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Full Length Research Paper

Intraspecific diversity of Egyptian and foreign new lines of chickpea based on Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and isozyme markers

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Four Egyptian and 21 foreign new lines of kabuli chickpea (*Cicer arietinum* L.) were analyzed by using biochemical molecular markers to gain insight into the genetic diversity between accessions. Polyacrylamide gel electrophoresis (PAGE) was applied on the total soluble seed proteins by using sodium dodecyl sulphate (SDS) and five isozyme systems to examine the storage and functional components, respectively. The storage protein markers alone were not enough to discriminate polymorphism among accessions of the different geographical origins. Isozyme data revealed 18 alleles belonging to 11 loci in the 5 systems. Unweighted pair-group method with arithmetic average (UPGMA) revealed that, the examined Egyptian accessions could strongly be originated from an ancestor that possessed little intraspecific diversity or lost, during domestication, much of its variation.

Key words: Kabuli, biochemical markers, electrophoresis, isozyme.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most important cool season food legume in the world after dry beans and peas (FAOSTAT, 2008). It serves as an important source of protein in human diet; as hypocholesteremic agent and plays an important role in the enrichment of soil fertility (Geervani, 1991). It is the unique cultivated species within the genus *Cicer*, and is mainly distributed in the Indian subcontinent, West Asia, North Africa and also introduced to Americas and Australia. Chickpea is a self-pollinated diploid with 2n = 2x = 16 (Arumuganathan and Earle, 1991) and has two main types of cultivars namely *kabuli* and *desi* that are grown globally and representing two diverse gene pools. *Kabuli* types (white flower, large and cream colored seeds) are generally grown in the Mediterranean region including Southern Europe, Western Asia and Northern Africa, whereas the *desi* types (purple flowers, small angular and dark seeds) are mainly grown in Ethiopia and Indian subcontinent (Taylor and Ford, 2007).

In the past, agricultural systems and plant breeding have narrowed the genetic base of cultivated chickpea (Robertson et al., 1997). This promoted the search for additional sources of genetic diversity that might be useful in gene-bank management and prospective breeding experiments for example, tagging of germplasm and establishment of core collections (Atta et al., 2009). Such target is achieved by various studies based on different approaches either quantitative for example, grain yield, number of seeds per plant, biological yield, number of pods (Rao et al., 2007; Alwawi et al., 2009; Kan et al., 2010) or qualitative for example, biochemical and molecular markers (Bhagyawant and Srivastava, 2008; Talebi et al., 2008; Gujaria et al., 2011; Kahrizi et

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al., 2012). However, the intraspecific diversity of the chickpea has always been questionable. At a time in which the high heritability and phenotypic diversity are correlated with the researches established based on quantitative data, a controversy is observed in those based on qualitative data. The evidence is that, application of both biochemical and DNA-based markers on chickpea sometimes showed low levels of intraspecific diversity (Ahmad et al., 1992; Ghafoor et al., 2003) and other times showed high levels (Labdi et al., 1996; Sant et al., 1999; Hameed et al., 2009). These have mainly been explained as a result of the self pollinating nature and mutations, respectively.

New lines have continuously been added to the gene pole of chickpea and the choice of marker system is largely dependent on the intended application, costs involved in development and the ease of use (Gujaria et al., 2011). Because of their validity and simplicity; biochemical protein markers are still efficient tools used to address the intraspecific diversity. In this respect, seed protein and isozyme profiles as revealed by polyacrylamide gel electrophoresis (PAGE) are successfully used to resolve taxonomic problems and describing the genetic diversity within many crop plants for example, *Cicer* (Ahmad and Slinkard, 1992), *Pisum* (Ghafoor and Arshad, 2008); *Lens* (Yüzba-io-lu et al., 2008), wheat (Kakaei and Kahrizi, 2011a) and rapeseed (Kakaei and Kahrizi, 2011b). Seed proteins are considered as practical and reliable methods because seed storage proteins and nucleotide sequences are largely independent of environmental fluctuations (Rostami-Ahmadvandi et al., 2011). On the other hand, a number of studies have been made on chickpea genotypes by using seed proteins or isozymes for different purposes. Kazan et al. (1993) used isozyme loci to detect significant polymorphism and interspecific crosses of cultivated with wild species. Nisar et al. (2007) used sodium dodecyl sulphate (SDS)-PAGE of total seed protein to study genetic relationships and reported many cases of out-breeding. Hameed et al. (2009) analyzed total seed proteins of 8 *kabuli* mutants, identified 5 genotypes and reported their biochemical fingerprints. However, most of these studies were made on Pakistani materials by using single technique. The present work aimed at evaluating genetic diversity of 25 new Egyptian and foreign *kabuli* chickpea lines by using SDS-PAGE of seed proteins and isozyme markers.

