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***Full Length Article***

# Morphological and Molecular Genetic Diversity Analysis of Chickpea Genotypes

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## Abstract

Chickpea (*Cicer arietinum* L.) is one of most important legume crops. However, the harvested yield is quite low than the potential yield, particularly in hot and dry environments. This demands development of genotypes for the target environments. However, genetic variability, its estimation and use are considered as the first step for the breeding program. In this study, 52 chickpea genotypes of diverse morphology and origin, were investigated for phenology, morphology, yield related traits and genetic diversity in dry Mediterranean environment. There was high degree of variability among the tested genotypes for phenology, morphology and yield related traits under field conditions. Genetic diversity among the tested chickpea genotypes was estimated with sequence related amplified polymorphism (SRAP) and simple sequence repeat (SSR) markers. In the principal component analysis (PCA), initial five PCs described 87% of the variance, where in the first two PCs contributed 39 and 21%, respectively. Based on geography and breeding year, main clusters (five) comprised of genotypes with arithmetic mean (UPGMA) analysis and unweighted (pair) group technique. Seventeen SRAP primers generated 677 amplified fragments (peaks), with mean polymorphic information content [PIC] of 94. Forty SSR primers showed good amplification, with average PIC of 0.40. Estimated and recorded heterozygosity ranged from 0.34–0.63 and 0.18–0.78, respectively. The UPGMA analysis on SRAP and SSR data grouped the genotypes according to geography and pedigree. Based on the field performance and genetic diversity data, maximum diversity was observed among 8 genotypes, which may be selected for further breeding programs. 

**Keywords:** Chickpea; Genetic diversity; Morphology; Sequence-related amplified polymorphism; Simple sequence repeat

## Introduction

Chickpea (*Cicer arietinum* L.) is one of the most important grain legume crops. Its grain contains 41–51% carbohydrates, 3–6% oil, 17–24% protein, and appreciable amount of several minerals including magnesium, manganese, potassium, zinc and iron (Ibrikci *et al*., 2003). However, the harvested yield is quite low than the potential yield, particularly in hot and dry environments (Farooq *et al*., 2017a, b). This demands development of genotypes for the target environments.

Chickpea genotypes harbor large variation for plant architecture, phenological behavior, and tolerance against biotic and abiotic stresses (Duranti and Gius, 1997; Farooq *et al*., 2017a, b).

For target-oriented breeding programs, characterization of the genotypes helps improve the breeding efficiency in response to direct selection on the trait of interest. Such characterizations can be done using morphological, molecular markers and biochemical attributes (Varshney *et al*., 2005; Khan *et al*., 2016). Efficiency of the breeding programs for crop improvement can be substantially enhanced adapting molecular markers technique. As the molecular markers are directly linked with the traits, and accelerate the new group of genotypes, particularly when it is difficult to score traits of different characters otherwise. For chickpea, hundreds of markers are employed for analysis of genetic variability among genotypes (Sethy *et al*., 2006; Nayak *et al*., 2010).

Simple sequence repeats (SSR) markers are the most widely used in breeding, given its genome-wide coverage, high reproducibility, multi-allelic type and co-dominant inheritance (Gupta and Varshney, 2000). However, for primer design, SSR markers need nucleotide information (Sun *et al*., 1998). This limitation has been overcome with advent of sequence related amplified polymorphism (SRAP) markers (Segovia-Lerma *et al*., 2003).

In our previous study, we evaluated 26 chickpea genotypes, for genetic variability and grouped them based largely on the genetic background and/or origin using six

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SRAP and four AFLP primer combination (Khan *et al*., 2016). However, to the best of our knowledge, no information is available on use of SSR and SRAP markers to assess genetic variability in a huge assembly of genotypes of chickpea together with field assessment in dry environment of Saudi Arabia. This study was, therefore, conducted to estimate the genetic variability in fifty two chickpea genotypes, of diverse origin and morphology, using SRAP and SSR markers, and evaluate the phenology, morphology and yield related traits in dry Mediterranean environment of Saudi Arabia for selection of superior chickpea genotypes to use in the future breeding programs.

## Materials and Methods

### Plant Material, Experimental Site and Crop Husbandry

Fifty two genotypes of chickpeas having diverse origin and morphology, were used in the study (**Error! Reference source not found.**). Seeds of the tested genotypes were collected from the Legume Research Unit, Plant Production Department, College of Food and Agriculture, King Saud University, Riyadh, Saudi Arabia. This study was carried out at the Educational Research Farm (24.72° E, 46.62° N), College of Food and Agricultural Sciences, King Saud University, Riyadh, Saudi Arabia during the winter season 2014–2015.

