Pak. J. Bot., 46(6): 2113-2120, 2014.

SSR MARKERS LINKED TO SEED SIZE AND SEED WEIGHT IN LOCAL AND EXOTIC CHICKPEA GERMPLASM REPORTED FROM PAKISTAN

ZAKIA AHMAD[1], MOHAMMAD NISAR[1*], ABDUL SAMAD MUMTAZ[2], ABDUL GHAFOOR[3] AND SHAHID ALI[4]

[1]*Department of Botany, University of Malakand, Chakdara Dir (Lower), Khyber Pakhtunkhwa Pakistan*
[2]*Department of Plant Sciences Quaid-i-Azam University, Islamabad, Pakistan*
[3]*Resources Institute (PGRI), National Agricultural Research Center, Islamabad, Pakistan*
[4]*Center for Biotechnology and Microbiology, University of Swat, Odigram Swat, Pakistan*
**Corresponding author e-mail: mnshaalpk@yahoo.com /shahidaligenedoctor@gmail.com*

Abstract

In advance research studies the PCR based molecular markers have a great contribution in genome analysis and marker-assisted selection. In the present study, we have utilized RAPD and SSR markers for linkage analysis with chickpea yield contributing traits. For that purpose, we were obtained 70 chickpea (*Cicer arietinum* L) accessions from Plant Genetic Resource Program (PGRP), National Agriculture Research Centre, Islamabad, Pakistan. It was found that 100 seed weight was highly significantly correlated with seed size quantitatively. For linkage analysis, 20 RAPD and 30 SSR makers were utilized. In the makers, 5 RAPD and 15 SSR were polymorphic and showed significant levels of coefficient of variation. None of the RAPD primer was linked to seed size and seed weight while, SSR markers TA72 and TA130 showed linkage and sorted chickpea accession on the basis of seed weight and seed size. The majority of accessions of USA origin was observed with maximum100 seed weight (30-57gm) and medium to large (7.2-9.9mm) size seeds including one of the accession 2562 of Pakistani origin also with large size seeds. Hence, the linkage of these makers and the use of large and medium size seeds may enhance the yield of chickpea plant in Pakistan.

Key words: Chickpea, Seed weight, Seed size, RAPD and SSR markers, Linkage analysis.

Introduction

In Pakistan the legumes are considered the best option to support large population food requirements after animal proteins (Akbar *et al.,* 2011). Chickpea (*Cicer arietinum* L.) is a significant sustenance legume of the temperate and semi-arid tropics used by the people all over the world due to its high nutritious value. The production of chickpea however, is not up to the actual demand of the present day densely populated regions like Pakistan because no true work has been observed so far to improve the yield due to an extensive slit existing between its actual yield and potentiality (Pankaj, *et al*., 2001 and Sharif, 2004). Therefore, it is worthwhile to evaluate different growth parameters in order to increase the yield of a crop.

Seed size having a direct relationship with seed weight in chickpea is one of the important growth parameters to consider for increasing the yield (Narayanan *et al*., 1981; Vadivelu & Ramakrishnan, 1983; Dahiya *et al*., 1985; Upadhyaya *et al*., 2006). The genetical studies and understanding of the pattern of inheritance of traits in crop plants are required to develop seed of a specific size to meet market demand (Hossain, 2010). There is a great variation existing in seed size of chickpea Desi and Kabuli types, but sometimes Kabuli types appeared as small as the size found in Desi type and the latter has attained a larger size of Kabuli type (Kumar & Singh 1995). The earlier workers include Sharma *et al*., (1969); Sandhu & Singh, (1972); Gupta *et al*., (1972); Katiyar *et al*., (1970) and Wadud & Yaqoob, (1989) reported that grain yield has a positive relationship with 100 seed weight.

The grain yield and many related traits correlation coefficient showed a direct and indirect relationship among these traits, hence on the basis that the breeder can select the most effective traits to release varieties (Ulukan *et al*., 2003, Yucel *et al*., 2006). In most of the previous research the linkage of the two valuable quantitative traits (100 seed weight and seed size) was examined in the chickpea F2 generation and recombinant inbred line (RIL) populations developed for QTL analysis using the molecular markers for future marker-assisted breeding strategies (Hossain, 2010). It is difficult and time consuming procedure to select and identify the yield contributing components by developing certain lines. In addition, for selection of better yield producing parents, marker assisted selection (MAS) is considered now more useful tool than conventional breeding methods to improve the production rate of chickpea plant. Marker assisted selection is most effectively used as an alternate method to replace the traditional breeding programs (Allahverdipoor *et al.,* 2011). In advance research studies the PCR based molecular markers have a great contribution in genome analysis and marker-assisted selection (Datta *et al.,* 2010). There are many studies where PCR based marker RAPD has been opted for assessment of genetic diversity and germination patterns (Pervaiz *et al.,* 2010; Mumtaz *et al.,* 2010; Jan *et al.,* 2011; Shinwari *et al.,* 2011; Akbar *et al.,* 2011) Recently, the technology of molecular marker has been greatly developed for plant breeding. In this way the SSR techniques can be used for direct selection of desirable traits, when linked them with traits of interest (Edwards & Mogg, 2001). The present study is proposed to check the level of genetic correlation of seed size and seed weight and it was further hypothesized that, is there any sort of linkage of the quantitative traits (yield contributing traits) with the molecular markers: RAPD and SSR markers.