MATERIALS AND METHODS

Seeds of the examined 25 chickpea genotypes (*C. arietinum* L.) are derived from crosses made at the International Center for Agricultural Research in Dry Area (ICARDA) and were provided by both Agricultural Research Centers in Giza, Egypt (four lines) and in Alepo, Syria (21 lines). To extract seed proteins, 0.5 g of mature seeds of each accession were mixed with an equal weight of pure, clean, sterile fine sand and powdered using a mortar and pestle and homogenized with 1 M Tris-HCl buffer, pH 8.8 in clean Eppendorf tube and left in refrigerator overnight, the extract was centrifuged at 3.000 rpm for 10 min (Badr et al., 1998). The supernatant (protein extract) was transferred to new tubes and immediately used for electrophoresis or kept in deep-freeze until use. For electrophoresis, 10 µl of the extract were mixed with 5 µl of a treatment buffer. Electrophoresis was carried out by the modified discontinuous SDS-PAGE (DISC SDS-PAGE) method (Laemmli, 1970) using 12% (w/v) acrylamide separating gel (0.375 M Tris-HCl buffer, pH 8.8) and 4% (w/v) acrylamide stacking gel (0.125 M Tris-HCl, pH 6.8). The electrode buffer was Tris-glycine (2.25 g Tris and 10.8 g glycine per 750 ml buffer solution, pH 8.3) with 0.1% (w/v) SDS. Gels were stained overnight in 0.1% (w/v) Coomassie Brilliant Blue-R250 solution containing 40% (v/v) methanol and 10% (v/v) trichloracetic acid; then destained and photographed.

The examined isozymes were α-and β-esterase (Est.), acid phosphatase (Acph.), alcohol dehydrogenase (Adh.) and aldehyde oxidase (Ao.). For their extraction, three mature seeds of each accession were germinated under the same incubation conditions that is, in pots holding 2500 g of air-dried soil in a greenhouse for 6 to 8 weeks with suitable irrigation; 0.25 g of fresh leaves of the seedlings was homogenized in 1 ml extraction buffer (1 M Tris-HCl, pH 8.8) using a mortar and pestle, centrifuged at 3000 rpm for 5 min, the supernatant was kept at -20°C until use. For isozymes separation, 10% (w/v) native-polyacrylamide gel electrophoresis method was used (Stegemann et al., 1985). For electrophoresis, 50 µl of extract was mixed with 20 µl of treatment buffer and 50 µl of this mixture was applied to the well. In gels staining, protocols of Scandalios (1964) were used for α and β-Est., Wendel and Weeden (1989) for both Ao and Acph, and Weeden and Wendel (1990) for Adh. Gels were washed two or three times with tap water, fixed in ethanol, 20% glacial acetic acid (9:11 v/v) for 24 h and photographed.

In the numerical analysis, the computer program package NTSYS-pc 2.2 (Rohlf, 2005) was used in all operations. The supplementary data editor program NTedit 2.2 was used to prepare the raw data matrix of computations. The produced bands by SDS-PAGE and isozymes techniques were scored as one data set. The presence or absence of each band was coded as 1 and 0, respectively. The similarity matrix of genotypes was generated by SIMQUAL module based on DICE coefficient (Dice, 1945) that is, 2a/ (2a + b + c), where a = number of common bands in two genotypes, b = total number of bands in genotype "1" and b = total number of bands in genotype "2". Clustering was performed using unweighted pair-group method with arithmetic average (UPGMA) and represented in a phenogram by using SAHN and TREE modules, respectively. To test reliability, Mantel's test of significance (Mantel, 1967) was used as follows:

The correlation coefficient "*r*" value was obtained by computing the phenogram cophenetic (ultramatric) value matrix by using COPH module and compared with Dice distance matrix by using MXCOMP module (Rohlf, 2005).

RESULTS AND DISCUSSION

The SDS-PAGE of seed protein data revealed that, a total number of 30 detectable seed protein bands (subunits) are observed in the produced SDS-PAGE. Molecular mass (Mr) of the storage protein subunits are ranged from 106 to 24 kDa. Ghafoor et al. (2003) and Nisar et al. (2007) reported low intraspecific diversity in chickpea based on SDS-PAGE of seed protein profiles and concluded that, the storage seed protein is a very conservative trait and that, the seed-protein profiles are

