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Simple sequence repeat markers associated with agro-morphological traits in chickpea (*Cicer arietinum* L.)

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Abstract

Chickpea (*Cicer arietinum* L.), a cool season grain legume, serves as an important cheap source of protein and energy in developing countries, and plays an important role in the enrichment of soil fertility. In order to identify simple sequence repeat (SSR) markers associated with agro-morphological traits in chickpea, 44 genotypes comprising cultigen, landraces, internationally developed improved lines and wild relatives, were phenotyped for several agro-morphological traits in a randomized complete block design with three replications at Urmia Rainfed Research Station. High genetic variability was observed among the chickpea genotypes for the studied agro-morphological traits. In molecular experiment, genetic variability among genotypes was assessed by using SSR markers. One hundred alleles were generated at 16 SSR loci, with a mean number of alleles per locus of 6.25. Using general linear model (GLM) and applying multiple testing corrections, 10 SSR loci associated with the genes controlling the studied agro-morphological traits were identified. Some identified markers were associated with more than one trait. The identified markers could be of great interest in marker assisted selection (MAS) in chickpea breeding programmes.

Key words: association mapping, *Cicer arietinum*, false positive, genetic diversity, molecular markers.

Introduction

Chickpea as a third major cool season grain legume in the world has an important role in poor people's diet. The cultivated chickpea (*Cicer arietinum* L.) is grown on 11 million hectares with 9.77 million ton production (http://faostat.fao.org). The world average yield (0.88 t ha^{-1}) is far below potential seed yield of about 5 t ha^{-1} (Ahmad et al., 2005) due to complex field environment with its heterogeneous conditions and global climatic changes (Mittler, Blumwald, 2010), lack of well adapted cultivars for autumn or winter sowing, poor farm management and susceptibility to several biotic and abiotic stresses.

Ascochyta blight, caused by the fungus *Ascochyta rabiei*, is a damaging disease of chickpeas in Iran (Kanouni et al., 2010). The fungus has the potential to attack all above ground parts of the plant which may cause 100% yield loss (Rajesh, Muehlbauer, 2008) and is most prevalent in the areas where cool, cloudy and humid weather occurs during the crop season (Kanouni et al., 2010). Developing ascochyta blight resistant varieties is the most economically and environmentally sound means of controlling the disease.

Cold stress generally affects chickpea growth and development. Low temperature has been considered as a primary limiting factor in most regions and has been therefore the focus of most research works. Chickpea is the least cold tolerant crop among the cool-season food legumes. The severity of cold stress depends on the rate of cooling, minimal temperature and its duration. There is significant variability for cold tolerance in chickpea germplasms at early growth stage (Saeed et al., 2010).

Molecular breeding has revolutionized conventional breeding techniques in all areas within the last twenty years. It has played a significant role in chickpea breeding programs by integrating marker assisted selection (MAS) within empirical selection (Chaturvedi, Nadarajan, 2010). As a prerequisite, identifying tightly linked markers is important for the successful deployment of molecular marker technology within a breeding programme. Molecular markers linked with quantitative trait loci (QTL)/major genes for traits of interest are being routinely identified in many crops by utilizing genetic linkage map developed on the second filial generation (F2) or recombinant inbred lines (RILs) populations. Genetic linkage maps have been developed for chickpea in different research works (Jain, Chattopadhyay, 2010; Gaur et al., 2011), and QTLs

controlling different traits such as double podding and other morphological traits (Gaur et al., 2011), as well as drought tolerance (Jain, Chattopadhyay, 2010) have been identified. Identification of markers associated with important traits in a group of genotypes through association mapping offers an alternative means, as has been used in several plants species (Vijayan et al., 2005; Roy et al., 2006; Darvishzadeh et al., 2008). Roy et al. (2006) by using the phenotypic data of 55 elite wheat genotypes for 14 different agronomic traits and molecular data produced by 20 simple sequence repeat (SSR), 2 selectively amplified microsatellite polymorphic loci (SAMPL) and 8 amplified fragment length polymorphism (AFLP) primer combinations (in total 519 deoxyribonucleic acid (DNA)-based molecular markers), reported some molecular markers associated with traits via simple or multiple linear regression analysis. In their study, a total of 131 SSR, 43 SAMPL and 166 AFLP markers showed significant associations with at least one of the 14 studied traits. Darvishzadeh et al. (2008) by using multiple linear regression analysis identified putative AFLP markers associated with partial resistance to phoma black stem in 60 sunflower mutant lines. Vijayan et al. (2005) genotyped 44 mulberry genotypes with inter-simple sequence repeat (ISSR) markers and identified putative markers associated with leaf yield attributing traits. The potential of association mapping to identify and characterize loci/genes associated with different complex traits is highly affected by admixture of populations (Zhang et al., 2012). Therefore, knowledge on the population structure is a prerequisite in association mapping and can be used to avoid identifying false positive correlations between markers and traits (Pritchard et al., 2000; Pritchard, Donnelly, 2001).