All chickpea genotypes were planted on November 14, 2014 using a hand drill with planting geometry of 60 cm × 20 cm; and plot size of 6 m × 3 m was maintained. Experiment was laid out in randomized complete block design having 4 replications. Nitrogen (N), potassium (K) and phosphorus (P) were applied at 50, 60 and 45 kg $ha^{-1}$, using ammonium sulphate, calcium super phosphate and potassium sulfate as sources, respectively. Whole of P, K and 1/3 of N was applied at sowing. Remaining dose of nitrogen was applied at flowering and pod development stage in two equal splits. Weeds were controlled by manual hoeing. The chickpea genotypes were harvested at harvest maturity during last week of April.

### Field Performance

Days to 50% flowering and days to maturity were noted from planting to the appearance of at least 50% flowers, and ripening of 95% pods, respectively by visual observation. Data related to morphological traits were recorded form each plot. Five plants were selected randomly. Plant height was recorded from plant base to upper tip of leaf using a meter rod. The branches sprouted from the leaf axils, from upper half of main stem, were counted and recorded as primary branches; whereas the branches sprouted from the form leaf axils of basal primary branches were noted as secondary branches. Leaves from separated from all of five selected plants and the leaf area was recorded with leaf area meter (LI 3000C).

From each plot, yield related traits and yield was taken by harvesting plant at maturity. The number of pods from five plants was counted, and grains per pod were recorded. Bundles of two middle rows were tied up and biological yield was recorded after weighing. To obtain grain yield, the harvested plants were threshed, grains were separated from straw and weighed. From the grains of each plot, 100-grain weight was recorded. Harvest index was estimated as a ratio of grain yield to biological yield and was expressed in percentage.

### Genetic Diversity and Molecular Characterization

**DNA extraction:** Fully developed fresh leaves of each genotype were threshed and were immediately dipped in liquid nitrogen. The leaf DNA was extracted following modified SDS protocol (Hoelzel, 1998). Electrophoresis and spectrophotometry were used for the determination of concentration and quality of extracted DNA on 1% (agarose gel) and Nano drop spectrophotometer, respectively.

**Sequence related amplified polymorphism (SRAP):** Sixty pairs were chosen for SRAP based on their consistency for the amplification of PCR and regenerated polymorphism by considering eight chickpea genotypes following Li and Quiros (2001). For each PCR, 20 µL reaction volume 1 × GoTaq Green Master mix (Promega; Madison, WI, USA) contained template of DNA (50 ng), 0.1 µM for each reverse and forward primers, to make volume 20 µL, nuclease free water was used. For PCR amplification, first denaturation was done for 5 min at 94°C after fixing thermal cycler. This process was followed by denaturation for 1 min at same temperature by using 5 cycles and settle down for 1 min at 35°C and elongating for same time at 72°C. Temperature for annealing was rise to 50°C for time of 1 min to complete last step of elongation for 7 min at 72°C for the remaining 30 cycles. With 0.5 µL Gene Scan 500 LIZ size standard (Applied Biosystems P/N 4322682) and 8.5 µL Hi-Di formamide (Applied Biosystems P/N 4311320) for fragrant analysis, (1 µL) volume of PCR (amplified product) was added. The mixture was loaded and denatured on sixteen capillary systems of Applied Biosystems 3130*xl* Genetic analyzer was used for loading and denaturation of mixture.

**Simple sequence repeats (SSR):** For polymorphic markers screening, 40 SSR markers were selected. PCRs was accomplished by using 20 µL reaction volume encompassing 1X Go Taq Green Master Mix (Promega; Madison, WI, USA), individual primer of 0.1 µM, DNA template of 50 ng and nuclease water (free) was used to attain volume of 20 µL. For PCR amplification, thermal cycler profile was set and arranged in pattern including 5 min denaturation at 94°C. This procedure was subsequently followed by denaturation at same temperature for 45 s using thirty five cycles. Same process was repeated

