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Full Length Article

Field Performance and Genetic Diversity of Chickpea Genotypes

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Abstract

Chickpea (*Cicer arietinum* L.) is an important legume crop widely grown across the globe. In this study, twenty six chickpea genotypes, of diverse origin, were evaluated for field performance and genetic diversity. Significant differences in morphological characters of tested chickpea genotypes were observed at vegetative and reproductive stages under field conditions. Amplified fragments length polymorphism (AFLP) and sequence-related amplified polymorphism (SRAP) markers, used in this study, showed considerable genetic diversity among the tested genotypes. With markers, 716 AFLP and 1164 polymorphic SRAP loci were analyzed using four AFLP and six SRAP primer combinations. The values for polymorphism information content for SRAP and AFLP were more than 0.6 and 0.9, respectively. In addition, many sub-clusters within genotypes were noted, which indicated high diversity level in the tested genotypes. Clustering of chickpea genotypes was largely based on genetic background and/or origin. Both morphological and molecular data helped to quantify the genetic diversity in tested chickpea genotypes and may be useful for the use in breeding program aimed at yield improvement. © 2016 Friends Science Publishers

Keywords: Amplified fragments length polymorphism; Chickpea; Crop improvement; Genetic diversity; Sequence-related amplified polymorphism

Introduction

Chickpea (*Cicer arietinum* L.) is an important legume crop widely distributed and cultivated throughout the world including South/North America, North Africa, West/Central Asia, South Europe, and Australia (Ladizinsky and Alder, 1976; Singh and Ocampo, 1997). Its grains are very nutritious due to the presence of proteins, carbohydrates, vitamins and minerals (Wang *et al*., 2010). Moreover, chickpea also occupies high biological value due to its well-balanced amino acids content and low levels of anti-nutritional factors than other grain legumes like pigeon pea, black gram and green gram (Friedman, 1996; Santiago Cardoso *et al*., 2001; Kaur and Singh, 2007). Nonetheless, chickpea production is being constrained due to several biotic and abiotic stresses worldwide. Estimation and use of genetic diversity from the available genetic resources is a key factor for a successful breeding program (Renganayaki *et al*., 2001) aimed at improving crop performance under biotic and abiotic stresses. Variance of relatively highly heritable quantitative genetic markers provides estimates of genetic diversity, which may be utilized for selecting parents in the breeding programs.

Genetic diversity is thus very essential and useful for the conservation of genetic resources and cultivar improvement through breeding. Generally, diversity is estimated by measuring variation in morphological parameters (Upadhaya *et al*., 2007) or using molecular markers (Sharma *et al*., 1995). Diversity assessment based on morphological traits is usually limited as environmental factors influence the expression of quantitative traits. As molecular markers are highly efficient and insensitive to environmental factors, these are being used more efficiently to differentiate within and between species and populations (Kumar, 1999). Several DNA-based markers are being used to quantify the genetic diversity within plant species. Amplified fragments length polymorphism (AFLP) (Segovia-Lerma *et al*., 2003), simple sequence repeats (SSR) (Sharma *et al*., 1995), inter-simple sequence repeats (ISSR) (Reddy *et al*., 2002), restriction fragment length polymorphism (RFLP) (Banerjee *et al*., 1999) and random amplified polymorphic DNA (RAPD) (Ratnaparkhe *et al*., 1998; Talebi *et al*., 2008) have been successfully used for assessing molecular diversity in a number of plant species. Although SSR markers are highly polymorphic, but require nucleotide information for primer design (Sun *et al*., 1998); nonetheless AFLP (Segovia-Lerma *et al*., 2003), and sequence-related amplified polymorphism (SRAP) (Ariss and Vandemark, 2007; Castonguay *et al*., 2010) have overcome this limitation.

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Assessment of chickpea genotypes on morphological and chemical composition has been reported widely (Amjad *et al*., 2006; Khattak *et al*., 2006); however, little efforts have been done to access the genetic variation among chickpea genotypes at morphological as well as molecular levels using newly developed molecular markers. This study was, therefore, conducted to assess the genetic variation among chickpea genotypes at morphological and molecular levels in field conditions using AFLP and SRAP molecular marker techniques.

Materials and Methods

Field Performance

This 2-year study was carried out at Dirab Research and Experimental Station (24º 43' 34" N, 46º 37' 15" E), King Saud University, Riyadh, Kingdom of Saudi Arabia during winter season 2011-12 and 2012-13. The experimental soil was sandy clay loam (pH= 8.15; electrical conductivity= 2.1 dS m^{-1}). Seeds of 26 chickpea genotypes, used in the study, were obtained from International Center for Agricultural Research in Dry Areas (ICARDA) (Table 1).