The objectives of the present study were to characterize the population structure within chickpea genotypes and to identify SSR markers associated with agro-morphological traits in chickpea using association mapping by general linear model (GLM) procedure.

Materials and methods

Plant material and phenotypic data. A set of 44 genotypes of chickpea out of 225 genotypes (Saeed et al., 2010) comprising cultigen, landraces, internationally developed improved lines and wild relatives (Table 1), were planted in a randomized complete block design with three replications at Urmia Rainfed Research Station. The accessions were planted in two rows with 4 meters length and 30 centimetres width on 12 October, 2009. Traits including number of stems (NS), plant height (PH), cold tolerance (CT), days from the first effective raining after sowing to 50% flowering (DF), days from the first effective raining after sowing to 90% maturity (DM), susceptibility to ascochyta blight disease (SABD), 100-seed weight (100 SW) and seed yield (YLD) were recorded (Table 1). For the visual screening of cold tolerance, we used a scale of 1–9, where 1 – tolerant, no visible symptoms of damage; 3 – tolerant, slight foliar damage (11–20% leaflets show withering) and up to 20% branches show withering and drying, no plant killing; 5 – intermediate, 41–60% leaflets and 21–40% branches show withering and drying, up to 5% plant killing; 7 – susceptible, 81–99% leaflets and 61–90% branches show withering and drying, 26–50% plant killing; and 9 – highly susceptible, 100% plant killing (International Center for Agricultural Research in the Dry Areas (ICARDA) manual, 2008). Pathological tests were performed by using the method described by Kanouni et al. (2010).

Table 1. Mean and standard error values of studied agro-morphological characteristics in 44 genotypes of chickpea comprising cultigen, landraces, internationally developed improved lines and wild relatives, evaluated in a randomized complete block design with three replications at Urmia Rainfed Research Station

No.	Genotype	Origin	Pedigree	Type	Sub group[b]	NS		DF (days)		DM (days)		SABD		CT		PH cm		100 SW g		YLD kg ha^{-1}	
						$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
1	ILC533	Egypt	not traced	Kabuli	mixed	2	1.00	185	0.33	225	1.53	7	1.33	9	0.59	52	6.11	16.1	4.38	145.0	37.48
2	ILC 3279	Former USSR	landrace	Kabuli	red	2	0.00	184	0.67	226	1.53	2	0.33	8	0.00	51	0.88	28.2	0.09	728.0	141.91
3	ILC 8262	Spain	selection from ILC 3470	Kabuli	red	4	0.67	182	0.67	220	3.33	2	0.33	3	0.00	51	2.19	26.4	0.41	4190.4	1322.33
4	ILC 8617	ICARDA	ILC482 (mutation)	Kabuli	mixed	3	0.33	180	0.33	225	1.20	2	0.33	3	0.00	44	2.19	27.3	0.49	842.0	186.25
5	ILWC 81	Turkey	*C. reticulatum* IG	wild	red	5	0.33	174	4.00	220	2.96	2	2.19	2	0.33	46	8.41	15.0	5.72	266.0	62.80
6	ILWC 106	Turkey	*C. echinuspermum* IG	wild	red	6	0.88	179	2.52	220	2.96	2	0.33	2	0.33	31	1.00	19.5	1.96	251.8	43.44
7	ILWC 139	Turkey	*C. judaicum* IG	wild	mixed	7	1.00	172	4.67	220	2.96	2	0.67	2	0.33	18	4.48	18.2	2.01	197.6	33.72
8	ILWC 181	Turkey	*C. reticulatum* IG	wild	mixed	3	0.33	179	0.33	220	3.33	2	0.58	2	0.33	23	0.00	15.2	3.66	31.8	20.88
9	ILWC 235	Turkey	*C. echinuspermum* IG	wild	red	5	0.33	176	3.93	220	3.18	2	0.88	2	0.33	16	5.78	19.5	1.53	583.0	160.32
10	Shahindezh	Iran	landrace	Desi	red	3	0.00	176	0.58	221	0.88	3	0.00	8	0.00	38	0.33	18.2	0.64	622.0	148.35
11	Germezi Nukhud	Iran	landrace	Desi	red	3	0.00	175	0.33	220	1.15	3	0.00	8	0.00	42	1.33	19.5	0.99	590.2	121.09
12	Mahabad	Iran	landrace	Desi	red	3	0.00	176	1.15	220	1.20	3	0.00	4	0.00	44	2.40	18.3	0.72	691.2	170.64
13	Kaka	Iran	not traced	Desi	red	4	0.33	186	3.33	220	1.67	2	0.67	4	0.33	64	8.84	12.0	1.58	110.8	29.69