**Table 1:** Pedigree/origin of chickpea genotypes used in the study

| S. No. | Genotypes | Pedigree | S. No. | Genotypes | Pedigree/Origin |
|---|---|---|---|---|---|
| 1 | ILC482 | Improved Check | 27 | FLIP08-28C | X2002TH 55/S00754 X FLlP98-175C |
| 2 | FLIP05-11C | X2000TH 39/FLlP98-29CXS99001 | 28 | FLIP06-35C | 002TH 281FLlP98-28C X FLlP98-079C |
| 3 | FLIP07-7C | X03TH-51/(S00787XSEL01ter73616XFLIP98-22C | 29 | FLIP06-161C | X98TH58/(Malik1 XILC7795XFLlP94-92C) XS9Q2~3. |
| 4 | FLIP05-46C | X2000TH 39/FLlP98-29CXS99001 | 30 | FLIP06-18C | 2002-'FH-2-1-ffi6&7-8T-X-FI:IP9M61C |
| 5 | FLIP05-156C | X2001TH23/(FLlP98-132CXS99093XFLlP98-9C | 31 | ILC3279 | Improved Check |
| 6 | FLIP07-31C | X03TH-153/FLlP98-133CXFLlP98-117C. | 32 | FLIP06-144C | X200HH 85/S15042XFLlP97-25C |
| 7 | FLIP07-34C | X03TH-153/FLlP98-133CXFLlP98-117C. | 33 | FLIP06-38C | X2002TH 281FLlP98-28C X FtlP98-079C |
| 8 | FLIP05-22C | X2000TH 21/FLlP98-64CXFLlP98-47C | 34 | FLIP06-91C | X2002TH 118/FLlP98-64CXFLlP98-12CXSeI99TER85448) X FLlP97 -026C |
| 9 | FLIP03-128C | XOOTH 51/FLlP98-52CXFLlP98-47C | 35 | FLIP06-2C | X2002TH 51FLlP98-130C X FLlP97 -219C |
| 10 | FLIP07-75C | X03TH-25/(S00834XFLlP98-121C)XCA 9783009 | 36 | FLIP06-53C | X2002TH 55/S00754 X FLlP98-175C |
| 11 | FLIP82-150C | X79TH101/ILC 523 X ILC 183 (Improved check) | 37 | FLIP06-10C | X2002TH 8/S00787 X FLlP98-028C |
| 12 | FLIP08-23C | X01TH4/(FLlP98-134CXseI99ter8507 4)XFLlP97 -22C | 38 | FLIP87-59C | X85TH27411LC3843XFLlP82-130C (Improved Check) |
| 13 | FLIP05-22C | X2000TH 21/FLlP98-64CXFLlP98-47C | 39 | FLIP03-27C | X98TH86/[(ILC267XFLlP89-4C)XHB-1jXS95345 |
| 14 | FLIP06-116C | X2002TH130/(ILWC112XS85485)X FLlP98-38C | 40 | X05TH37 | ICARADA |
| 15 | FLIP06-19C | X2002TH 21/S00787 X FLlP97-261C | 41 | SEL2083 | Italy |
| 16 | FLIP07-22C | X03TH-152/FLlP97-131CXFLlP97-111C. | 42 | SEL2490 | Syria |
| 17 | FLIP05-27C | X2000TH88/(FLlP84-92CXFLlP90172C)X FLlP98-47C | 43 | SEL2556 | Cyprus |
| 18 | FLIP88-85C | X85 TH14311LC 629 x FLIP 82-144C | 44 | SEL2558 | Italy |
| 19 | FLIP07-32C | X03TH-153/FLlP98-133CXFLlP98-117C. | 45 | SEL2560 | Syria |
| 20 | FLIP06-40C | X2002TH 391ELlp97-149C X FOP91PJ2ZC | 46 | SEL2604 | Cyprus |
| 21 | FLIP05-160C | X2001 'FH 24/(S98588XS99093)XS99358 | 47 | SEL2616 | Turkey |
| 22 | FLIP05-153C | X2001TH16/(FLlP9725CXS98588)XS99481 | 48 | SEL2627 | Jordan |
| 23 | FLIP06-74C | X2002TH921S00701 X FLlP98-38C | 49 | SEL2709 | ICARADA |
| 24 | FLIP05-40C | X2000TH35/FLlP98-29CXS99442 | 50 | SEL2712 | ICARADA |
| 25 | FLIP08-12C | XOOTH90/(FLlPB4-145CXS95338)XFLlP98-10C | 51 | SEL2714 | ICARADA |
| 26 | FLIP08-58C | X02TH3/FLIP 98- 28C X FLIP 97-102C | 52 | SEL2717 | ICARADA |

ICARADA = International Center for Agricultural Research in the Dry Areas

at annealing temperature (55°C) for same time period and at 72°C for 60 s with final step of half hour at 72°C. For electrophoresis, 1 µL product (PCR amplified) was passes through dilution process and 0.5 then added with 0.5 µL of Gene Scan 500 LIZ size standard (Applied Bio systems) and 8.5 µL of Hi-Di (Formamide; Applied Biosystems). This product was loaded and denatured by using sixteen capillary systems of (Applied Biosystems) 3130*xl* Genetic Analyzer.

### Statistical Analysis

The SAS version (9.3) was used to analyze the recorded data. The mean data were normalized and analyzed using the Euclidian distance. A dendogram was developed with these distances and by using UPGMA in PAST 3.11 (Hammer *et al*., 2001). To avoid the scaling effect, qualitative data were standardized using data-transformation techniques and clusters were made, based on Euclidean distance, to study the similarities among the genotypes. Collected data was arranged in binary format (1) for presence or (0) for allele absence; and Gene Mapper (analysis) software (v3.7) was employed to accomplish fragment analysis of SSR and SRAP.