Material and Methods

Plant material: A total of 70 local and exotic accessions obtained from Plant Genetic Resource Program (PGRP), National Agriculture Research Centre, Islamabad, Pakistan. Recommended agricultural practices were carried out till harvest. The plants were grown in the field of Malakand University in Randomized Complete Block Design (RCBD) in replicate for the purpose to achieve pure lines. The plants were harvested in 2011-2012.

Categories: The accessions were categorized into three classes: (i) small (ii) medium (iii) and large based on the length and width of each seed and the size was measured by the procedure defined by Ahirwar, 2012. The data obtained in centimeter (cm) was then converted into millimeter (mm) for valid comparisons. The samples of seeds were categorized into different size ranges from 3 to 9.9 mm (Table 1). The mean values of 100 seed weight, however were taken from already scored data and the correlation coefficient was estimated by following Ahmad *et al.,* 2012.

Polymerase chain reaction: For PCR reaction, DNA was extracted from 70 accessions using Kang *et al.,* 1998 described a protocol. The DNA was quantified with the help of spectrophotometer (Manning, 1991). For RAPD analysis the PCR reaction was optimized with initial denaturation temperature 94ºC for 3 minutes, annealing 34-40 ºC for 1 minute and final extension temperature 72ºC for 5-7-minutes. While, for SSR markers the PCR conditions were the same with exception of annealing temperature, which was 50-56 for 1 minute. Data of both RAPD and SSR markers were scored and the binary data matrixes were developed. The matrix was subjected for statistical analysis, i.e., Standard deviation, t-test and correlation coefficient by statistical software "STATISTICA version 6" (Nisar *et al*., 2008). For linkage analysis the matrix was analyzed using cluster analysis and Two-way Joining Tree (McCune & Grace, 2002).

Results

Correlation: The correlation study was carried out among 100 seed weight, seed size and unique SSR locus 1 and 2. It was found that 100 seed weight was highly significantly correlated with seed size; unique locus 1 and 2, at 0.50, 0.64 and 0.69 levels respectively. Furthermore, seed size was also highly significantly correlated with both unique loci at 0.596 and 0.615 levels, respectively with p≥0. 001 (Table 2).

Frequency distribution: Out of 70 accessions, 40% were having seed size ranged 3-4 mm and categorized into small seed size germplasm. While 44% were ranged from 4.2–7.2 mm and 16% ranged from 8-9.9 mm seed length, they were categorized into medium and large size respectively. The cumulative frequency was calculated, which showed that 84% were small and medium sized ranged from 4.2–7.2 mm in length (Table 3: Fig. 1). It is evident from table 4 that medium and large size chickpea accessions have attained maximum 100 seed weight, ranged from 29.26-57.18gm.

Genetic diversity: The accessions were tested through 20 RAPD and 30 SSR markers for estimation of genetic diversity in the collected lines based on loci presence and absence. Out of which 5 RAPD and 15 SSR markers were polymorphic, while the remaining markers did not consider for further analysis due to their poor amplification, reproducibility and monomorphic nature. Among RAPD markers, UBC 733b, UBC 181 showed 89.80 and 77.43% allele polymorphism respectively (Table 5). While among the SSR markers CaSTMS15, CaSTMS2, TA194 and TA71 indicated 97.88%, 82.24%, 71.19% and 70.46% allele polymorphism respectively regarding genetic variability among the accessions (Table 6).

Table 1. A seed size distribution based on length and width of chickpea accessions.

S.#	Seed size (mm)	Seed class Distribution	Representative accessions	Country of Origin
1.	3- 4	Small	1898, 1936, 1998, 2023, 2188, 2237, 2272, 2273, 2278, 2532, 2544	Pakistan
			2595, 2611, 2616, 2629, 2650, 2831, 3011, 3015, 3016 3033, 3035, 3041, 3057	USA
2.	4.2- 7.2	Medium	1995, 2234, 2235, 2236, 2430, 2441, 2473, 2497, 2499, 2531, 2553	Pakistan
			2654, 2819, 2859, 3017, 3020, 3021, 3023, 3024, 3027, 3031, 3032, 3037, 3039, 3040, 3042, 3044, 3045, 3046, 3058, 3059, 3062, 3063, 3065, 3066	USA
3.	8 ≥ 9.9	Large	2558, 2562	Pakistan
			2855, 3022, 3026, 3043, 3047, 3054, 3056, 3061 3064	USA

Table 2. Correlation of 100 seed weight, seed size and SSR loci in chickpea70 accessions.

Traits	100 seed weight	Seed size
100 seed weight	1.000	0.504**
Seed size	0.504**	1.000
Unique SSR –locus1	0.644**	0.596**
Unique SSR -locus 2	0.69**	0.615**

**p≥0.001 denoted the correlation is highly significant

Table 3. The frequencies and cumulative frequencies of seed size categories in chickpea 70 accessions.