Table 1 continued

No.	Genotype	Origin	Pedigree	Type	Sub group[b]	NS		DF (days)		DM (days)		SABD		CT		PH cm		100 SW g		YLD kg ha^{-1}	
						$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE
14	Piruz	Iran	not traced	Desi	green	4	0.33	173	0.88	220	1.86	2	0.67	3	0.33	37	1.53	17.4	4.86	514.8	99.87
15	Ghazvin	Iran	landrace	Kabuli	green	4	0.33	172	0.88	222	0.58	2	1.00	6	0.00	56	4.84	35.8	0.29	2411.0	693.61
16	X 96TH3 K3	ICARDA	FLIP 91-150C × FLIP 93-93C	Kabuli	green	3	0.00	181	2.91	221	16.04	2	0.67	5	0.00	67	6.57	32.7	0.46	2830.4	852.97
17	X 95TH5 K10	ICARDA	FLIP 91-149C × FLIP 93-194C	Kabuli	mixed	3	0.33	176	3.38	220	2.67	2	0.33	6	0.00	60	1.73	31.9	1.62	2970.4	696.41
18	Sel 93TH2 4416	ICARDA	ILC482 × NEWC 36	Desi	mixed	3	0.67	169	4.37	220	1.67	6	1.45	5	0.00	39	6.74	25.5	2.08	1975.0	461.79
19	Sel 93TH2 4477	ICARDA	ILC3470 × ILC 8617	Desi	pink	4	0.33	170	4.37	220	2.00	6	1.20	4	0.00	53	5.17	27.0	0.89	1784.4	413.45
20	Sel 95TH1 716	ICARDA	ILC482 × ILWC182	Desi	pink	3	0.33	170	3.51	220	1.76	5	0.67	3	0.00	48	6.00	27.3	0.78	3123.4	745.54
21	Sel 95TH1 744	ICARDA	ILC482 × NEWC 36	Desi	pink	4	0.67	169	3.84	220	1.53	8	1.20	5	0.33	31	2.67	28.2	1.05	528.2	77.75
22	Sel 95TH1 745	ICARDA	ILC482 × NEWC 36	Desi	pink	4	0.33	168	4.37	220	1.76	8	1.15	3	0.33	39	4.41	27.3	1.79	615.4	167.21
23	Sel 96TH1 1403	ICARDA	ILC482 × NEWC 36	Desi	blue	4	0.58	168	4.37	220	1.86	8	1.76	2	0.33	30	6.49	26.4	0.91	314.0	20.11
24	Sel 96TH1 1404	ICARDA	ILC482 × NEWC 36	Desi	blue	4	0.33	168	4.33	220	1.67	8	1.20	3	0.33	36	2.89	26.6	0.36	302.2	57.04
25	Sel 96TH1 1406	ICARDA	ILC482 × NEWC 36	Desi	blue	4	0.00	170	5.03	220	2.40	8	1.76	3	0.33	35	5.03	28.7	1.45	802.4	193.57
26	Sel 96TH1 1439	ICARDA	ILC482 × ILWC 182	Desi	mixed	4	0.00	168	4.00	225	1.53	4	0.58	2	0.33	52	6.77	27.3	0.12	1932.8	414.53
27	Sel 96TH1 1484	ICARDA	ILC482 × NEWC 36	Desi	yellow	4	0.33	170	7.02	220	3.51	8	1.45	3	0.33	30	5.03	26.5	2.38	663.8	176.68
28	Sel 96TH1 1485	ICARDA	ILC482 × NEWC 36	Desi	yellow	4	0.33	172	5.03	220	2.96	7	0.67	2	0.33	34	2.52	26.2	2.91	945.0	218.51
29	Sel 96TH1 1488	ICARDA	ILC482 × NEWC 36	Desi	yellow	3	0.33	172	6.77	220	3.51	7	0.58	5	0.33	31	4.48	24.5	3.80	302.6	60.88
30	Sel 98TH1 744	ICARDA	ILC482 × NEWC 36	Desi	mixed	3	0.33	172	4.18	220	2.19	7	0.58	3	0.33	35	2.60	30.1	1.65	1234.4	331.10
31	Sel 98TH1 1518	ICARDA	not traced	Desi	yellow	4	0.00	173	4.04	220	2.00	7	0.33	3	0.33	36	5.81	26.4	2.42	1130.6	301.70
32	Azad	Iran	FLIP85-122C/ FLIP82-150C// FLIP 86-77C	Kabuli	mixed	3	0.33	176	4.04	224	1.76	2	0.33	5	0.33	53	2.03	29.7	0.24	2480.8	407.61
33	Flip 93-261C	ICARDA	X90TH566/ FLIP87-69C × LWC219	Kabuli	mixed	3	0.33	175	4.67	222	2.67	6	0.33	5	0.33	53	4.58	26.5	0.21	961.4	244.24
34	Flip 98-258C	ICARDA	not traced	Kabuli	aqua	3	0.00	184	1.53	225	0.00	2	0.33	6	0.00	53	1.20	34.4	1.06	2464.4	735.73
35	Flip 99-34C	ICARDA	X96TH8/ FLIP91-150C × FLIP91-105C	Kabuli	aqua	3	0.00	188	0.33	224	1.53	2	0.33	8	0.00	50	3.38	33.4	0.51	2496.6	1073.60
36	Flip 99-66C	ICARDA	X96TH61/(F91-159XILC1278) × F91-149C	Kabuli	mixed	3	0.00	174	1.33	224	0.58	2	0.00	4	0.00	42	2.40	32.4	0.10	966.0	209.61
37	Flip 01-06C	ICARDA	X98TH23-1-BH-20/ILC1799 × FLIP92-148	Kabuli	aqua	4	0.33	178	0.67	224	0.58	2	0.33	8	0.00	58	4.91	28.3	1.07	1987.6	563.67
38	Flip 03-46C	ICARDA	X99TH 13/ FLIP93-62C × FLIP93-50C	Kabuli	aqua	3	0.00	188	1.33	226	1.76	2	0.33	8	0.00	65	4.48	25.6	0.00	797.4	179.66
39	Flip 03-112C	ICARDA	X00TH51/ FLIP 98-52C × FLIP 98-47C	Kabuli	aqua	3	0.00	178	1.76	224	0.58	2	0.33	5	0.00	54	1.00	29.1	0.00	893.6	227.56
40	× 2001TH 45	ICARDA	S 99093 × S 98006	Kabuli	aqua	3	0.00	178	0.00	228	0.33	6	0.00	5	0.00	48	0.33	35.6	0.91	1084.0	278.19
41	× 2003TH 21	ICARDA	(S 00791 × FLIP 98-23C) × ICCV2	Kabuli	aqua	3	0.00	172	4.67	225	0.58	2	1.33	8	0.00	62	3.18	32.7	0.43	3086.2	938.44
42	× 2003TH 164	ICARDA	FLIP 98-50C × FLIP 97-90C	Kabuli	mixed	3	0.00	184	0.00	225	0.00	2	1.33	7	0.00	60	2.40	30.4	0.61	846.0	195.44
43	*C. oxyodon* L.-4[a]	Iran	*C. oxyodon* L.	wild	green	1	0.00	169	0.33	220	0.00	2	0.00	2	0.00	76	1.73	–		–	
44	*C. oxyodon* L.-9[a]	Iran	*C. oxyodon* L.	wild	green	1	0.00	169	0.33	220	0.00	2	0.00	2		78	1.86	–		–	