All chickpea genotypes were sown, during second week of November during both experimental years, with a hand drill in 30 cm spaced rows in a plot measuring 3 m × 0.9 m following randomized complete block design with four replications. Phosphorus (P) and nitrogen (N) was applied at 150 and 200 kg ha^{-1}, respectively. Ammonium sulphate and calcium super phosphate were used as sources of N and P, respectively. Whole of P and 1/3 of N were applied as basal dose. The remaining N was at flowering and pod filling stages in two equal splits.

Days to 50% flowering (DF) and days to 95% maturity (DM) were noted from sowing to 50% flower and 95% maturity, respectively by visual observation. Morphological and yield related traits were recorded following the standard procedure. Plant height was recorded with a meter rod from the base of plant to the tip of upper most leaf. The branches per plant (BPP) were counted manually at maturity. All genotypes were harvested at harvest maturity to record the grain yield. For this purpose, 20 randomly selected plants were harvested from each plot; pods were threshed to record number of grains per plant and grain yield. All grains (from 20 plants) were weighed on an electric balance to work out grain yield, which was adjusted to 12% moisture. Analysis of variance (ANOVA) technique was used to determine the significance among treatments by using *MSTATC* software (Michigan State University, East Lansing, MI, USA).

Molecular Characterization

For molecular analysis, DNA was isolated according to method previously described by Alghamdi *et al*. (2012). Six SRAP primer combinations showed consistently reproducible polymorphisms when tested on six randomly selected genotypes, and thus were used to analyze all studied genotypes (Table 2). All SRAP reactions were performed following standard protocol as detailed in Alghamdi *et al.* (2014). Thermal cycler (TC-5000, Bibby Scientific - UK) was used for amplification. AFLP analysis was done following the protocol as described in PE Biosystems plant mapping kit (Applied Biosystems, Foster city, CA, USA), using a modified procedure of Vos *et al.* (1995) as detailed in Alghamdi *et al.* (2014). GeneMapper Analysis Software v3.7 (ABI) was used to perform fragment analysis as detailed in Alghamdi *et al.* (2014). The threshold for allele calling was set at 200 relative florescence units (rfu) according to Wooten and Tolley-Jordan (2009). Data obtained from RFLP and SRAP analysis were analyzed using the Jaccard similarity coefficient as described by Jaccard (1908). Correlation among morphological traits, RFLP, AFLP and SRAP data was calculated, followed by construction of distance matrix for each assay and comparison between those matrices following a Mantel matrix correspondence test.

Results

Field Performance

Tested chickpea genotypes differed significantly for DF, plant height, DM, BPP, grains per plant and grain yield. Genotypes xO5TH174 (with 58 days) and xO5TH162 (with 61 days) were early flowering; whereas genotypes xO5TH32 and xO5TH35 matured earlier than other genotypes (Table 3). Dwarf plants were noted in genotype xO5TH20; whereas genotype xO5TH137 had the maximum plant height (Table 3). The chickpea genotype x05TH33 had maximum BPP (Table 3). However, genotype x05TH172 had the maximum grains per plant (93.3) and grain yield per plant (35.8 g; Table 3).

Principal components analysis grouped the six morphological traits into various components; with the first three components describing 79.58% of the total variations (Table 4). First principle component demonstrated 39.44% of the total variation, and included important traits like number of grains per plant and grains yield per plant (Table 4). These traits may be used as selection criterion to develop high performance chickpea genotypes (Table 4). Second component showed 23.02% of the total variation, which comprised of DF, DM and plant height. Third component revealed 17.12% of the total variation and contained number of BPP (Table 4).