S. No.	Seed size distribution	Frequency (%)	Cumulative frequency	Ranged
1.	Small	40	40	3-4 mm
2.	Medium	44	84**	4.2-7.2 mm
3.	Large	16	100	8-9.9 mm

Table 4. The accessions of medium and larger size with maximum 100-seed weight.

S. No.	Accession No.	Origin	Mean/100 seed weight	Seed size (mm)	Category
1.	3056	USA	57.18	9.9	Large
2.	3037	USA	56.66	4.2	Medium
3.	3054	USA	53.16	9.9	Large
4.	3043	USA	46.19	9.9	Large
5.	3040	USA	44.27	4.2	Medium
6.	3065	USA	39.37	7.2	Medium
7.	3027	USA	37.65	4.2	Medium
8.	3026	USA	37.63	9.9	Large
9.	3063	USA	37.11	7.2	Medium
10.	3059	USA	35.51	7.2	Medium
11.	3058	USA	32.53	7.2	Medium
12.	3021	USA	31.78	7.2	Medium
13.	3023	USA	31.72	7.2	Medium
14.	2654	USA	31.15	4.2	Medium
15.	3020	USA	31.12	7.2	Medium
16.	3047	USA	30.92	8	Large
17.	3064	USA	30.70	8	Large
18.	2562	Pakistan	30.33	8	Large
19.	3022	USA	29.81	8	Large
20.	3061	USA	29.26	9.9	Large

Table 5. Sequences of the RAPD primers used in the present study for molecular analysis of chickpea germplasm.

S. No.	Primer	Sequence	Bands	Mean	Std. Dev.	*t*-value	Coef. Var.
1.	UBC 733b	GGGAAGGGAG	17	1.11	1.00	9.32	89.80**
2.	UBC 181	ATGACGACGG	17	1.26	0.97	10.81	77.43**
3.	OPA4	AATCGGGCTG	9	1.77	0.64	23.12	36.18
4.	OPA9	GGGTAACGCC	6	1.86	0.52	29.95	27.94
5.	OPG13	CTCTCCGCCA	16	1.94	0.34	48.44	17.27

St. Dev. = Standard deviation, Coef. Vr. = Coefficient of variation

Linkage analysis of SSR markers: The observed results with regard to linkage analysis of 20 RAPD and 30 SSR markers revealed that only SSR markers TA72 and TA130 were linked with 100 seed weight and seed size (Figs. 2, 3). To check the linkage distances of the accessions with respect to the linked SSR markers, the banding profile of each utilized maker was scored. The presence of alleles (bands) was scored as "1" and absence of allele noted as "0". For estimation of linkage distance the scored data was put in binary data matrix to develop dendrogram based on Un-weighted Pairs Group Mean Average (UPGMA). Based on genetic distance with respect to genetic disagreement, 70 accessions were grouped into two lineages, L- I and L- II at a linkage distance 0.6. While at a linkage distance 0.4 the lineage-I comprised of cluster-1, based on the presence and absence of alleles, enclosed category 2 (Medium) and category 3 (large) seeds with presence of both the alleles for seed weight and size. Cluster-2 of the same lineage included the seeds of small size represented by category 1 indicated the allele for seed size only. On the other hand cluster- 3 of lineage- II enclosed medium (category-2) and large (category-3) size accessions with presence of allele for seed weight only. Similarly cluster- 4 of lineage- II, grouped medium, large and small categories of seeds which did not score any allele for 100 seed weight and seed size (Fig. 4). The linkage distance of the accession was retested through Cross validated interpretation of two- way clustering, which showed the same pattern of linkage (Fig. 5).

Table 6. Sequences of the SSR primers used in the present study for molecular analysis of chickpea germplasm.

S. No.	Name	Sequences forward/Reverse	Bands	M.Wt (bp)	*t*-value	Coef. Vr.
1.	CaSTMS2	ATTTTACTTTACTACTTTTTTCCTTTC AATAAATGGAGTGTAAATTTCATGTA	2	114/110	10.173	82.24**
2.	CaSTMS15	CTTGTGAATTCATATTTACTTATAGAT ATCCGTAATTTAAGGTAGGTTAAAATA	1	159	8.547	97.88**
3.	CaSTMS21	CTACAGTCTTTTGTTCTTCTAGCTT ATATTTTTTAAGAGGCTTTTGGTAG	1	60	12.689	65.94**
4.	TA72	GAAAGATTTAAAAGATTTTCCACGTTA TTAGAAGCATATTGTTGGGATAAGAGT	1	198	39.256	21.31
5.	TA130	TCTTTCTTTGCTTCCAATGT GTAAATCCCACGAGAAATCAA	1	219	13.134	63.7**
6.	TA194	TTTTTGGCTTATTAGACTGACTT TTGCCATAAAATACAAAATCC	2-3	204/190	4.887	71.19**
7.	TA71	CGATTTAACACAAAACACAAA CCTATCCATTGTCATCTCGT	1	202	11.874	70.46**
8.	TA22	TCTCCAACCCTTTAGATTGA TCGTGTTTACTGAATGTGGA	1	228	18.262	45.81
9.	TA200	TTTCTCCTCTACTATTATGATCACCAG TTGAGAGGGTTAGAACTCATTATGTTT	1	296	19.238	43.49
10.	TA46	TTTATTGCAATAAAACTCATTTCTTATC TTCTTTTTGTGTGAAAAAAAAATATAGTA	1	239	16.613	50.36**
11.	TA135	TGGTTGGAAATTGATGTTTT GTGGTGTGAGCATAATTCAA	1	192	19.238	43.49
12.	TR1	CGTATGATTTTGCCGTCTAT ACCTCAAGTTCTCCGAAGT	1	224	13.134	63.7**
13.	TR7	GCATTATTCACCATTTGGAT TGTGATAATTTTCTAAGTGTTTT	1	204	23.125	36.18
14.	TR29	GCCCACTGAAAAATAAAAAG ATTTGAACCTCAAGTTCTCG	2	220/270	39.256	21.31
15.	TR31	CTTAATCGCACATTTACTCTAAAATCA ATCCATTAAAACACGGTTACCTATAA	1	217	15.906	52.6**