NS – number of stems, DF – days from the first effective raining after sowing to 50% flowering, DM – days from the first effective raining after sowing to 90% maturity, SABD – susceptibility to ascochyta blight disease, CT – cold tolerance, PH – plant height, 100 SW – 100-seed weight, YLD – seed yield; [a] – accession number, [b] – see Figure 3

Molecular experiments. Genomic DNA was extracted from the young leaf tissues of 2-week-old seedlings using the modified method described by Dellaporta et al. (1983). Nineteen polymorphic SSRs were used for genotyping, from the available composite collections of International Crops Research Institute for the Semi-Arid-Tropics (ICRISAT) and International Center for Agricultural Research in the Dry Areas (ICARDA) (Varshney et al., 2007). Among them, 12 tri-nucleotide repeat motifs markers of 113–496 bp allele size with annealing temperature of 56–59°C (Winter et al., 1999), and 4 di-nucleotide repeat motifs markers of 201–464 bp allele size with annealing temperature of 56°C (Sethy et al., 2003) showed polymorphism. The selected markers used in the current study were single locus, co-dominant microsatellite markers whose usefulness in chickpea has already been reported (Udupa et al., 1999; Pandian et al., 2000; Cingilli et al., 2005; Varshney et al., 2007). Polymerase chain reaction (PCR) were performed in a volume of 20 µl containing 2.5 µM of each SSR primer, 0.4U of Taq DNA polymerase ("Life Technologies", USA), 100 µM of each dNTP ("Promega", France), 2 µl (10×) PCR buffer, 2 mM $MgCl_2$ ("Promega", France), ddH_2O and 25 ng template DNA, using a 96-well "Eppendorf Mastercycler Gradient" (Type 5331, Eppendorf AG, Germany). Amplification was carried for 35 cycles, each consisting of a denaturation step at 94ºC for 1 min, annealing at 54–59ºC (depending on the primers sequence) for 1 min and an extension step at 72ºC for 1.5 min. An initial denaturation step at 94ºC for 3 min, and a final extension step of 10 min at 72ºC were also included. The reaction products were mixed with an equal volume of formamide dye (98% formamide, 10 mM EDTA (ethylenediaminetetraacetic acid), bromophenol blue and xylene cyanol) and resolved in 3% agarose gel (0.5 × TBE buffer (tris-borate-EDTA)), stained with ethidium bromide (1 µg ml^{-1}) and photographed under UV (ultraviolet) light. Compared to polyacryl-amide-gel electrophoresis, or automated analysis, agarose-gel electrophoresis is the most-appropriate and safe technology for routine analyses of these types of markers.