For fragment scaling, threshold level was fixed at (200 relative fluorescence unit; rfu), '1' was assigned to observe peaks at 200 rfu or more. Whereas '0' indicated lesser value. Fragment size analysis was accomplished from 100-500 bp ranges. Fragments having frequency greater than 1 – (3/N), where (N indicated individual number samples) were removed to account for the estimation bias (as much as 5%) (Lynch and Milligan, 1994). SSR alleles, showing variation for each primer pair, were separated after analysis using the Excel Microsatellite Toolkit (Park, 2001). The expected heterozygosity, polymorphic information contents (PIC) value for individual primer pair and percentage discrimination power (DP) were also taken. DP was attained through division of amplified (polymorphic) alleles for each primer by sum of all polymorphic primer (Khierallah *et al*., 2011). To find out allelic variability PIC of each primer was calculated with formula.

$$\mathrm{PIC} = 1 - \sum_{j=1}^{n} {P_{ij}}^2$$

Here, $P_{ij}$ reflects $i^{th}$ allele frequency for marker $j$ and summation sign extends over n alleles (Anderson *et al*., 1993).

For individual SRAP and SSR markers, PIC was calculated and Jaccard's similarity coefficient was used for analysis of data generated from (SRAP and SSR) markers (Jaccard, 1908). These similarity coefficients and UPGMA in PAST 3.11 were employed to develop dendogram (Hammer *et al*., 2001).UPGMA-based polygenetic tress was produced using PAST.

## Results

### Field Performance

Analysis of variance showed that, genotypes under study differed significantly for morphological, phenological and yield related traits (Table 2). The tested chickpea genotypes had wide range of values for plant height, leaf area, days to

**Table 2:** Analysis of variance for the morphological, phenological and yield related traits in chickpea genotypes

| SOV | DF | PH | PB | SB | LA | DF | DM | PP | GP | GW | YP | GY | Hi |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotypes | 51 | 455.08** | 0.1* | 2.35** | 22.54** | 373.58** | 324.68** | 0.075** | 85.64** | 36.37** | 8.54** | 214.75** | 0.01** |
| Rep | 2 | 121.88 | 5.61 | 0.50 | 8.33 | 129.73 | 303.68 | 0.014 | 163.23 | 0.011 | 48.71 | 120.68 | 0.05 |
| Error | 102 | 25.01 | 0.072 | 0.100 | 0.012 | 16.07 | 30.45 | 0.005 | 17.19 | 0.010 | 1.48 | 0.258 | 0.00045 |

SOV = Source of variation, DF = Degree of freedom, PH = Plant height, PB = Number of primary branches, SB =Number of secondary branches, LA = Leaf area, DF= Days to 50% flowering, DM = Days to maturity, PP = Number of pods/plant, GP = Number of grain/pod, GW = 100-grain weight, YP= Grain yield/plant, BY= Biological yield/plant, HI = Harvest index

*, ** Significant at $p \leq 0.05$ and $p \leq 0.01$, respectively

**Table 3:** Descriptive statistics of morphological, phenological and yield related traits of chickpea genotypes

| Traits | Mean | SD | Min | Max |
|---|---|---|---|---|
| Plant height (cm) | 50.10 | 12.02 | 33.93 | 89.57 |
| Number of primary branches | 1.70 | 0.21 | 1.37 | 2.50 |
| Number of secondary branches | 4.88 | 0.87 | 3.63 | 6.87 |
| Leaf area ($cm^2$) | 10.03 | 2.74 | 4.52 | 16.43 |
| Days to 50% flowering | 109.68 | 11.12 | 89.43 | 134.57 |
| Days to maturity | 147.45 | 10.32 | 128.43 | 169.70 |
| Grain filling periods (days) | 37.81 | 4.50 | 24.27 | 50.63 |
| Number of pods/plant | 22.65 | 5.44 | 14.40 | 36.90 |
| Number of grains/pods | 0.97 | 0.15 | 0.70 | 1.30 |
| 100-grain weight (g) | 21.65 | 3.48 | 15.50 | 30.23 |
| Grain yield/plant (g) | 11.54 | 1.72 | 4.34 | 22.28 |
| Biological yield (g) | 25.69 | 8.72 | 13.81 | 45.76 |
| Harvest index (%) | 0.43 | 0.05 | 0.32 | 0.53 |

SD= Standard deviation, Min= minimum and Max=maximum values

50% flowering and maturity, pods number per plant, 100-grain weight and grain yield. However, range of values was narrow for primary branches per plant, secondary branches per plant and number of grains per pod (Table 3).

Multivariate analysis indicated, first 5PCs generated for 83.27% of summation of variance. The first through fifth PCs contributed 39.06, 20.99, 8.88, 7.46 and 6.86% of the total variance recorded in the study. The highest values in PC1 were recorded for grain yield (0.38), biological yield (0.35), 100-grain weight (0.35), plant height (0.35), number of secondary branches (0.32), harvest index (0.31) and pods per plant (0.30). However, in PC2, maximum values were noted for days to flowering and maturity (0.52 and 0.55), respectively. In PC3, for leaf area, maximum value (0.75), which was followed by number of pods per plant (0.24), days to maturity (0.23) and plant height (0.21) (Table 4).