M. Wt. = Molecular weight maker, Coef. Vr. = Coefficient of variation

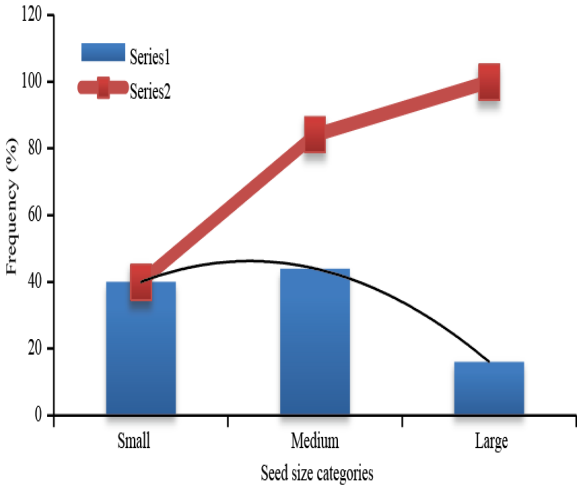

Fig. 1. A comparative graph between frequency (%) and cumulative frequency of seed size grouping in chickpea local and exotic accessions. Series-1= frequency (%), series-2= cumulative frequency (%).

Discussion

Chickpea crop is recently organized by number of prodigious disorders, including multiple disease, stress and other environmental stresses which directly affecting upon the yield (Jenkins, 2011). The day to day increasing population made chickpeas as foremost wealth and important food legume for people because of its higher nutritional and medicinal value can be used for future food security in the country. Therefore, it is imperative to adopt suitable strategies to develop high yielding varieties which are the main objective of the breeders (Singh & Auckland, 1975; Byth *et al*., 1980; Lal & Tomer, 1980). The significant correlation in yield contributing traits has been always helpful in the establishment of trait improvement. In correlation studies 100 seed weight was found positively highly significantly correlated with grain yield (Ahmad *et al*., 2012). Seed weight a valuable quantitative trait was also proposed as an accurate measure of chickpea seed size (Upadhyaya *et al*., 2006). Therefore, to produce seed of an ideal size, the yield may also be improved (Hossain, 2010). In present investigation 100 seed weight was found a stable trait and positively highly significantly correlated with seed

size, also reported by Bicer, 2009. It is evident from the study that medium and large size chickpea accessions have conquered maximum 100 seed weight, ranged from 30-57gm. Thus the use of large and medium size seeds may enhance the yield. Similar findings were obtained Stougaard & Xue, 2005 and Royo *et al.,* 2006, in wheat by estimating 18% increase in yield while using seeds of larger size and 16% decrease with the use of small size seeds. Among the total evaluated germplasm a high (44%) frequency (%) was recorded for medium size chickpea lines followed by smaller and larger type of seeds. Similarly the cumulative frequency of the large size accessions was intended to calculate as 100%, such as (%) however indicating an excellent future trend in chickpea yield enhancement.

In linkage analysis of 20 RAPD and 30 SSR markers, only SSR markers TA72 and TA130 have shown linkage with 100 seed weight and seed size respectively. In addition, among RAPD markers UBC181 and UBC733b have shown 89.80% and 77.43% allele polymorphism respectively, which was calculated 97.88%, 82.24%, 71.19% and 70.46% using SSR markers CaSTMS15, CaSTMS2, TA194 and TA71 respectively. Thus the significant coefficient of variation found among the accessions based on loci presence and absence determined stability by measuring genetic diversity in chickpea accessions.

The dendrogram however constructed based on UPGMA percent disagreement characterized by the presence and absence of specified alleles using TA72 and TA130 resulted in groups of 70 accessions into four clusters. This grouping was based either on the presence of loci for both the selected traits (100 seed weight and seed size) or for a single trait only (100 seed weight or seed size). In addition, cluster-1 was examined the group of medium and large size seeds with the presence of both loci for allele 1 and 2, indicated that the accessions with high (%) of seed weight have also shown larger seed size irrespective of Desi or Kabuli type. Similar markers were also reported by Hossain, 2010, who determined that the inheritance of 100 seed weight was not influenced by any environmental factor and the small sized seed character is due to maternal effect which prohibited the full expression of larger seed size. These results were accorded with the findings of Rastogi, 1979; Malhotra *et al*., 1997 and Kumar & Singh, 1995. While Niknezad *et al*., 1971 observed larger seed size, dominant over small size.

