large seed size |
| G26 | BGD 9920 | *Desi* | Extra-short duration genotype, Cicerreticulatum derivative |
| G27 | BGD 9706 | *Desi* | Short duration & high yielding genotype |
| G28 | BGD 9730 | *Desi* | Short duration & high yielding genotype |
| G29 | BGD 9608 | *Desi* | Short duration & high yielding genotype |
| G30 | BGD 99101 | *Desi* | Short duration & high yielding genotype |

**Scoring STMS data and statistical analysis :** Clearly resolved, unambiguous polymorphic bands were scored visually for their presence or absence. The scores were obtained in the form of a matrix with '1' and '0', which indicate the presence and absence of bands in each genotype, respectively. Polymorphism information content (PIC) or expected heterozygosity scores for each SSR marker was calculated based on the formula $Hj = 1-\Sigma pi^2$, where pi is the allele frequency for the i-th allele. The genetic associations between accessions were evaluated by calculating the Jaccard's similarity co-efficient for pair wise comparisons based on the proportions of shared bands produced by the primers (Jaccard, 1908). Similarity matrix was generated using

**Table 2.** List of STMS primers used for diversity analysis in 30 chickpea genotypes and their PIC values

| Sr.No. | Name of the primers | PIC values* |
|---|---|---|
| 1 | CAM1068 | 0.283 |
| 2 | CAM1402 | 0.309 |
| 3 | CAM0111 | 0.489 |
| 4 | CAMO443 | 0.500 |
| 5 | CAMO446 | 0.098 |
| 6 | CAMO464 | o.469 |
| 7 | CAMO598 | 0.445 |
| 8 | CAMO886 | 0.500 |
| 9 | HIB17 | 0.497 |
| 10 | HIF14 | 0.347 |
| 11 | ICCM0249 | 0.469 |
| 12 | HIF05 | 0.480 |
| 13 | HIF12 | 0.499 |
| 14 | TA176 | 0.402 |
| 15 | CAM1903 | 0.000 |
| 16 | CAM4317 | 0.000 |
| 17 | CAMO639 | 0.000 |
| 18 | CAMO658 | 0.000 |
| 19 | CAMO919 | 0.000 |
| 20 | CAM1052 | 0.000 |
| 21 | CAMO1093 | 0.000 |
| | Total | |
| | Mean | 0.409 |

the SIMQUAL programme of NTSYS-pc software, version 2.02 (Rohlf, 1998). The similarity coefficients were used for cluster analysis and dendrogram was constructed by the Un-weighted Pair-Group method of Arithmetic Averages (UPGMA) as described in Sneath and Sokal (1973).

**Relationship between Mahalanobis's generalized distance (D) and distance based on STMS markers :** Correlation between Mahalanobis's generalized distances (D) based on multivariate analysis of morphological data and distance based on STMS markers were calculated for each pair of distance measures obtained for both locations. The significance of r was tested by comparing the calculated t value with n-2 degrees of freedom for $\leqslant 0.05$.

$$t = \frac{r}{\sqrt{1-r^2}} \cdot \sqrt{n-2}$$

Where, n= total number of pairs of distance measures.

## RESULTS AND DISCUSSION

Analysis of variance was carried out for 12 morphological traits recorded at the IARI Centre, Dharwad and at the Genetics Division, IARI, New Delhi to partition the total variation due to treatments (genotypes) and other sources. The results indicated that the mean sum of squares due to genotypes were significant for all the characters at Dharwad ($E_1$) and at New Delhi ($E_2$).

Thirty chickpea genotypes were used to construct dendrogram based on and the results are presented in **Fig 1 & 2**. These genotypes were grouped into eight clusters at $E_1$ and among them, Cluster 1 was the largest consisting of 17 genotypes followed by Cluster II with 6 genotypes and the remaining 5 clusters were all solitary clusters (III, IV, VI, VII, and