Statistical analysis. The SSR data were scored as codominant markers in order to distinguish homozygotes and heterozygotes for each locus. Population structure was analysed using a model-based Bayesian approach in the software package *Structure 2.3.4* (Pritchard et al., 2000). Five independent runs were performed, setting the number of sub populations (K) from 1 to 10, burn in time and Markov chain Monte Carlo (MCMC) replication number both to 100,000, and a model for admixture and correlated allele frequencies. The K value was determined by Ln P(D): the log probability of data in the *Structure* output and ΔK: an ad hoc quantity based on the second order rate of change of the likelihood function with respect to K (Evanno et al., 2005). The appropriate value of K was determined when the estimate of Ln P(D) reached a minimum stable value. Inferred ancestry estimates of individuals (Q-matrix) were derived for the selected subpopulation (Pritchard et al., 2000). Association mapping was performed to analyse marker-trait association by structured association approach using ancestry coefficient (Q values) estimates as covariate in a general linear model (GLM) function using *TASSEL 2.1*. Multiple testing corrections were performed by adjusting maker probability values for multiple tests run by a permutation test in the *TASSEL 2.1* software.

Results

Analysis of variance revealed significant differences among genotypes for the studied traits (Table 2). The coefficient of variation (CV) ranged from 2.22 to 89.13. However, for the majority of traits the values were less than 20% (Table 2).

Table 2. Mean of square and some statistical parameters for 44 genotypes of chickpea comprising cultigen, landraces, internationally developed improved lines and wild relatives

Source of variation	NS	DF (days)	DM (days)	CT	SABD	PH cm	100 SW g	YLD kg ha^{-1}
Block	1.1031**	626.58**	267.96**	2.06**	5.58 ns	708.93**	95.82**	12057874.70**
Genotype	1.23**	42.38**	31.89 ns	1.67**	6.39**	364.27**	77.32**	661609.50**
Mean	3.38	179.80	224.60	1.87	3.88	40.57	26.79	637.97
CV	19.58	2.48	2.22	19.75	39.09	15.60	11.92	89.13
Max	5.00	189.30	229.00	6.00	6.70	57.00	35.70	1663.30
Min	2.00	173.60	207.00	1.30	1.30	22.30	15.00	71.00
SE	±0.07	±0.54	±0.49	±0.07	±0.17	±1.11	±0.51	±70.26

NS – number of stems, DF – days from the first effective raining after sowing to 50% flowering, DM – days from the first effective raining after sowing to 90% maturity, CT – cold tolerance, SABD – susceptibility to ascochyta blight disease, PH – plant height, 100 SW – 100-seed weight, YLD – seed yield; CV – coefficient of variation, SE – standard error

Mean data revealed high range for most of the studied traits (Table 1). Number of stems per plant ranged from 1 to 7, days from the first effective raining after sowing to 50% flowering – from 168 to 188 days, SABD – from 2 (partial resistance) to 8 (highly susceptible), cold tolerance – from 2 (tolerant) to 9 (highly susceptible), plant height – from 16 to 78 cm, 100-seed weight – from 12 to 35.8 g, seed yield – from 31.8 to 4190.4 kg ha^{-1} (Table 1).

Genetic diversity among 44 chickpea genotypes was assessed by using 19 microsatellite (SSR) loci (Fig. 1). Three loci were dropped due to missing data (>5%). A total number of 100 alleles were detected by 16 SSR primers with an average of 6.25 alleles per locus.

Detailed information on the levels of genetic diversity of genotypes is available elsewhere (Saeed et al., 2011). Analysis of population structure distinguished 6 subspecific populations (Fig. 1).