**Cluster Analysis-based on Agro-morphological Traits**

The cluster analysis showed that at a distance value of 0.5, the dendrogram grouped the 52 genotypes into five clusters; one genotype (FLIP05-46c) could not be grouped into a cluster and was detached individually (Fig. 1). Six genotypes present in 1st cluster, namely, FLIP07-34C, FLIP06-116C, FLIP08-58C, FLIP05-160C, SEL2616 and SEL2604. The second cluster had nine genotypes (FLIP08-23C, FLIP06-35C, FLIP06-18C, ILC3279, FLIP06-38C, FLIP06-161C, FLIP06-144C, FLIP06-91C and FLIP07-7C). This cluster was divided further to form two sub-clusters at a distance value of 0.4. In this regard, genotypes FLIP08-23C, FLIP06-35C, FLIP06-18C and ILC3279 were considered the most similar genotypes at the distance value of 0.20. However, in this cluster, genotype FLIP06-38C was far away from all other genotypes, with similarity index value of 0.28.

The third cluster had 10 genotypes (FLIP05-156C, SEL2490, FLIP08-58C, FLIP05-22C, FLIP88-85C, FLIP0640C, FLIP08-12C, FLIP06-74C, SEL2627 and SEL2717). This cluster was further subdivided into three sub-clusters at a (0.38) distance value. The fourth cluster had 16 chickpea genotypes *viz.* FLIP03-128, FLIP05-27C, FLIP07-32C, SEL2709, X05TH37, FLIP05-153C, FLIP87-59C, FLIP03-27C, FLIP87-59C, FLIP03-27C, SEL2714, FLIP05-27C, SEL2714, FLIP05-23C, ILC482, SEL2558, SEL2083, SEL2556, FLIP06-2C and FLIP06-53C. The genotype X05TH37 (0.28 similarity index) was grouped with genotypes SEL2709 and FLIP07-32C at a (0.24) similarity index. The fifth cluster had seven genotypes (FLIP07-31C, FLIP0511C, FLIP07-31C, FLIP05-46C, SEL2712 and SEL2560). The genotypes FLIP07-31C and FLIP0511C (0.24 similarity) were clustered with genotype FLIP07-31C at a (0.30; similarity index). The most diverse genotype in this group was FLIP82-150C making group with genotypes FLIP05-46C, SEL2712 and SEL2560 at genetic distance value of 0.45 and making group with two genotypes FLIP06-19C and FLIP07-22C at a 0.48 distance value.

**Genetic Diversity and Molecular Characterization**

Sixty SRAP primers combinations were investigated on a panel of eight genotypes to determine the best amplification status for these primers, to be employed

**Table 4:** Multivariate analysis of morphological, phenological and yield related traits of chickpea genotypes

| Factor | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| Plant height (cm) | 0.35 | -0.16 | 0.21 | -0.44 | 0.15 |
| Number of primary branches | 0.17 | 0.37 | -0.25 | 0.41 | 0.08 |
| Number of secondary branches | 0.32 | -0.16 | -0.09 | 0.23 | 0.38 |
| Leaf area ($cm^2$) | 0.09 | -0.22 | 0.75 | 0.39 | -0.07 |
| Days to 50% flowering | -0.16 | 0.52 | 0.16 | 0.07 | 0.26 |
| Days to maturity | -0.09 | 0.55 | 0.23 | 0.02 | 0.23 |
| Grain filling periods (days) | 0.06 | 0.04 | 0.12 | 0.13 | 0.01 |
| Number of pods/plant | 0.30 | 0.29 | 0.24 | -0.09 | -0.49 |
| Number of grains/pods | 0.35 | 0.11 | 0.18 | -0.26 | 0.28 |
| 100-grain weight (g) | 0.35 | 0.05 | -0.37 | 0.09 | -0.18 |
| Grain yield/plant (g) | 0.38 | 0.22 | -0.02 | -0.007 | -0.44 |
| Biological yield | 0.35 | 0.050 | -0.07 | -0.24 | 0.36 |
| Harvest index | 0.31 | -0.16 | -0.0003 | 0.53 | 0.13 |
| Variance | 33.76 | 25.31 | 12.50 | 8.18 | 7.20 |
| Cumulative variance | 33.76 | 59.07 | 71.56 | 79.75 | 86.93 |