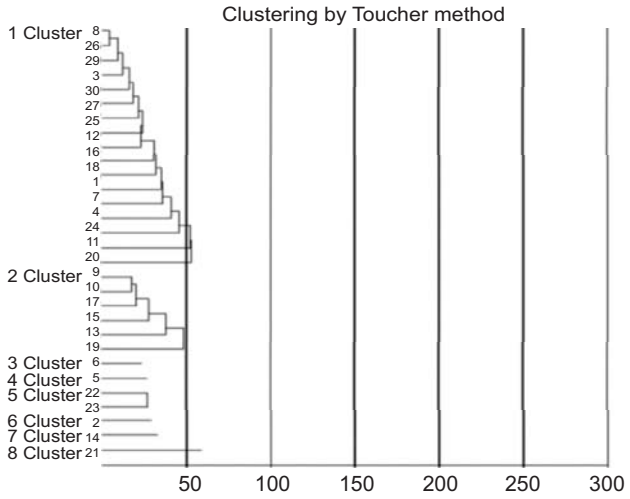


**Fig. 1.** Dendrogram depicting morphological diversity among 30 genotypes of chickpea at $E_1$

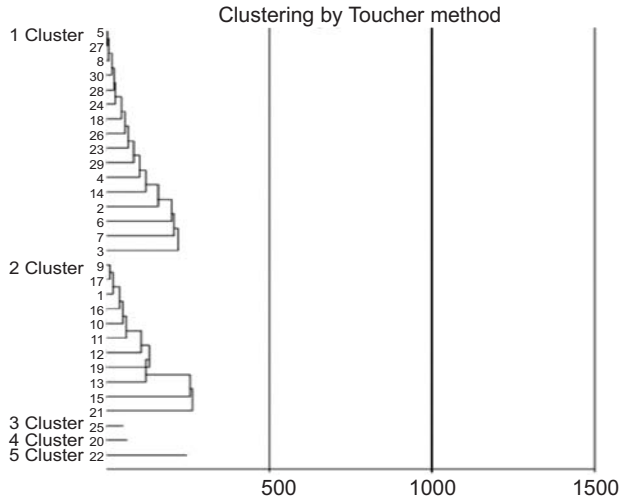


**Fig. 2**. Dendrogram depicting morphological diversity among 30 genotypes of chickpea at $E_2$

VIII) with a single genotype each. In case of $E_2$, genotypes were grouped into 5 clusters with Cluster I having maximum number of 16 genotypes followed by cluster II with 11 genotypes and all the remaining three clusters as solitary clusters. This indicated the presence of appreciable amount of diversity among the genotypes under study. The formation of solitary clusters may be due to total isolation preventing the gene flow or intensive natural / human selection for diverse adoptive complexes. These genotypes may be very unique and useful in breeding point of view.

The average inter-cluster $D^2$ values ranged from 6.74- 19.06 in $E_1$ and was maximum between cluster VI and VIII (19.06) followed by VII and VIII (19.01). Thus in case of $E_1$, ICC 4958 and BG 5023 showed maximum divergence with Kari kadale. In $E_2$, average inter-cluster $D^2$ values ranged from 16.88- 42.52 and was maximum between cluster IV (ICCV 5) and V (BGD 9617), followed by III (BG 1054) and IV (ICCV5).

It is concluded that, *Kari kadale*, a landrace from Karnataka

**Table 3.** Average inter & intra cluster D-square values at $E_1$

| Cluster | I | II | III | IV | V | VI | VII | VIII |
|---|---|---|---|---|---|---|---|---|
| I | 6.20 (2.50) | 10.19 (3.19) | 8.65 (2.94) | 7.99 (2.82) | 10.36 (3.21) | 8.30 (2.88) | 9.53 (3.08) | 14.59 (3.81) |
| II | | 6.67 (2.58) | 10.56 (3.24) | 10.55 (3.24) | 14.31 (3.78) | 14.45 (3.80) | 9.31 (3.05) | 14.65 (3.82) |
| III | | | 0.00 (0.00) | 6.74 (2.59) | 7.41 (2.72) | 12.90 (3.59) | 13.35 (3.65) | 7.67 (2.76) |
| IV | | | | 0.00 (0.00) | 9.37 (3.06) | 10.19 (3.19) | 12.88 (3.58) | 10.56 (3.24) |
| V | | | | | 5.21 (2.28) | 13.93 (3.73) | 16.19 (4.02) | 9.71 (3.11) |
| VI | | | | | | 0.00 (0.00) | 11.58 (3.40) | 19.06 (4.36) |
| VII | | | | | | | 0.00 (0.00) | 19.01 (4.36) |
| VIII | | | | | | | | 0.00 (0.00) |

*Values in the parenthesis indicate average intra & inter cluster D values.