Molecular Characterization

A total of 6 SRAP primers were selected on the basis of their ability to show consistent PCR amplification and reproducible polymorphism using six genotypes. These six SRAP primers were used for similarity distance among the tested genotypes. Primers were labeled and

Table 1: Pedigree of chickpea genotypes used in the study

Genotype	Pedigree
xO5TH13	FLIP 97-90 x FLIP 97-126C) x FLIP 02-43C
xO5TH15	FLIP 97-165 x FLIP 97-28C) x FLIP 01-25C
xO5TH20	FLIP 98-160 x FLIP 95-68C) x FLIP 02-36C
xO5TH32	S01169 x FLIP 97-90C)X FLIP 02-41C
xO5TH35	S01203 X FLIP 97-205C) X FLIP 01-29C
xO5TH36	S01205 X FLIP 97-229C) X FLIP 00-72C
xO5TH37	S01228 X FLIP 98-229C)X FLIP 01-28C
xO5TH43	FLIP 87-59C X FLIP 99-34C) X FLIP 006C
xO5TH52	ILC 5258 X S 01107)X FLIP 98-178C
xO5TH68	Leb.Market-1X UC 15)X FLIP 02-35C
xO5TH71	FLIP 93-93C X UC 27) X FLIP 00-6C
xO5TH77	ILP 1929 X FLIP 00-14C
xO5TH86	FLIP 86-6C X FLIP 00-14C
xO5TH104	FLIP 01-28C X FLIP 00-06C
xO5TH108	FLIP 01-16C X FLIP 00-14C
xO5TH113	FLIP 98-113C X FLIP 0014C
xO5TH137	FLIP 98-178C X F5LM (5745)
xO5TH162	FLIP 00-14C X ICCV-92337
xO5TH172	FLIP 02-35C X ICCV-94304
xO5TH174	FLIP 00-14C X ICCV-92337
xO5TH182	ICCV 03301 X FLIP 00-14C
xO5TH183	ICCV 03304 X FLIP 00-06C
xO5TH184	ICCV 03307 X FLIP 97-85C
xO5TH193	ICCV 03109 X FLIP 97-85C
FLIP 82-150c	X79TH101/ILC 523 X ILC 183
FLIP82-150C	X79TH101/ILC 523 X ILC 183

Table 2: Name and sequence of SRAP primers used in the study

Primer name	Forward 5'-3'	Primer name	Reverse 5'-3'
ME1	TGAGTCCAAACCGGATA	EM1	GACTGCGTACGAATTAAT
ME2	TGAGTCCAAACCGGAGC	EM2	GACTGCGTACGAATTTGC
		EM3	GACTGCGTACGAATTGAC
		EM4	GACTGCGTACGAATTACG
		EM5	GACTGCGTACGAATTACT
		EM6	GACTGCGTACGAATTAGC

genotyping was carried out on ABI 3130*xl* 16 capillaries sequencer (Applied Biosystems). PCR products were analyzed using *Genemapper* software. Chromatograms showing polymorphic fragments are shown in Fig. 1. A total of 1164 amplified bands were obtained with average of 194 bands for each primer combination showing 100% polymorphism (Table 5). Primer combination ME1/EM1 had the lowest fragments; whereas primer combination ME2/EM6 produced the maximum (Table 5). The PIC value measured from all the SRAP primers was high and ranged from 0.655 to 0.978 showing reasonable discrimination power and high level of molecular diversity (Table 5).

Among the tested genotypes, the genetic similarity, ranged from 0.02-0.60. Highest similarity was obtained between genotype pairs xO5TH36 and xO5TH43 (Fig. 2). However, the genetic differences ranged from 10-60% among the tested chickpea genotypes. At the lowest range of similarity, tested chickpea genotypes were divided into two main groups i.e., group A and group B.

Table 3: Phenological, morphological and yield-related traits of tested chickpea genotypes (two year means)

Genotype	Days to 50% flowering	Days to maturity	Plant height (cm)	Branches per plant	Grains per plant	Grain yield per plant (g)
xO5TH13	85	127;	56	5.8	28.6	07.5
xO5TH15	84	127	50	6.6	51.8	15.6
xO5TH20	81	124	46	6.5	57.3	15.7
xO5TH32	81	122	56	6.3	48.3	12.2
xO5TH35	78	122	55	6.3	49.0	13.2
xO5TH36	82	125	56	6.2	52.8	14.2
xO5TH37	85	127	51	7.3	28.3	09.0
xO5TH43	80	127	55	7.2	52.3	15.6
xO5TH52	78	130	56	6.9	57.7	14.9
xO5TH68	71	133	63	5.8	81.9	25.4
xO5TH71	83	133	62	7.0	62.4	19.2
xO5TH77	79	126	52	6.7	54.2	13.9
xO5TH86	79	128	56	5.8	46.9	11.2
xO5TH104	81	129	53	6.4	51.8	13.3
xO5TH108	79	127	53	5.8	82.4	24.0
xO5TH113	79	130	58	6.0	48.0	12.6
xO5TH137	90	132	71	5.2	54.3	16.9
xO5TH162	61	128	54	5.8	59.3	17.5
xO5TH172	72	125	53	6.0	93.3	35.8
xO5TH174	58	124	52	4.5	74.7	24.0
xO5TH182	64	126	50	6.4	46.1	15.8
xO5TH183	66	125	53	5.8	58.8	12.5
xO5TH184	78	128	54	5.6	83.9	23.2
xO5TH193	70	132	48	7.4	88.4	22.8
FLIP 82-150c	86	132	53	7.9	65.3	15.6
FLIP82-151C	86	133	51	7.4	47.4	11.4
Mean	77.54	127.77	54.50	6.3	58.7	16.6
LSD (p 0.05)	3.7	2.3	3.6	0.8	10.6	3.20