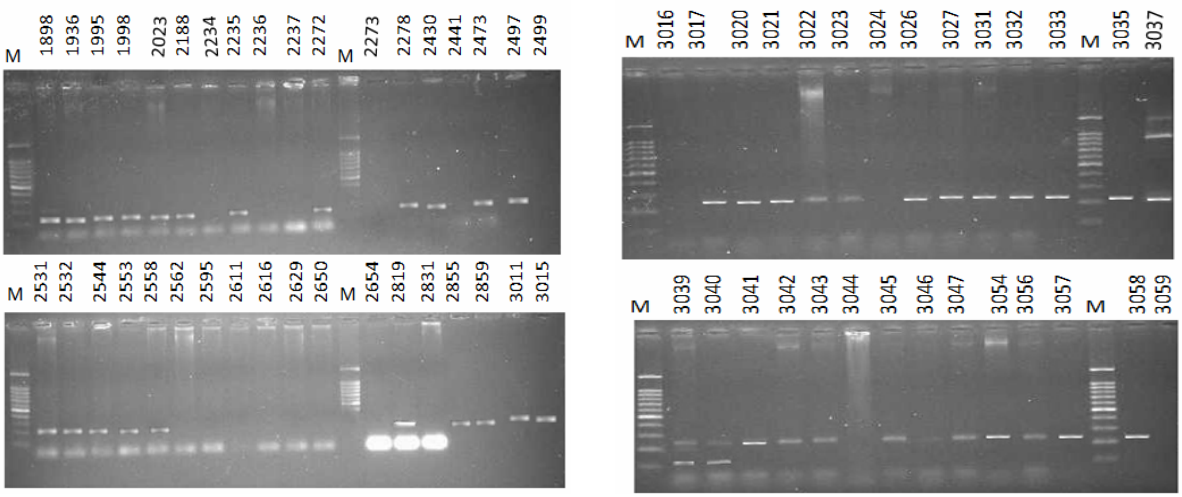

Fig. 2. SSR-PCR amplification Products of chickpea local and exotic accessions using SSR primer TA72 (198bp) with 50 bp ladder.

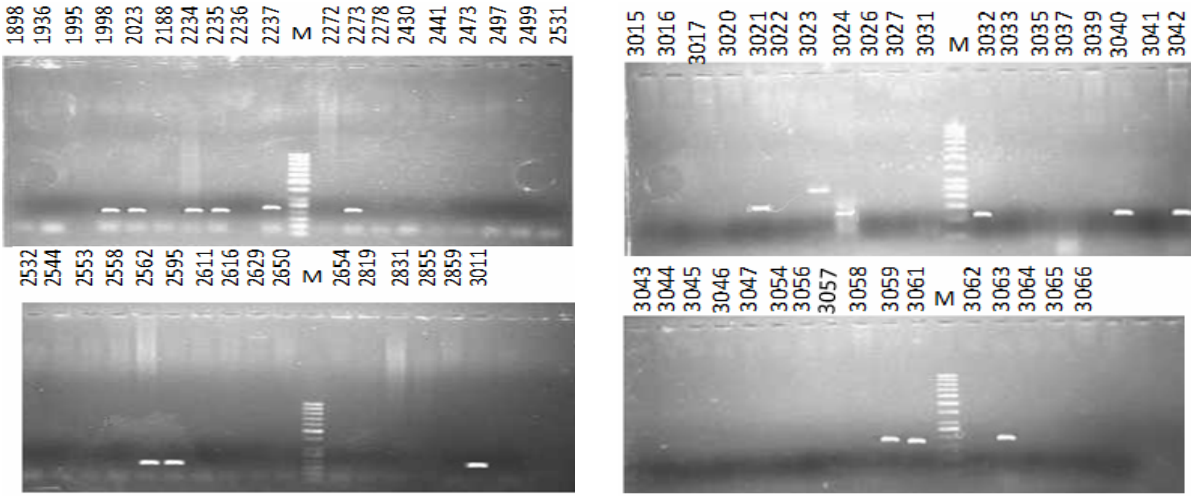

Fig. 3. SSR-PCR amplification Products of chickpea local and exotic accessions using SSR Primer TA130 (219bp) with 100bp ladder.