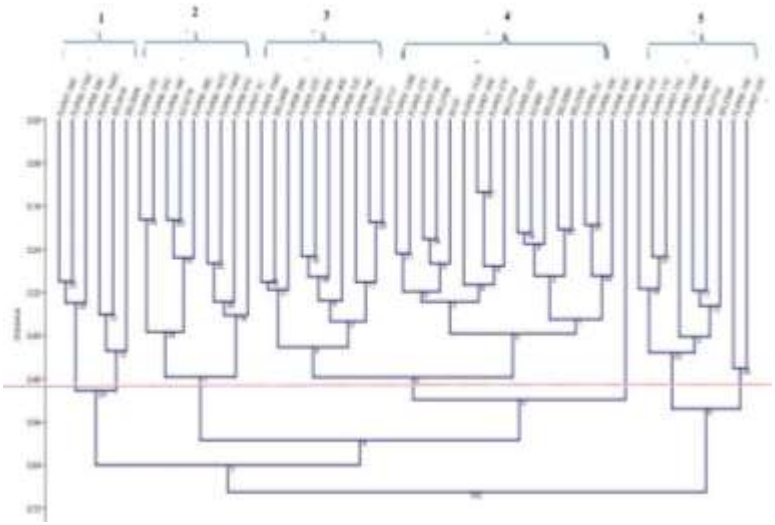

**Fig. 1:** UPGMA Cluster analysis of chickpea genotypes based on Euclidean distance

for genetic diversity assessment amongst 52 genotypes. Seventeen primer pairs showed good amplification with PCR and consistent reproducible polymorphism, which were selected to amplify the 52 genotypes.

These primers produced (638) total amplified fragments, range from 8 for SRAP07-28 to 81 for SRAP09-01 (Table 5). Total 7851 bands were produced from the 17 SRAP primer pairs across the 52 genotypes, having mean of 461.82 bands/primer. Maximum value (987) of bands were generated by the SRAP11-18 primer pair, followed by 924 bands by the SRAP09-01 primer pair, whereas the fewest bands (140) were generated by SRAP07-28. The polymorphic percentage for each primer varied between 94 and 100, with an average polymorphism value of 98.35%. The lowest polymorphism value (94%) was recorded for the SRAP06-13 primer pair (Table 5).

The PIC values, for all primers was calculated as percentage of polymorphic alleles, were high and varied between 81 and 98%. The primer pairs SRAP09-01, SRAP10-18 and SRAP11-18 recorded a PIC value of 98%, followed by the primer pairs SRAP04-18, SRAP04-21, and SRAP06-09, which had a PIC value of 97%, whereas the minimum PIC value (81%) was recorded for the primer pair SRAP10-18 (Table 5).

The UPGMA analysis was used to cluster the genotypes of chickpea by using SRAP data (Fig. 2). At Jaccard's (0.26) similarity index, four main clusters were formed; three genotypes (FLIP06-40C, FLIP88-85C, and FLIP08-58C) failed to form any cluster and were separated individually. Cluster I encompassed about 79% of the total genotypes, while the second and the third clusters contained two genotypes each; four genotypes (SEL2709, SEL2712, SEL2717, and SEL 2714) were completely different from the other genotypes and were grouped in the fourth cluster. Cluster I was further divided into seven sub-clusters at 0.4 similarity index, which represents 75% of the similarity distance from 0.01 to 0.52 (Fig. 2).

Seven out of 40 SSR primers showed multi-allelic polymorphism and were used in the study, whereas the

**Table 5:** Summary of SRAP primer combinations results across 52 chickpea genotypes

| *Primers combinations | Total bands | Polymorphic loci | Polymorphisim (%) | PIC (%) |
|---|---|---|---|---|
| SRAP08-22 | 518 | 28 | 100 | 95 |
| SRAP08-25 | 253 | 20 | 95 | 91 |
| SRAP04-18 | 502 | 53 | 96 | 97 |
| SRAP04-21 | 260 | 40 | 100 | 97 |
| SRAP06-09 | 863 | 53 | 100 | 97 |
| SRAP06-11 | 314 | 26 | 100 | 94 |
| SRAP06-12 | 564 | 44 | 100 | 96 |
| SRAP06-13 | 434 | 39 | 94 | 96 |
| SRAP06-16 | 342 | 25 | 100 | 93 |
| SRAP06-17 | 266 | 18 | 100 | 91 |
| SRAP06-25 | 506 | 37 | 100 | 95 |
| SRAP09-01 | 924 | 81 | 100 | 98 |
| SRAP06-28 | 234 | 17 | 100 | 91 |
| SRAP07-28 | 140 | 8 | 100 | 81 |
| SRAP09-18 | 178 | 11 | 90 | 88 |
| SRAP10-18 | 566 | 60 | 97 | 98 |
| SRAP11-18 | 987 | 78 | 100 | 98 |
| Total | 7851 | 638 | --- | --- |
| Average | 461.82 | 37.53 | 98.35 | 93.88 |

*SRAP08-22 means forward and reversed primer, respectively
PIC = Polymorphic information content