Fig. 1: SRAP markers electropherograms of six chickpea genotypes using ME2/EM2 primer combination analyzed in the GeneMapper software

Group A had all the genotypes except xO5TH20, xO5TH193 and xO5TH113, which constituted group B (Fig. 2). At 30% similarity, group A was further divided into two sub-groups i.e., sub group A and sub group B. At 34% similarity reference, the sub group A was further separated into individual genotypes except one genotype while the sub-group B formed two sub-sub cluster containing 3 and 11 genotypes, respectively (Fig. 2).

AFLP profiling of tested chickpea genotypes revealed polymorphic bands ranging in size from 100-500 nucleotides. For AFLP, four primer combinations were applied, which yielded 716 amplified bands with an

Table 4: Eigen values, individual and cumulative percentage variations and eigen vectors explained by three principal components based on morphological traits in chickpea genotypes

	F1	F2	F3
Eigen value	2.761	1.61	1.20
Variability (%)	39.44	23.01	17.12
Cumulative %	39.44	62.45	79.58
Eigen vectors			
Days to 50% flowering	-0.368	0.45	0.082
Days to maturity	0.071	0.625	0.314
Plant height	0.103	0.629	-0.426
Branches per plant	-0.327	0.041	0.675
Grains per plant	0.5	0.034	0.404
Grain yield per plant	0.558	0.031	0.256

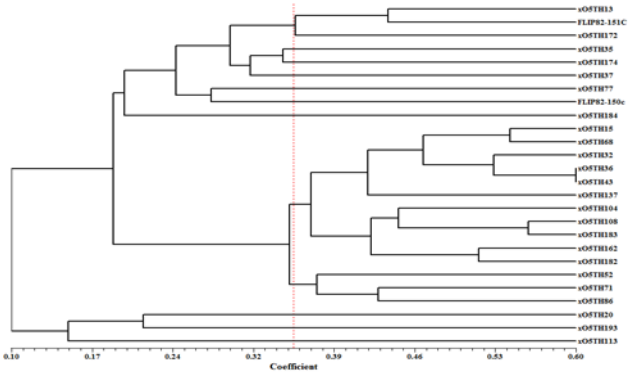

Fig. 2: Dendrogram of tested chickpea genotypes generated from SRAP markers by Jaccard's coefficient and UPGMA clustering method

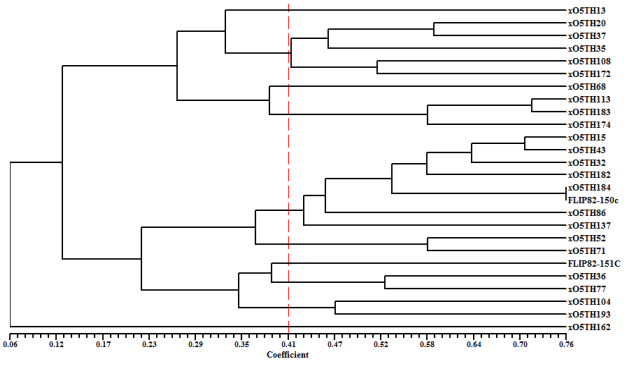

Fig. 3: Dendrogram of tested chickpea genotypes generated from AFLP markers by Jaccard's coefficient and UPGMA clustering method

average of 179 bands for each primer combination (Table 5). The bands scored were highly polymorphic for each combination. Primer combination E_{CT}/M_{CTG} produced the maximum fragments whereas primer combination E_{TA}/M_{CTC} produced the minimum fragments (Table 5). PIC value measured for individual primer combination was high and ranged from 0.907 to 0.960 (Table 5). This high value showed that AFLP markers were highly informative and were able to discriminate the chickpea genotypes with high discrimination power. Genetic similarity among tested genotypes ranged from 0.06 to 0.76; among these, maximum similarity was found between genotype pairs xO5TH184 and FLIP82-150c (Fig. 3). Cluster analysis divided the tested genotypes into two groups at 12% similarity level. Group A was further separated into two subgroups at 25% similarity, while group B separated into two groups at 23% similarity level. At 62% similarity level, chickpea genotypes are separated into individual cluster except six genotypes xO5TH113, xO5TH183, xO5TH184, FLIP82-150C, xO5TH15 and xO5TH43 (Fig. 3). There were significant correlations of AFLP and SRAP data with morphological matrix (Table 6).