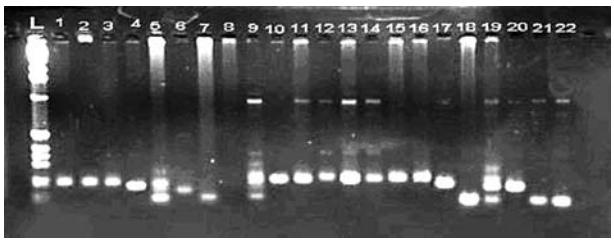
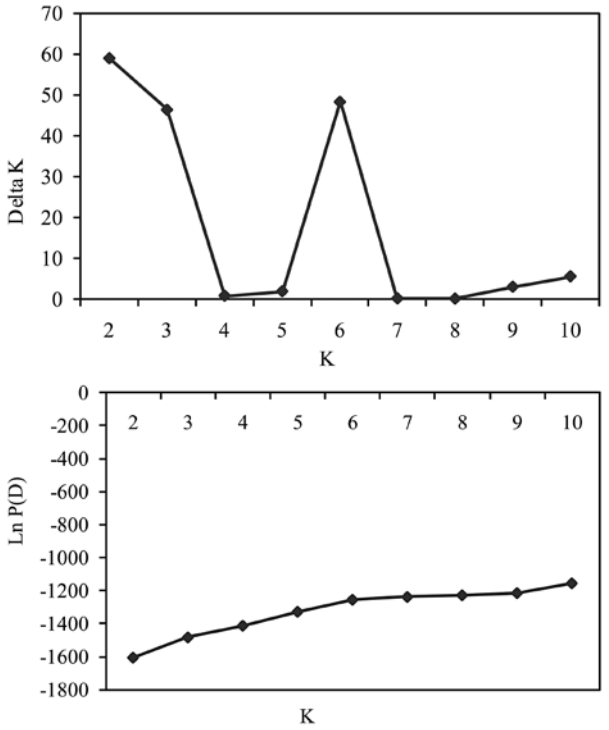


Figure 1. Polymorphism detected by simple sequence repeat (SSR) primer Ta 135; L – 1 kb molecular ladder (genotype codes see in Table 1)

The six subgroups matched rather to the six germplasm groups, landraces, wild relatives, improved lines (Sel 96TH: Desi type), improved lines (Sel 96TH: Kabuli type), improved lines (Flip$_s$) (Figs 2 and 3). Of all genotypes, 70.46% were assigned into the corresponding subgroups, and the remaining ones were categorized into the mixed subgroups based on their Q values (Table 1 and Fig. 3).

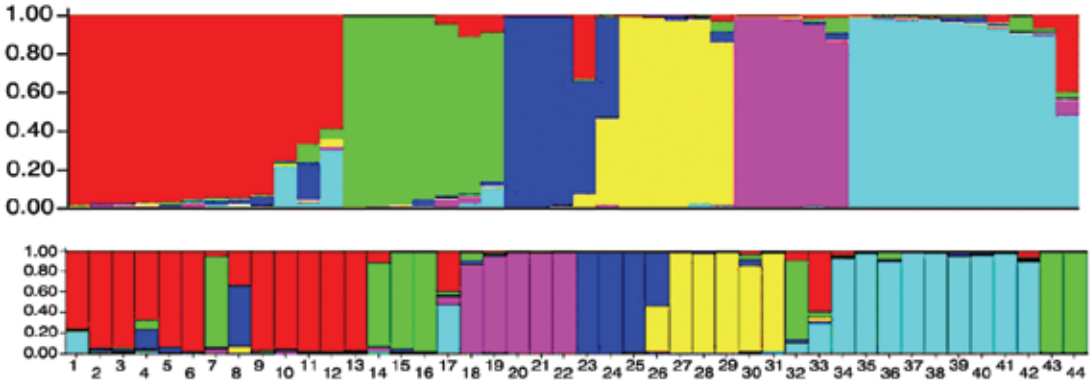
By applying GLM procedure and multiple testing corrections in the *TASSEL 2.1* software, 3, 2, 1, 3, and 1 SSR loci were identified to be associated with genes controlling stem number, days from first effective raining after sowing to 90% maturity, 100-seed weight, plant height, and cold tolerance, respectively (Table 3). None of identified markers for yield and partial resistance to ascochyta blight disease passed the stringent bar of statistical significance, and all *P*-values were greater (Table 3). The phenotypic variance explained by loci (R^2) ranged from 8% to 75% (Table 3).