**Table 4.** Average inter & intra cluster D-square values, at $E_2$

| Cluster | I | II | III | IV | V |
|---|---|---|---|---|---|
| I | 11.71 (3.42) | 35.91 (5.99) | 16.88 (4.10) | 31.80 (5.63) | 27.85 (5.27) |
| II | | 12.87 (3.58) | 35.13 (5.92) | 19.61 (4.42) | 37.18 (6.09) |
| III | | | 0.00 (0.00) | 37.68 (6.13) | 17.39 (4.17) |
| IV | | | | 0.00 (0.00) | 42.52 (6.52) |
| V | | | | | 0.00 (0.00) |

*Values in the parenthesis indicate average intra & inter cluster D values.

state showed maximum divergence with the high yielding cultivars and short duration breeding lines in $E_1$ (**Table 3 and 4**). Therefore, crossing of *Kari kadale* with the high yielding cultivars released for general cultivation in warm season environments of southern India is expected to be of great promise in improving the genetic yield potential of these existing varieties further and thereby increasing the chickpea production & productivity in such terminal stress environments.

**Genetic diversity :** Molecular markers are a useful complement to morphological and physiological characterization of cultivars because they are plentiful, independent of tissue or environmental effects and allow cultivar identification early in plant development. The usefulness of these markers for germplasm characterization has been demonstrated in sweet potato, soybean, rapeseed, rice, *Phaseolus* (Gupta *et al.,* 2000), *Vigna radiata* (Sangiri *et al.,* 2007) and also chickpea (Upadhyaya *et al.,* 2008; Sefera *et al.,* 2011).

In the present study 21 out of the 33 primers analyzed were polymorphic. Polymorphism information content (PIC) value of each STMS marker is a measure of marker diversity. PIC provides an estimate of discriminatory power of a locus by taking into account not only the number of alleles expressed but also the relative frequency of those alleles. In the present study the PIC values were in the range of 0.098 to 0.500 and it was highest for the STMS primer CAMO443 and CAMO886 (0.500) and the lowest (0.098) for the primer CAMO446. The higher the PIC value, more informative is the STMS marker. Hence the primer used in our present study was highly informative.

The 30 genotypes could be clearly distinguished using the 21 STMS loci. The PIC value is influenced by the occurrence of variants per locus as well as relative distribution of the alleles. These values are lower than those observed by Udupa *et al.* (1999) who reported an average number of alleles of 14.1 per locus and an average PIC of 0.86 for 12 SSR loci in 78 genotypes of chickpea including 72 landraces, 4 cultivars and 2 wild species of the primary gene pool (*i.e. C. reticulatum* and *C. echinospermum*). Although allele number is very much dependent on sample size, the possible explanation for the low observed PIC value in our study could be that most of the genotypes were breeding lines derived from a limited number of parental lines or hybrids, whereas many of the studies cited above used a larger number of genotypes from geographically diverse areas including landraces and wild relatives. However, the materials used here still reveal a considerable amount of genetic variation. Thus the microsatellite technique proved to be a useful system for managing our experimental line. This indicated that these primers might be an effective and useful tool to determine the genetic differences among the accessions and to study the phylogenetic relationship. These results are in agreement with the observations of several workers about the potential utility of STMS in characterizing chickpea germplasm (Bharadwaj *et al.,* 2010; Huttel *et al.*, 1999, Winter *et al.,* 1999, Flandez-galvez, 2003 and Choumane *et al.,* 2000).

The genetic similarity coefficients between different pairs of 30 chickpea genotypes based on 21 STMS markers are presented in the **Table 5**. They ranged from 0.214 to 0.850. The highest similarity index (0.850) was observed between BGD72 and Annigeri-1 whereas BGD 9920 and ICC 92944 showed the lowest similarity index (0.214). The similarity coefficient values obtained for each pair wise comparison of STMS markers among the 30 chickpea genotypes were used to construct a dendrogram & the results are presented in **Fig. 3**. The 30 geno-types formed 7 clusters at nearly 52% similarity levels. Similarity index values arrived from the polymorphic data gave the amount of relatedness between individuals. Lower the similarity between the genotypes, better the scope to include them for breeding programme. The formation of 7 clusters through hierarchial cluster analysis of STMS data revealed that the presence of genetic diversity at molecular level was high among the different accessions of the genotypes used for the study.