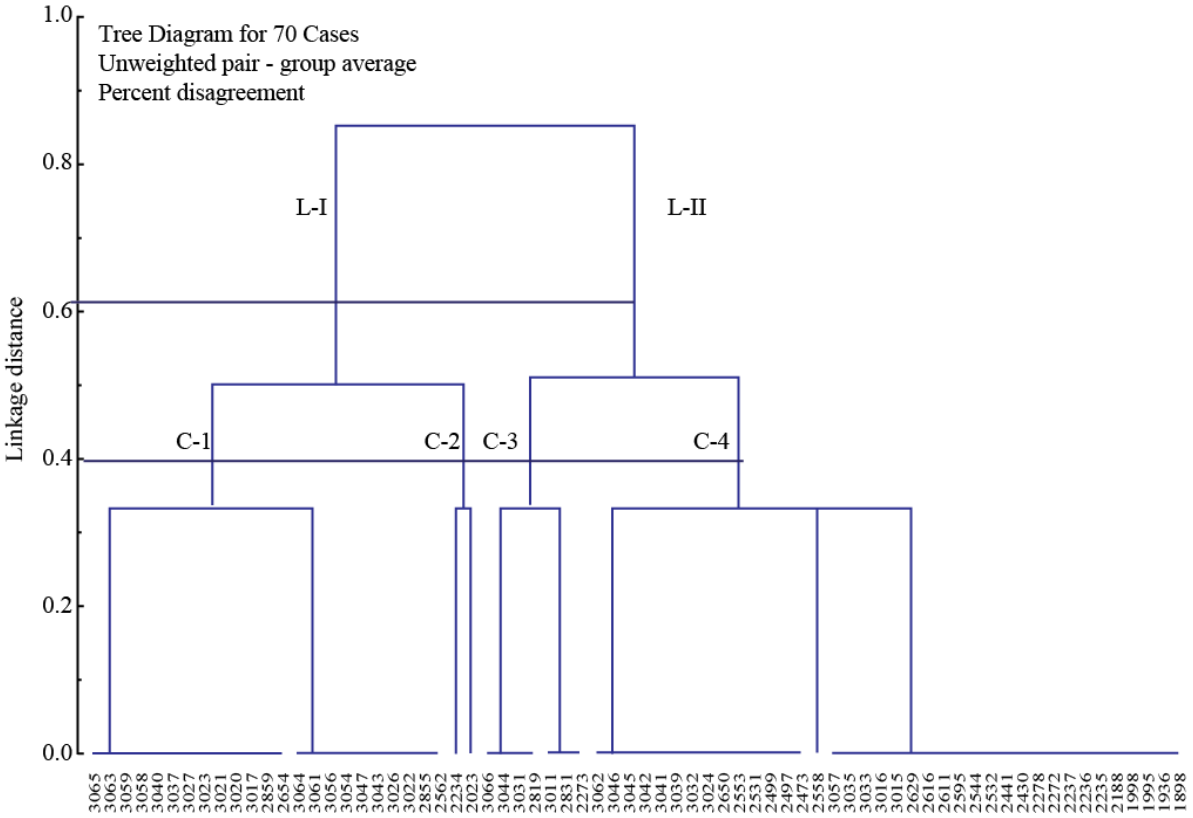

Fig. 4. Dendrogram of 70 chickpea accessions based on SSR data for 100 seed weight and seed size using UPGMA Percent disagreement.