Among the identified SSR loci, two SSR loci were common whereas the others were specific. The SSR locus TS84 was common for the majority of traits including stem number, days from first effective raining after sowing to 90% maturity, 100-seed weight, plant height, and cold tolerance. The phenotypic variance explained by this marker (R^2) in different traits ranged from 31% to 68%. The locus Ta142 was common for stem number and plant height.



Note. Ln P(D) is the log likelihood of the observed genotype distribution in k clusters and is an output by *Structure* simulation, ΔK – an ad hoc quantity based on the second order rate of change of the likelihood function with respect to *K*.

Figure 2. Bilateral charts to determine the optimal number of K identified by *Structure* program



Notes. Numbers on the y-axis indicate the membership coefficient. The colour of the bar indicates the six groups identified through the *Structure* program (red – landraces, green – wild relatives, blue and yellow – improved lines (Sel 96TH1: Desi type), pink – improved lines (Sel 96TH1: Kabuli type), aqua – improved lines (Flip$_s$). Accessions with the same colour belong to the same subgroup.

Figure 3. Genetic relatedness of 44 genotypes of chickpea comprising cultigen, landraces, internationally developed improved lines and wild relatives with 16 simple sequence repeats (SSRs) as analysed by the *Structure* program

Table 3. Single sequence repeats (SSR) loci identified for studied agro-morphological traits in chickpea using association mapping by general linear model (GLM) procedure

Trait	Locus	*F*-marker[a]	*P*-marker[b]	*P*-adj marker[c]	R^2
NS	Ta118	3.3918	0.009	0.064 ns	0.4809
NS	Ta135	6.3815	0.000	0.001*	0.7494
NS	Ta142	9.0165	0.000	0.001*	0.5652
NS	Tr43	2.8113	0.019	0.251 ns	0.5002
NS	TS84	11.6027	0.000	0.001*	0.68
DM	NCP6	2.8917	0.017	0.309 ns	0.2852
DM	NCP7	7.5115	0.010	0.028*	0.0822
DM	Ta135	2.6583	0.028	0.443 ns	0.2921
DM	Tr43	2.5068	0.032	0.595 ns	0.2334
DM	TS84	4.2344	0.003	0.026*	0.3132
SABD	NCP19	3.6472	0.038	0.444 ns	0.0652
CT	Taa58	2.8947	0.029	0.401 ns	0.2566
CT	Tr43	3.3142	0.007	0.081 ns	0.3292
CT	TS84	4.3087	0.002	0.010*	0.3719
PH	Ta118	2.3423	0.048	0.732 ns	0.3114
PH	Ta142	4.7652	0.003	0.015*	0.3397
PH	Tr43	4.2329	0.002	0.007*	0.4345
PH	TS84	4.1408	0.003	0.036*	0.4234
100 SW	NCP6	2.3848	0.041	0.692 ns	0.289
100 SW	Tr29	2.7239	0.041	0.543 ns	0.1618
100 SW	Tr43	3.3994	0.007	0.074 ns	0.2748
100 SW	TS84	6.1875	0.000	0.001*	0.4126
YLD	NCP6	2.8295	0.019	0.292 ns	0.4951
YLD	Tr7	2.6011	0.091	0.945 ns	0.1952
YLD	TS84	3.1343	0.014	0.134 ns	0.5262

Notes. NS – number of stems, DM – days from the first effective raining after sowing to 90% maturity, SABD – susceptibility to ascochyta blight disease, CT – cold tolerance, PH – plant height, 100 SW – 100-seed weight, YLD – seed yield; [a, b] – *F*-statistics and *p*-values for the requested *F*-tests, [c] – the marker *p*-value adjusted for multiple tests (Ge et al., 2003); R^2 – the portion of total variation explained by the marker but not by the other terms in the model.

Discussion

High genetic variability was observed among the chickpea genotypes for the studied agro-morphological traits. High genetic variability observed among advanced breeding lines indicates the efforts underway in ICARDA to widen the genetic base of chickpea for various traits. A higher variation for a character in the breeding materials correlates with a greater ability for its improvement through selection. The CV for the majority of traits was less than 20%. In general, CV value higher than 20% is considered to be high; however, it may be possible to ignore the high CV values when F test is significant and this item is found in several published research works (Okwuagwu et al., 2008; Kandic et al., 2009). The inconsistent CV values reported in many studies might be due to physio-genetic characteristics and degree of compatibility of the plant material, low number of sampled individuals per genotype in plot, low number of replications per genotype and/or variable environments in which the trial was carried out (Okwuagwu et al., 2008).