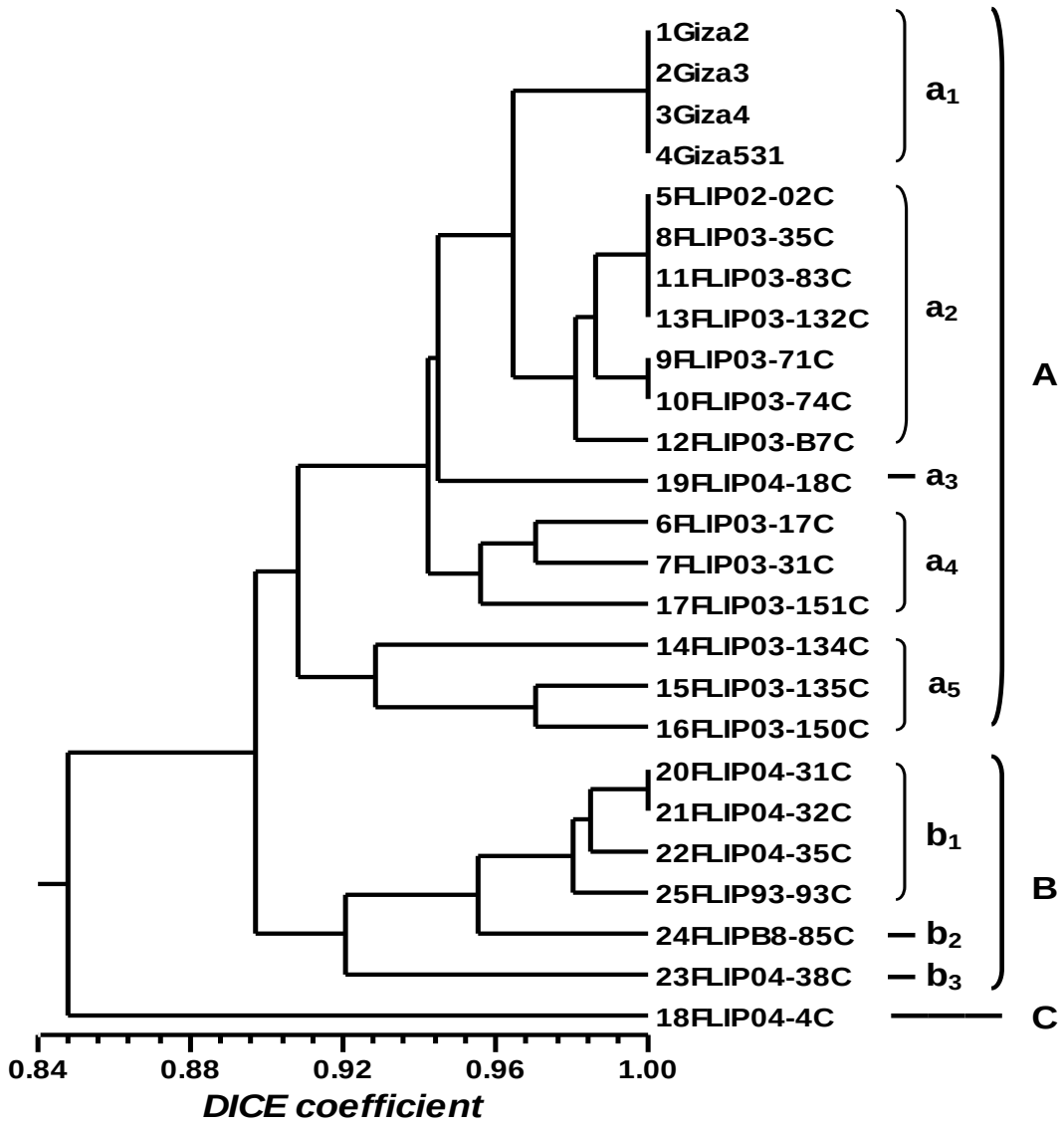

Figure 1. UPGMA phenogram showing genetic diversity of the chickpea genotypes based on combination of SDS-PAGE and isozymes characters. Serial numbers are added on left of the correspondent genotype name. Capital letters indicate the large groups, lowercase letters the subgroups.

largely species-specific. In the present study, the most common observation based on SDS-PAGE data is the limited intraspecific variations in electrophoretic profiles of the foreign genotypes and their complete lack among those of the Egyptian. The observed variations are among the genotypes FLIP 04-38C, 03-135C, 03-134C, 03-17C, 03-31C, 03-151C, B8-85C, 03-150C, 04-4C and 04-35C. The remaining 15 both Egyptian and Syrian genotypes showed relatively monomorphic profile either in numbers or in position of bands on the gel. These observations are compatible with those of Ghafoor et al. (2003) and Nisar et al. (2007). Absence of discriminate polymorphism between accessions of the different geographical origins reveals that, the ancestral characters of both gene pools are the same, which supports the view of Rouamba et al. (2001) who in their work on *Vicia faba* clarified that, weak geographical differentiation of populations may have resulted from commercial exchange between countries of the same language.