From the present investigation the genotypes from cluster VII (BGD 128, BG 1103, BG 1088, BG 2024, BG 1108, BG 1054) appears to be most divergent and farthest apart from the genotypes of cluster I (ICC 92944). Pre-breeding using the genotypes of cluster VII with those of cluster I would lead to a greater realization of superior segregates as well as help in broadening the genetic base. Clusters III and IV noticed overlapping of both *desi* type and *Kabuli* types, but in remaining clusters *viz.,* V and VI with *desi* types and VII with *Kabuli* types, distribution of genotype in these clusters was based on seed type. This confirms the unequal distribution of *desi* and *Kabuli* in

**Table 5.** Similarity coefficients among thirty chickpea genotypes based on 21 SSR primer

| | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | G10 | G11 | G12 | G13 | G14 | G15 | G16 | G17 | G18 | G19 | G20 | G21 | G22 | G23 | G24 | G25 | G26 | G27 | G28 | G29 | G30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G1 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| G2 | 0.57 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| G3 | 0.38 | 0.44 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| G4 | 0.50 | 0.57 | 0.64 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| G5 | 0.60 | 0.67 | 0.40 | 0.59 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | |
| G6 | 0.46 | 0.65 | 0.58 | 0.58 | 0.54 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | |
| G7 | 0.46 | 0.40 | 0.59 | 0.52 | 0.55 | 0.61 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | |
| G8 | 0.55 | 0.54 | 0.54 | 0.61 | 0.71 | 0.70 | 0.71 | 1.00 | | | | | | | | | | | | | | | | | | | | | | |
| G9 | 0.46 | 0.59 | 0.52 | 0.59 | 0.55 | 0.85 | 0.62 | 0.71 | 1.00 | | | | | | | | | | | | | | | | | | | | | |
| G10 | 0.48 | 0.42 | 0.48 | 0.48 | 0.50 | 0.63 | 0.80 | 0.65 | 0.57 | 1.00 | | | | | | | | | | | | | | | | | | | | |
| G11 | 0.65 | 0.50 | 0.64 | 0.64 | 0.59 | 0.58 | 0.67 | 0.68 | 0.59 | 0.68 | 1.00 | | | | | | | | | | | | | | | | | | | |
| G12 | 0.50 | 0.57 | 0.44 | 0.44 | 0.46 | 0.52 | 0.40 | 0.48 | 0.52 | 0.42 | 0.44 | 1.00 | | | | | | | | | | | | | | | | | | |
| G13 | 0.65 | 0.57 | 0.44 | 0.57 | 0.52 | 0.52 | 0.59 | 0.48 | 0.46 | 0.61 | 0.64 | 0.39 | 1.00 | | | | | | | | | | | | | | | | | |
| G14 | 0.50 | 0.44 | 0.39 | 0.56 | 0.46 | 0.46 | 0.41 | 0.54 | 0.46 | 0.54 | 0.56 | 0.56 | 0.56 | 1.00 | | | | | | | | | | | | | | | | |
| G15 | 0.59 | 0.58 | 0.36 | 0.46 | 0.61 | 0.48 | 0.54 | 0.50 | 0.54 | 0.50 | 0.52 | 0.41 | 0.65 | 0.46 | 1.00 | | | | | | | | | | | | | | | |
| G16 | 0.57 | 0.44 | 0.39 | 0.44 | 0.46 | 0.36 | 0.46 | 0.42 | 0.35 | 0.48 | 0.64 | 0.39 | 0.57 | 0.44 | 0.65 | 1.00 | | | | | | | | | | | | | | |
| G17 | 0.50 | 0.57 | 0.33 | 0.44 | 0.59 | 0.46 | 0.59 | 0.48 | 0.46 | 0.48 | 0.44 | 0.33 | 0.57 | 0.35 | 0.81 | 0.57 | 1.00 | | | | | | | | | | | | | |