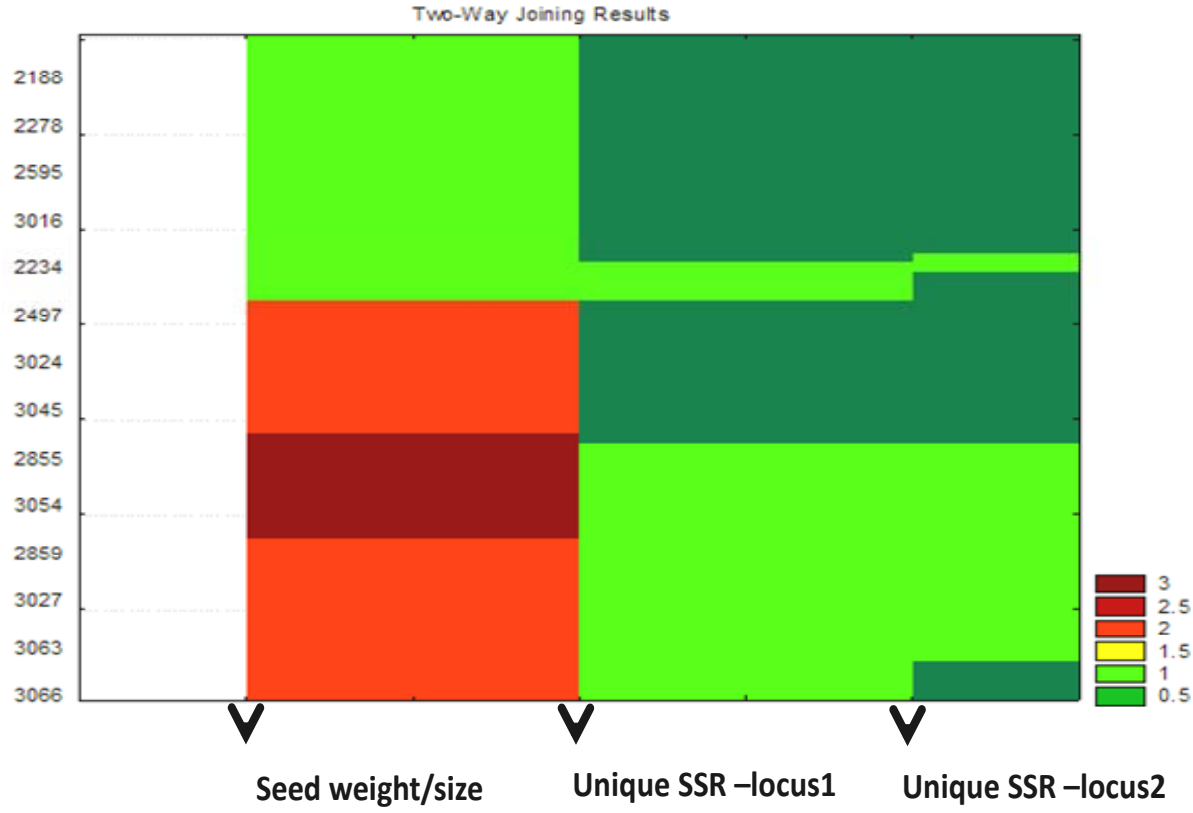

Fig. 5. Cross validated interpretation of two- way clustering in chickpea 70 accessions based on binary data matrix of SSR makers.

Conclusion

Seed weight and seed size are important growth parameters and having a direct relationship with each other because in chickpea, seeds of quality, size ranged from medium (7.2 mm) to large (8-9.9 mm) irrespective of Desi or Kabuli type have given comparatively better and higher yield in the evaluated germplasm. Thus the use of molecular markers in linkage analysis of yield contributing quantitative traits may provide a better chance to isolate medium to large size chickpea seeds to improve the production rate of the crop.

References

Ahirwar, J.R. 2012. Effect of seed size and weight on seed germination of Alangium lamarckii, Akola, India. *Res. J. Recent. Sci.* Vol. 1(ISC-2011): 320-322.

Ahmad, Z., A.S. Mumtaz, M. Nisar and N. Khan. 2012. Diversity analysis of chickpea (*Cicer arietinum* L.) germplasm and its implications for conservation and crop breeding. *J. Agric. Sci*., 3(5): 723-731.

Akbar, F., M.A. Rabbani, M.S. Masood and Z.K. Shinwari. 2011. Genetic diversity of sesame (*Sesamum indicum* L.) germplasm from Pakistan using RAPD markers. *Pak. J. Bot*., 43(4): 2153-2160.

Akbar, N., M. Iqbal, Ehsanullah, A. Iqbal and H.Z. Khan. 2011. Agro- qualitative response of two cultivars of chickpea to different sowing techniques under irrigated conditions of Faisalabad. *Crop & Environment*, 2(1): 19-23.

Allahverdipoor, K.H., B. Bahramnejad and J. Amini. 2011. Selection of molecular markers associated with resistance to *Fusarium* wilt disease in chickpea (*Cicer arietinum* L.) using multivariate statistical techniques. *AJCS*., 5(13): 1801-1809.

Bicer, B.T. 2009. The effect of seed size on yield and yield components of chickpea and lentil. *African Journal of Biotechnology,* 8(8): 1482-1487.

Byth, B.E., J.M. Green and G.C. Hawtin. 1980. ICRISAT/ ICARDA Chickpea Breeding Strategies. pp. 11-27 in Proceedings of the *Internationa* India. *Workshop on chickpea improvement,* 28 Feb-2Mar.1979. ICRISAT, Hyderabad, India.

Dahiya, B.S., I.S. Solanki and R. Kumar, 1985. Germination rate and its genetics in chickpea. *Intern. Chickpea Newslett*. 13: 6-8.

Datta, J., L. Nand, K. Mayank and P.G. Prasoon. 2010. Efficiency of three PCR based marker systems for detecting DNA polymorphism in *Cicer arietinum* L. and *Cajanus cajan* L. *Millspaugh. Genet. Eng. Biotech. J*., vol-5.

Edward, K.J. and R. Mogg. 2001. Plant genotyping by analysis of single plants nucleotide polymorphisms. In Plant genotyping- the DNA fingerprinting of Edited by R. Henry. CAB. *International, Wallingford, UK*. pp. 1-3.

Gupta, S., K.K. Ghosal and V.N. Gadgil. 1972. Haploid tissue culture of *Triticum aestivum* var. *Sonalika* and *Pisum sativum* var. B22. *Indian Agriculturist.* 16(3): 277-278.

Hossain. 2010. Inheritance of seed size in chickpea (*Cicer arietinum* L.) and identification of QTL based on 100-seed weight and seed size index. *Aust. crop Sciences*.

Jan H.U., M.A. Rabbani and Z.K. Shinwari. 2011 Assessment of genetic diversity of indigenous turmeric (*Curcuma longa* L.) germplasm from Pakistan using RAPD markers. *Journal of Medicinal Plants Research*, 5(5): 823-830.

Jenkins, L., K. Moore, G. Cumming, R. Gill and J. Thelander. 2011. Chickpea planting seed quality issues. *Australian Government Grains Research and Development Corporation*.

Kang, H.W., Y.G. Cho, U.H. Yoon and M.Y. Eun. 1998. A rapid DNA extraction method for RFLP and PCR analysis from a single dry seed. *Plant-Molecular Biology Reporter*, 16(1): p. 1-9

Katiyar, R.C., S.K. Ranjhan and K.S. Shuklam. 1970. Yield and nutritive value of *Clitoria ternatea* - a wild perennial legume for sheep. *Ind. J. Dairy Sci*., 23(2):79-81.