In this study wild *Cicer oxyodon* accessions showed cold tolerance and high partial resistance against ascochyta blight disease. These results are in agreement with the findings of Toker (2005) who observed high cold tolerance in wild relatives of *Cicer* such as *C. reticulatum*. Wild relatives of *Cicer* species (Singh et al., 2008) and landraces are a promising source of genes for stress tolerance. Inter-specific hybridization has opened up the possibility of transferring genes from wild to cultivated species (Singh, Ocampo, 1997). The present study used 16 pairs of SSR primers and subdivided the association population into six subpopulations using the model-based cluster method. Data from our genetic diversity (Saeed et al., 2011) and population structure analysis revealed that this association population showed a diverse genetic variation and, therefore, could be used for the association analysis. Among factors influencing marker-traits association, the admixture of populations is very important (Zhang et al., 2012). Many models were used to minimize the false-positive of association analysis produced from the admixture of populations. It has been showed that a K matrix incorporated into the GLM was sufficient to minimize false-positive associations (Zhang et al., 2012). Using GLM method several loci associated with genes controlling traits were identified. A great challenge in association mapping via GLM method is instability of the experiment-wise Type I error. When multiple hypothesis tests are performed, the experiment-wise Type I error is quickly increased, which results in false positives. To reduce the probability of false positives, multiple testing corrections were used for controlling experiment-wise Type I error. Multiple corrections test was used to control experiment-wise Type I error in several association mapping studies such as Vanniarajan et al. (2012). Cho et al. (2002) by constructing an intraspecific genetic linkage map on F_{10} population via 55 sequence-tagged microsatellite

sites (STMS), 20 random amplified polymorphic DNAs (RAPDs), 3 intersimple sequence repeats (ISSR) and 2 phenotypic markers, determined the map positions of genes controlling days to 50% flowering, double podding, seed number per plant and 100-seed weight traits in chickpea. Sharma et al. (2004) identified the STMS and sequence-tagged site (STS) markers closely linked to resistance gene against *Fusarium oxysporum* f. sp. *ciceris* race 3 in chickpea by QTL mapping.

Identification of molecular markers using unrelated germplasm collections instead of utilizing materials derived from planned crosses such as F2, RIL, doubled haploid (DH) populations has increased in most of the laboratories in recent years (Roy et al., 2006; Haji-Allahverdipoor et al., 2011; Vanniarajan et al., 2012; Zhang et al., 2012).This approach could identify markers with pleiotropic effects indicating that population wide analysis served as an effective tool in deciphering marker-trait associations. Detection of molecular markers influencing various traits could increase the efficiency of marker-assisted selection and enhance genetic progress.

Conclusion

The present study offers an approach for identifying a set of deoxyribonucleic acid (DNA) markers projecting significant association with genes controlling agro-morphological traits in chickpea. Using general linear model (GLM) and applying multiple testing corrections, several simple sequence repeat (SSR) markers were identified. Some identified markers associate with more than one trait. Detection of molecular markers associated with genes controlling different traits could increase the efficiency of marker-assisted selection (MAS) in chickpea breeding programs.

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Sėjamojo avinžirnio (*Cicer arietinum* L.) paprastųjų pasikartojančių sekų žymeklių ir agromorfologinių požymių asociacijų analizė

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Santrauka

Sėjamasis avinžirnis (*Cicer arietinum* L.), šaltojo sezono pupinis augalas, besivystančiose šalyse yra svarbus ir pigus baltymų bei energijos šaltinis, dirvožemį papildantis maisto medžiagomis. Siekiant identifikuoti paprastųjų pasikartojančių sekų (PPS) žymeklius, susijusius su sėjamojo žirnio agromorfologiniais požymiais, buvo tirta 44 genotipų kolekcija, sudaryta iš kultigeno, vietinių veislių, pagerintų selekcinių linijų ir laukinių ekotipų. Tyrimas atliktas Urmia tyrimų stotyje lietinimo sąlygomis, taikant atsitiktine tvarka išdėstyto viso bloko schemą, trimis pakartojimais. Nustatyta didelė sėjamojo avinžirnio agromorfologinių požymių genetinė įvairovė. Genotipų genetinė įvairovė nustatyta molekulinio tyrimo metu, naudojant PPS žymeklius. Šešiolikoje PPS lokusų nustatyta 100 alelių, vidutiniškai 6,25 alelio viename lokuse. Taikant bendrąjį linijinį modelį ir kartotines tyrimo korekcijas identifikuota 10 PPS lokusų, susijusių su genais, kontroliuojančiais tirtus agromorfologinius požymius. Kai kurie identifikuoti žymekliai yra susiję su daugiau nei vienu požymiu. Identifikuoti žymekliai gali būti naudingi vykdant žymekliais pagrįstą atranką avinžirnio selekcinėse programose.

Reikšminiai žodžiai: asociacijų analizė, *Cicer arietinum*, genetinė įvairovė, molekuliniai žymekliai.