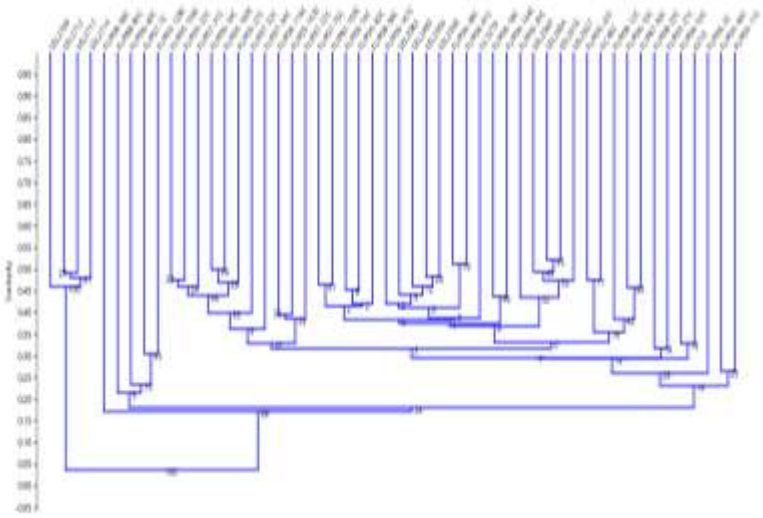

**Fig. 2:** Dandrogram of chickpea genotypes generated by Jaccard's coefficient and the unweighted pair group method with arithmetic average (UPGMA) clustering methods based on SRAP data

remaining were monomorphic for all genotypes. Primers LRU-FBSSR-29, LRU 45, and KSU-FBSSR-153 produced three alleles; LRU 19, LRU 25, and LRU 41 produced two alleles, while LRU 47 produced five alleles. PIC was calculated and expressed as percentage of fragments (polymorphic), was maximum for all combination of primer and ranged between 0.27 and 0.55%. The projected heterozygosity (He) ranged from 0.34 for LRU41 to 0.63 for KSU-FBSSR-153. The observed heterozygosity ($H_o$) for each primer ranged from 0.18 for LRU41, to 0.78 for LRU47. The highest DP (25%) was, recorded for the primer LRU-47, followed by LRU-FBSSR-29, LRU45 and KSU-FBSSR-153with 15%, while the rest of the primers had a value of 10% (Table 6).

Cutting the dendrogram, based on SSR data at an average similarity index of 0.39 generated 12 main clusters; 12 genotypes could not be grouped into any of the clusters and were separated individually. Number of genotypes in the clusters ranged from 2 in seven out of the twelve to 9 genotypes in one cluster. The remaining four clusters contained 3, 4, and 2 sub-calusters, containing 5 genotypes each (Fig. 3).

## Discussion

Agronomic and molecular assessment is used for exploration of genetic diversity amongst the genotypes and landraces - the first step for trait improvement through plant breeding programs (Farooq *et al*., 2017a, b). In this study, high variance values were recorded for various

**Table 61:** Summary of SSR data generated on chickpea genotypes

| Locus | Number of alleles | Number of bands across genotypes | PIC | $H_e$ | $H_o$ | DP (%) |
|---|---|---|---|---|---|---|
| LRU-FBSSR-29 | 3 | 58 | 0.35 | 0.42 | 0.38 | 15 |
| LRU 19 | 2 | 72 | 0.34 | 0.44 | 0.46 | 10 |
| LRU 25 | 2 | 51 | 0.28 | 0.35 | 0.24 | 10 |
| LRU41 | 2 | 32 | 0.27 | 0.34 | 0.18 | 10 |
| LRU45 | 3 | 83 | 0.51 | 0.59 | 0.62 | 15 |
| KSU-FBSSR-153 | 3 | 44 | 0.55 | 0.63 | 0.54 | 15 |
| LRU47 | 5 | 139 | 0.55 | 0.59 | 0.78 | 25 |
| Total | 20 | 479 | 0.40 | | | 100 |

LRU= legume research unit, KSU= King Saud university, $H_e$= Expected Heterozygosity, $H_o$= Observed Heterozygosity. PIC= polymorphism information content, Dp=discrimination power