Isozyme data revealed that, 18 electrophoretic bands (that are alleles) are detected in zymograms of the 5 isozyme systems analyzed. They belong to 11 putative loci, 4 for α-Est, 2 for each of β-Est, Acph, Ao, respectively, and 1 for Adh. Polymorphism is observed in 4 systems (α-Est; β-Est; Acph; Ao) including a total of 14 alleles. The monomorphic alleles are 4 that is, α-Est1, β-Est1, β-Est6 and Adh1. Specific alleles are only observed in the foreign gene pool, as α-Est3 and β-Est4 is recorded in FLIP 04-4C (No. 18; Figure 1), α-Est6 in FLIP

B8-85C and 93-93C (No. 24 and 25, respectively), Acph2 in FLIP 03-B7C and 04-18C (No. 12 and 19, respectively), that all are originating from Syria. Labdi et al. (1996) and Tayyar and Waines (1996) reported a low level of diversity based on the isozyme polymorphism. They explained their results in connection with the highly self pollinated nature of the species as well as the limited number of accessions of the wild species available for research purposes at ICARDA, respectively. Isozyme data of the present study disagree with such view as it reveals considerable diversity among the studied genotypes and are suitable to differentiate between many genotype groups.

The produced phenogram (Figure 1) based on the recorded 48 electrophoretic characters showed three major clusters that are A, B and C at a similarity level of 0.85. The 4 Egyptian genotypes Giza 2, 3, 4 and 531 that represent group a_1 are clustered together with no variation. However, they are nested in the Syrian genotypes at 85% homology. This result indicates that, the examined Egyptian chickpea accessions are domesticated from an ancestor that possessed little intraspecific diversity or lost during domestication some of its variation in the nucleic acids repeats and subsequently proteome. Similar phenomenon has previously been reported in *Vicia faba* by Shiran and Mashayekh (2004).

Except presence of the allele Acph2 instead of Acph1, the genotype FLIP 03-B7C (No. 12) has a common isozyme pattern with those of group a_2 (Figure 1). For this reason, it is distinguished among this group as a separate line and clustered with the remaining genotypes of group a_2 at the 97% homology. At the same time, presence of the allele Acph2 instead of Acph1 is also observed in FLIP 04-18C genotype (No. 19). However, this genotype is distinguished from both groups a_1 and a_2 in a separate group a_3 at the level of 94% homology mainly due to presence of the alleles α-Est5, β-Est2 and β-Est7. Thus, FLIP 03-B7C (No. 12) could strongly be the ancestor of FLIP 04-18C genotype (No. 19). On the other hand, these data revealed that, specific alleles are only observed in the foreign gene pool. The 2 alleles α-Est3 and β-Est4 are recorded in FLIP 04-4C (No.18); α-Est6 in FLIP B8-85C and 93-93C (No. 24 and 25, respectively); Acph2 in FLIP 03-B7C and 04-18C (No. 12 and 19, respectively), that all are originating from Syria.

The genotypes FLIP B8-85C and 04-38C (No. 24 and 23, respectively) are distinguished as the groups b_2 and b_3, respectively due to the fact that, both genotypes have the protein band with Mr of 64.12 kDa, that differentiate them from other genotypes, instead of another band with Mr of 64.86 kDa. These data provide the evidence that FLIP 04-38C (No. 24) could be the parent of the genotype FLIP B8-85C (No. 23). At the same time, FLIP 04-38C (No. 23) is characterized by presence of the band with Mr 36.20 kDa instead of another with Mr 36.60 kDa and absence of band with Mr of 102.0 kDa. The genotype FLIP 04-4C (No. 18) is distinguished from all other examined genotypes (Figure 1, group C) due to presence of the protein band with Mr of 42.90 kDa instead of that with Mr of 42.10 kDa. Thus, such protein subunit can be used for discrimination of this genotype. The relevant zymogram revealed that, the FLIP 04-4C (No. 18) genotype is characterized from all the examined genotype by each of the alleles α-Est3 and Ao2 that are existing instead of α-Est2 and Ao1, respectively; absence of α-Est4 and presence of β-Est4. Hence, the isozyme data reinforces that of SDS-PAGE regarding the discrimination of FLIP 04-4C genotype. It can be concluded that, these data confirms the importance of using more than one parameter of protein markers in examination the relationships and discrimination of the genotypes.

In conclusion, present findings agreed with the previous studies based on molecular assays in that: there is a close relationship between chickpea from different geographical regions and all of them must have evolved through the same maternal lineage. SDS-PAGE technique resolves only a small portion of genetic variation, which can be refers to the low sample size. As the genetic diversity is distributed mainly among accessions, more populations or accessions should be investigated to ensure the retention of allelic and genotypic diversity for the gene pool. The observed relatively higher degree of polymorphism based on isozyme data of this study than those in other reports appears to be due to more diverse material, which belonged to a different chickpea germplasm. Application of more than one type of markers on the same populations is further recommended in examination of the genetic relationships and discrimination of the genotypes.

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