Discussion

This study demonstrated significant variation in morphological and yield attributes among the tested chickpea genotypes (Table 3). This difference was owing to differences in the genetic makeup of tested genotypes, which can be potentially utilized in future breeding programs. Characterization and evaluation of genotypes, based on morphological traits and molecular markers, is crucial for their efficient conservation and further crop improvement (Talebi *et al*., 2008). Some morphological trait such as DF and grain yield discriminated genotypes more efficiently than others. Apart from grain yield, the time to reach flowering and maturity taken by a genotype are vital, as they are important for the adaptation of genotypes in various agro-ecological regions. Time to flowering is an important parts of the plant growth cycle, because of its strong association with grain yield (Kumar *et al*., 2011). This study was also able to identify some best performing genotypes suitable for large scale production in the Central Saudi Arabia. For instance, genotype "x05TH172" produced more grains per plant and provided highest grain yield per plant (Table 3) and may be used in breeding programs for chickpea yield improvement.

Recent programs of crop improvement require huge genetic diversity within the breeding material (van de Wouw *et al*., 2010). Understanding and estimation of genetic diversity with plant species aids to select diverse parental lines to develop the segregating populations having more variability (McCouch *et al*., 2013). Although, efforts has been done to increase the production of chickpea through conventional breeding approaches (Ahmad *et al*., 2010); nonetheless, genetic improvement through the use of these recent approaches is quite slow, possibly due to existence of huge genotype × environment interactions for grain yield and related traits (Kumar and Ali, 2006). However, as indicated in this study, use of molecular markers offers new perspectives for improving the chickpea production in diverse environments (Varshney *et al*., 2005). Molecular characterization of chickpea genotypes through SRAP and AFLP markers revealed a high level of

Table 5: Summary of molecular analysis using SRAP and AFLP markers on tested chickpea genotypes

SRAP					AFLP				
PC*	TF	PF	P%	PIC	PC	TF	PF	PP%	PIC
ME1/EM1	20	20	100	0.655	E_{CT}/M_{CTG}	246	246	100	0.960
ME1/EM2	138	138	100	0.938	E_{TA}/M_{CTC}	55	55	100	0.907
ME2/EM3	151	151	100	0.928	E_{TC}/M_{CTA}	241	241	100	0.953
ME2/EM4	34	34	100	0.880	E_{CC}/M_{CCT}	174	174	100	0.945
ME2/EM5	264	264	100	0.960	Total	716			
ME2/EM6	557	557	100	0.978	Mean	179			
Total	1164								
Mean	194								

*PC: Primer combination, TF: total amplified fragments, PF: polymorphic fragments, P%: polymorphism percentage, PIC: polymorphic information content

Table 6: Correlation among dissimilarity matrices of chickpea genotypes among different markers

Markers	SRAP	AFLP
Morphological	r=0.005 P=0.933	r=0.421 P=<0.0001
SRAP		r=0.564 p=<0.001

polymorphism among chickpea and proved to have high discrimination power in differentiating chickpea genotypes (Tables 4, 5). This high level of polymorphism, observed in this study, might be due to the use of more sensitive laser based genetic analyzer detection system, which even detected one base pair difference between the amplicons (Tavoletti and Iommarini, 2007; Altintas *et al*., 2008). Significant variation among chickpea genotypes using SRAP and AFLP marker revealed the high level of biodiversity in the tested genotypes (Fig. 1-3), which is quite useful for use in chickpea improving programs.

This study showed significant variation among tested chickpea genotypes, which may facilitate the selection of genotypes possessing great potential to produce better yield in diverse environments. Moreover, SRAP and AFLP markers are very useful to discriminate the relationship of various traits of different genotypes of chickpea. The significant correlations between AFLP and SRAP data with morphological matrix (Table 6) indicate the coherent pattern of genetic diversity and accuracy of the two marker systems to estimate and validate the genetic diversity statistics for studied genotypes.

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