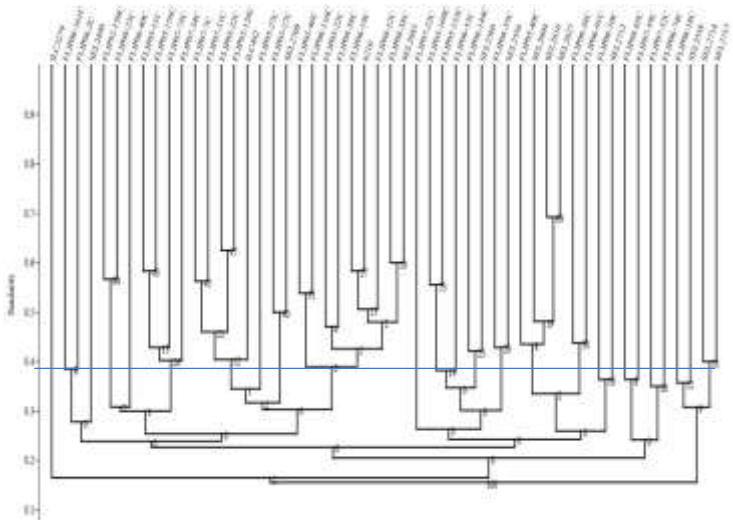

**Fig. 3:** Dandrogram of chickpea genotypes produced by Jaccard's coefficient and the unweighted pair group method with arithmetic average (UPGMA) clustering method based on SSR data

morphological, phenological and yield related attributes that includes plant height, leaf, pod number, days to (50%) flowering and maturity, grain yield etc., Quantitative traits with high variance values could be efficiently employed for direct selection of agronomical traits or identification and inclusion of genotypes of desirable traits in breeding program (Malik *et al*., 2014). Diversity in these traits indicate great scope of these genotypes for use in different breeding approaches (Malik *et al.*, 2010, 2014). For instance, the genotypes with early maturity may strive well in the environments with terminal heat and/or drought stresses (Farooq *et al*., 2017a, b). However, the variance values for number of primary branches followed by secondary and grains per pod were low, and therefore, selection for these traits among the chickpea genotypes might not be effective (Khan *et al*., 2011; Malik *et al*., 2014). in chickpea.

Molecular-data-based estimation of genetic variability exist among genotypes of chickpea is critical to confirm the morphological diversity and use in in the breeding approaches as well as in the preservation of genetic resources. The SRAP marker has great potential in assessing genetic variability for plant breeding (Aneja *et al*., 2012). These may enhance the efficiency of diversity estimation (Levi *et al*., 2011; Guo *et al*., 2012) and can predict the genetic diversity with a maximum polymorphism, greater value of discriminatory power and higher reproducibility degree (Alghamdi *et al*., 2012). In this study, the PIC values were high in range from 81 to 98%, with an average of 94%. This indicated that SRAP marker, used in this study, had higher polymorphism, which may contribute to improve information related to breeding efforts as have been noted in faba bean (Alghamdi *et al*., 2012). Interestingly, 677 polymorphic (fragments) were produced by 17 SRAP primer combinations within a size range of 100–500 bp, with an average of 37 fragments/primer pair.

Microsatellite markers are also effective for estimation of polymorphism and map the diversity among genotypes (Keneni *et al*., 2011; Sefera *et al*., 2011; Choudhary *et al*., 2012). In this study, genotypes showed maximum genetic diversity using 7 SSR (polymorphic primers), with a PIC value of 0.27–0.55%, with heterozygosity values between 0.34 and 0.63. Pattern of genetic variability degree of SSR markers and patterns of genetic diversity detected in chickpea genotypes may help the breeding methods (Tanya *et al*., 2011). These markers are particularly effective in situations where plants

have a low degree of polymorphism. It is generally noted that heterozygosity values are low and the PIC values are high in chickpea. Thus, information about genetics was alike to be found in chickpea.

Cluster analysis, supported by PCA and PCoA results, generated from phonological, morphological and yield related traits, and SRAP and SSR marker data, showed that scattering of genotypes across different clusters and components indicating wide genetic diversity among these genotypes, although some trends were attributable to the origin or pedigree of the genotypes. In this study, higher genetic variability observed in genotypes under consideration can be efficiently employed for mapping crosses of genome for desired traits including higher potential genotypes for yield and gene tagging. By PCoA and cluster analysis, groups of genetically diverse accessions are defined (Naghavi and Jahansouz, 2005).

Mean genetic uniformity acquired through SSR (0.27), SRAP (0.37), and variation related to chickpea genotypes used in this study offer bulk of information for pedigree selection in future for parental selection. Higher polymorphism percentage and PIC values obtained from SRAP markers are more than SSR. Both of these methods are highly informative and instructive as compared to storage proteins and isoenzymes, RAPD and AFLP markers to investigate the genetic association and diversity in chickpea genotypes (Labdi *et al*., 1996; Talebi *et al*., 2008a, b). Dendrogram developed on molecular markers are in line with that produced on the base of morphological traits. This indicates strong power of the molecular markers used in the study.

In conclusion, there was significant diversity and variation, among the tested chickpea genotypes, for phenological, morphological and yield related traits. Great genetic variability in the chickpea genotypes was characterized by higher level (degree) of polymorphism with SRAP and SSR markers. Based on the field performance and genetic diversity data, eight genotypes had the highest diversity, that can be selected to use in breeding strategies.

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