Kumar, S. and O. Singh. 1995. Inheritance of seed size in chickpea. *J. Genet. & Breed*., 49: 9104.

Lal, S. and Y.S. Tomer. 1980. Chickpea breeding programme at Hisar Proceedings of the International Workshop on Chickpea Improvement, Hyderabad, India. pp: 208-220, ICRISAT, Patancheru, India.

Malhotra, R.S., G. Bejiga and K.B. Singh. 1997. Inheritance of seed size in chickpea. *J. Genet. & Breed.,* 51: 45-50.

Manning, K. 1991. Isolation of nucleic acid from plants by differential solvent precipitation. *Analytical Biochemistry*, 195: 45-50.

McCune, B. and J.B. Grace. 2002. Analysis of ecological communities. Mjm Software Design, leneden Beach, OR, US.

Mumtaz, S.A.; M. Naveed and Z.K. Shinwari. 2010. Assessment of genetic diversity and germination pattern in selected cotton genotypes of Pakistan. *Pak. J. Bot*., 42(6): 3949-3956.

Narayanan, A., N.P. Saxena, A.R. Sheldrake. 1981. Varietal differences in seed size and seedling growth of pigeonpea and chickpea. *Indian- J. Agric. Sci*., 51: 389-393.

Niknezad, M., M. Khosh-Khur and S.R. Ghorashy. 1971. Inheritance of seed size in chickpea (*Cicer arietinum* L.). *Crop Sci*., 11: 768-769.

Nisar, M., A. Ghafoor, H. Ahmad, M.R. Khan, A.S. Qureshi, H. Ali and M. Aslam. 2008. Evaluation of genetic diversity of pea germplasm through phenotypic trait analysis. *Pak. J. Bot*., 40(5): 2081-2086.

Pankaj, K., P.S. Deshmukh, S.R. Kushwaha and Sunita-Kumari. 2001. Indian Society of Agricultural Science. Annals of Agricultural Research. pp. Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi, 110-112, India.

Pervaiz, Z.H., M.A. Rabbani, Z.K. Shinwari, M.S. Masood and S.A. Malik. 2010 Assessment of genetic variability in rice (*Oryza sativa* L.) germplasm from Pakistan using RAPD markers. *Pak. J. Bot*., 42(5): 3369-3376.

Rastogi, K.B. 1979. Genetic analysis of seed size in chickpea. *Indian-J. Agric. Sci*., 49: 42-44.

Royo, C., A. Ramdani, M. Moragues and D. Villegas. 2006. Durum wheat under Mediterranean conditions as affected by seed size. *J. Agron. Crop Sci*., 192: 257-266. (SCI).

Sandhu, T.S. and N.B. Singh, 1972. Correlation, path coefficient analysis and discriminant function selection in gram. *J. Res. Punjab. Agric. Uni. Ludhiana.* 9: 417-421.

Sharif, M. 2004. Wheat yield gap analysis: Future options for Pakistan: A report submit in partial fulfillment of the requirement for intership programme in Agricultural Economics B.Sc. (Hons.) Agriculture.

Sharma, A.K., R.R. Tiwari and A.S. Tiwari. 1969. Studies on Genetic and Environmental correlation in Gram. *Ind. J. Sci.,* 3(1): 34-46.

Singh, K.B. and A.K. Auckland. 1975. Chickpea breeding at ICRISAT Pages 3- 17 in Proceedings of a Workshop on Grain Legumes, ICRISAT, Hyderabad, India.

Shinwari, Z.K., S. Sultan and T. Mehmood. 2011. Molecular and morphological characterization of selected *Mentha* species. *Pak. J. Bot*., 43(3): 1433-1436.

Stougaard, R.N. and Q. Xue. 2005. Quality versus quantity: spring wheat seed size and seeding rate effects on *Avena fatua* interference, economic returns and economic thresholds. *Weed Res*., 45: 351-360. (SCI).

Ulukan, H. and S. Guler Keskin. 2003. A path coefficient analysis of yield and yield components in Faba bean (*Vicia faba* L.) genotypes. *Pak. J. Biol. Sci.,* 6: 1951-1955.

Upadhyaya, H.D., S. Kumar, C.L.L. Gowda and S. Singh. 2006. Two major genes for seed size in chickpea (*Cicer arietinum* L.) *Euphytica*., 147: 311-315.

Vadivelu, K.K. and V. Ramakrishna. 1983. Effect of seed size on quality attributes and yield of seeds in Bengal gram (*Cicer arietinum* L.). *Seed Res.,* 11: 177-181.

Wadud, A. and M. Yaqoob, 1989. Regression and correlation analysis in different cultivars of chickpea (*Cicer arietinum* L.) *Sarhad- J. Agric*., 5(2): 171-176.

Yucel, D.O., A.E. Anlarsal and C. Yucel. 2006. Genetic variability, correlation, and path analysis of yield and yield components in chickpea (*Cicer arietinum* L.). *Turkish J. Agric. & Forestry*, 30:183-188.

(Received for publication 15 July 2013)