| G18 | 0.38 | 0.64 | 0.39 | 0.50 | 0.52 | 0.41 | 0.35 | 0.48 | 0.46 | 0.32 | 0.44 | 0.39 | 0.44 | 0.50 | 0.58 | 0.50 | 0.57 | 1.00 | | | | | | | | | | | | |
| G19 | 0.36 | 0.42 | 0.42 | 0.61 | 0.39 | 0.50 | 0.39 | 0.41 | 0.39 | 0.46 | 0.37 | 0.28 | 0.54 | 0.48 | 0.39 | 0.32 | 0.42 | 0.42 | 1.00 | | | | | | | | | | | |
| G20 | 0.35 | 0.41 | 0.41 | 0.52 | 0.37 | 0.48 | 0.48 | 0.39 | 0.37 | 0.56 | 0.36 | 0.27 | 0.52 | 0.37 | 0.48 | 0.36 | 0.52 | 0.36 | 0.77 | 1.00 | | | | | | | | | | |
| G21 | 0.31 | 0.37 | 0.61 | 0.48 | 0.33 | 0.50 | 0.39 | 0.41 | 0.44 | 0.46 | 0.54 | 0.37 | 0.42 | 0.54 | 0.39 | 0.37 | 0.28 | 0.37 | 0.46 | 0.44 | 1.00 | | | | | | | | | |
| G22 | 0.29 | 0.30 | 0.63 | 0.50 | 0.31 | 0.52 | 0.58 | 0.43 | 0.46 | 0.60 | 0.50 | 0.39 | 0.50 | 0.56 | 0.46 | 0.39 | 0.39 | 0.30 | 0.48 | 0.52 | 0.74 | 1.00 | | | | | | | | |
| G23 | 0.44 | 0.39 | 0.56 | 0.63 | 0.46 | 0.46 | 0.46 | 0.60 | 0.52 | 0.48 | 0.70 | 0.44 | 0.44 | 0.68 | 0.46 | 0.50 | 0.35 | 0.50 | 0.38 | 0.32 | 0.67 | 0.62 | 1.00 | | | | | | | |
| G24 | 0.55 | 0.61 | 0.42 | 0.54 | 0.57 | 0.44 | 0.33 | 0.46 | 0.50 | 0.36 | 0.48 | 0.54 | 0.42 | 0.54 | 0.63 | 0.48 | 0.48 | 0.54 | 0.36 | 0.35 | 0.52 | 0.43 | 0.67 | 1.00 | | | | | | |
| G25 | 0.55 | 0.54 | 0.37 | 0.37 | 0.57 | 0.50 | 0.44 | 0.52 | 0.44 | 0.58 | 0.61 | 0.37 | 0.48 | 0.48 | 0.63 | 0.61 | 0.54 | 0.42 | 0.36 | 0.39 | 0.46 | 0.43 | 0.48 | 0.52 | 1.00 | | | | | |
| G26 | 0.29 | 0.48 | 0.36 | 0.42 | 0.44 | 0.50 | 0.38 | 0.40 | 0.44 | 0.46 | 0.42 | 0.21 | 0.48 | 0.42 | 0.39 | 0.36 | 0.42 | 0.48 | 0.52 | 0.39 | 0.46 | 0.42 | 0.42 | 0.40 | 0.52 | 1.00 | | | | |
| G27 | 0.38 | 0.57 | 0.39 | 0.44 | 0.46 | 0.58 | 0.46 | 0.48 | 0.52 | 0.48 | 0.44 | 0.29 | 0.50 | 0.39 | 0.52 | 0.44 | 0.57 | 0.57 | 0.54 | 0.46 | 0.42 | 0.39 | 0.44 | 0.48 | 0.54 | 0.79 | 1.00 | | | |
| G28 | 0.32 | 0.44 | 0.33 | 0.39 | 0.35 | 0.46 | 0.35 | 0.37 | 0.40 | 0.48 | 0.44 | 0.29 | 0.44 | 0.44 | 0.41 | 0.50 | 0.44 | 0.50 | 0.54 | 0.41 | 0.42 | 0.39 | 0.44 | 0.42 | 0.54 | 0.79 | 0.80 | 1.00 | | |
| G29 | 0.48 | 0.61 | 0.42 | 0.54 | 0.50 | 0.44 | 0.33 | 0.46 | 0.39 | 0.41 | 0.48 | 0.37 | 0.61 | 0.67 | 0.50 | 0.48 | 0.42 | 0.61 | 0.52 | 0.39 | 0.52 | 0.43 | 0.54 | 0.58 | 0.52 | 0.67 | 0.61 | 0.61 | 1.00 | |
| G30 | 0.41 | 0.55 | 0.36 | 0.48 | 0.44 | 0.39 | 0.38 | 0.35 | 0.32 | 0.46 | 0.42 | 0.26 | 0.62 | 0.48 | 0.50 | 0.48 | 0.55 | 0.55 | 0.52 | 0.50 | 0.46 | 0.42 | 0.37 | 0.46 | 0.46 | 0.68 | 0.62 | 0.62 | 0.75 | 1.00 |

these clusters. *Desi* and *Kabuli* groups were not clearly divided in UPGMA phylogenetic trees (**Fig. 3**). Some genotypes formed monophyletic groups in (cluster V, VI, VII) but others are paraphyletic (Cluster II and IV). In the study of Upadhyaya *et al.* (2008) with 2915 genotypes including 1668 *Desi* and 1167 *Kabuli* types, *Desi* and *Kubuli* genotypes are largely separated but include some paraphyletic members. This probably shows that these two groups have not been completely isolated and occasional hybridization between the two morphs might have occurred.

Within the 7 clusters the largest number of genotypes was in cluster IV. These genotypes may have a similar gene pool contributing to greater similar repetitive sequence during their course of evolution compared to other genotypes. Though conserved crossing between the most diverse genotypes among this pool would give out more adaptive ones which can be directly used in breeding programme compared to those obtained by crossing the genotypes of cluster I to VIII which is more of pre-breeding in nature. The high level of variability observed in microsatellite markers makes them suitable for application in identification of germplasm of local varieties, cultigens and cultivars (Udupa *et al.,* 1999).

In conclusion, most of the studies on polymorphism of molecular markers in chickpea indicate the presence of limited genetic variability in the cultivated species. However, our study shows that a few selected polymorphic STMS markers were enough to discriminate among chickpea cultivars studied. The present results will also help chickpea breeders in the selection of parent material in breeding programs.

**Assessment of relationship between Mahalanobis's generalized distance and distance based on STMS markers :** The extent of agreement between the dendrograms derived from morphological and STMS data was analyzed. In the present investigation, the correspondence between the dissimilarity matrix generated by STMS data and morphological data was evaluated by calculating product-moment correlation (r). There was no close correspondence between the dissimilarity matrix of STMS and morphological distance. The correlation (r) between morphological and STMS dissimilarity matrices was positive [r = 0.079] and non-significant ($p < 0.05$, 433 random permutations) for both the locations suggesting that the two systems gives different estimates of genetic relations among the genotypes. A similar disparity has been reported in rye grass (Roldan-Ruiz *et al.,* 2001). The lower agreement between phenotypic and molecular distances may be due to the fact that the variation observed at STMS level might have not been expressed at phenotypic level. Molecular markers access the diversity in the genomic regions with which they hybridize and this necessarily need not transform into

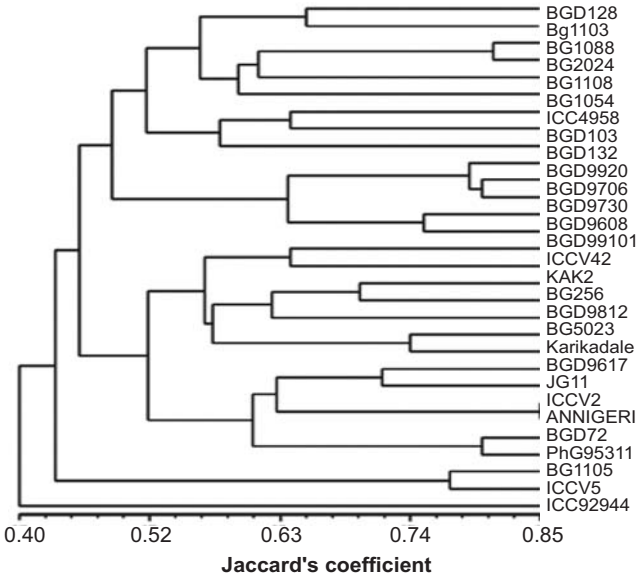


**Fig. 3.** Dendogram depicting molecular diversity using STMS markers among 30 genotypes of chickpea

phenotypic diversity. Furthermore a larger component of phenotypic diversity is due to 'G x E' component which is very low when estimating molecular diversity. Nevertheless, the diversity pattern obtained by molecular markers is a better realistic estimate provided a large repertoire of markers are used which are spread across the whole genome. Since only a Limited numbers of markers were analyzed in this study and the distribution of the markers studied was not enough to cover the whole genome of chickpea, a future line of work would be use the genome wide markers now avialbale in chickpea after publication of the chickpea genome sequence Varshney *et al.* (2013) . The variation between the genotypes based on morpho-logical traits was less as compared to the clustering pattern of STMS for the reason that the accessions of the same species grouped in a single cluster thus there is less agreement between the diversity pattern of phenotypic traits & molecular markers.

The present investigation also gives an insight of the inter-relationships among the genotypes and highlights the urgency for effective supplementation of pedigree data and other morphological data.

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