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Evaluation of microsatellite-based genetic diversity, protein and mineral content in chickpea accessions grown in Kyrgyzstan

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The genetic diversity of 23 chickpea accessions representing Kyrgyz landraces and cultivars, ICARDA breeding lines, Spanish and Turkish cultivars was characterized using nine microsatellite (SSR) markers which generated a total of 122 alleles. The number of alleles (Na) per locus varied from 9 to 20. The observed heterozygosity (Ho) ranged between 0.05 and 0.43 (average 0.13) whereas both the expected heterozygosity (He) and polymorphic information content (PIC) ranged from 0.71 to 0.90 (average 0.83). Analysis of molecular variance (AMOVA) showed that 62% of the total genetic variation was found within accessions while the remaining 38% was found among accessions. Principal coordinate analysis (PCoA) indicated the presence of two groups. The two Kyrgyz cultivars were found apart from these groups. Cluster analysis generally confirmed the results of PCoA and also separated the Kyrgyz cultivars from the subcluster formed by Kyrgyz landraces and the subclusters formed by breeding lines from ICARDA along with landraces from Turkey and Spain. In addition, protein content and mineral concentration were determined. Protein content and mineral concentrations for Ca, S, Mg, P, K, Fe, Mn, Cu and Zn varied significantly among accessions. The results show that Kyrgyz germplasm provides a source of diversity for improvement of chickpea.

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Chickpea (*Cicer arietinum* L.) is a self-pollinated diploid food legume species ($2n = 2x = 16$) which belongs to the genus *Cicer* L. (Leguminosae, Cicereae). The genus includes nine annual and 35 perennial species (van der Maesen 1987). The estimated genome size of chickpea is about 740 Mb (Arumuganathan and Earle 1991). Chickpea is the third most important grain legume protein source in the world with a gross production of 10.9 million t grown on 12 million ha. It ranks third among grain legumes after soybeans (261.6 million t, 102.4 million ha) and dry beans (23.2 million t, 30 million ha). The crop is mainly cultivated in southern Asia and in the Mediterranean regions of northern Africa and southern Europe. The major chickpea producer is India with an annual production of 7.5 million t and an area of 8.2 million ha (FAO 2010).

Southeastern Turkey and Syria are regarded as the primary Vavilov's center of origin for cultivated chickpea, while the secondary center of origin of chickpea is Ethiopia (van der Maesen 1987). Cultivated chickpea includes desi and kabuli types, which are recognized by their specific morphological characteristics. Kabuli types have white flowers and large, cream-colored seeds. They are mostly grown in the Mediterranean basin and central Asia (Castro et al. 2011). Desi types are mainly grown in the east Africa and the Indian subcontinent (Khan et al. 2010). The large-seeded type reached India via the Afghan capital about two centuries ago. This 'route' explains the given name 'Kabuli' or kabuli chana (chana = chickpea) in Hindi (van der Maesen 1972).

There have been many investigations on the nutritional quality of food legumes (Iqbal et al. 2006; Sharma et al. 2013), their chemical composition and mineral element content (Ereifej et al. 2001), as well as physiochemical, cooking, instrumental textural and roasting characteristics (Kaur et al. 2005), phytic acid and mineral micronutrients (Bueckert et al. 2011), calcium concentration in chickpea seeds (Abbo et al. 2000), and evaluation of the micronutrient composition (Thavarajah and Thavarajah 2012).

Chickpea provides essential amino acids to the human diet. The seed protein of chickpea has higher nutritional value than that of other grain legumes due to its amino acid composition (Gupta and Kapoor 1980). The protein quality is also higher than in pulses such as pigeonpea, black gram (or black lentil), green gram or mungbean (Kaur et al. 2005). Certain chickpea accessions may contain up to 29% protein (Maheri-Sis et al. 2008). In addition, chickpea is a good source of minerals. Calcium and

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iron are essential nutrients but generally limited in the diet of low income farmers and are particularly important for children, and pregnant and nursing women in developing countries (Singh 1985; Samarah et al. 2010). Chickpea seeds also provide other important minerals to the human diet, e.g. magnesium, zinc, manganese, selenium and chromium (Wood and Grusak 2007; Jukanti et al. 2012).

Legume crops are grown in 49 874 ha in Kyrgyzstan (StatCom 2013). Common bean (*Phaseolus vulgaris*), soybean (*Glycine max*) and pea (*Pisum sativum*) along with chickpea are the most important grain legumes grown by Kyrgyz farmers. Common bean is cultivated as an export crop, and chickpea, soybean and pea for local use as staple food and livestock feed. Chickpea is mainly grown in the southern part of Kyrgyzstan; i.e. in the provinces of Osh, Jalal-Abad and Batken, where the climate is continental. Chickpea can also be grown in arid regions of the country.

The protein content in the human diet is insufficient in Kyrgyzstan (StatCom 2009). Food security and the nutritional status of the Kyrgyz population was improving for several years after the independence but the level of food and nutrition insecurity remains high, particularly in the poor parts of the society (Babu and Reidhead 2000). This problem remains essential since animal protein is expensive. Hence, increasing food legume production and productivity will assist the improvement of the nutritional status of the Kyrgyz people. There is, however, only a few chickpea cultivars registered in the national list and the local farmers still grow landraces.

Several molecular techniques have been used for assessment of genetic diversity in crops and all have their strengths and weaknesses (Weising et al. 2005; Nybom et al. 2014). However, DNA markers requiring known genomic sequence information (e.g. microsatellites, expressed sequence tags and single nucleotide polymorphisms) are the most valuable tools for molecular breeding as they provide highly abundant data (Crouch and Ortiz 2004). Microsatellites (also known as simple sequence repeats or SSR) are widely used in genetic diversity studies due to their high reproducibility, multi-allelic and co-dominant nature (Weising et al. 2005; Nybom et al. 2014). Microsatellite markers have been developed and used for measuring genetic diversity in chickpea (Upadhyaya et al. 2008; Sefera et al. 2011; Choudhary et al. 2012; Keneni et al. 2012).

The aim of this study was to estimate the level of neutral genetic diversity in chickpea landraces and cultivars vis-à-vis that of breeding lines from the International Center for Agricultural Research in Dry Areas (ICARDA) introduced to this country, and a few landraces grown in the Mediterranean area (Spain and Turkey) with SSR markers and to evaluate the extent of useful diversity, namely protein and mineral content in their grains.

MATERIAL AND METHODS

Plant material and DNA extraction

We investigated the genetic diversity and nutritional value of landraces and cultivars from Kyrgyzstan. In addition, accessions from Turkey, Spain and breeding lines from ICARDA were included for comparison with the Kyrgyz material. The thirteen Kyrgyz landraces were collected in 2012 from farmers' fields. Two cultivars released by Kyrgyz breeders and five ICARDA breeding lines were obtained from the Kyrgyz Livestock and Pasture Research Institute of the Kyrgyz National Agrarian University. One accession from Turkey was made available by the Seed Association of Kyrgyzstan, and two Spanish accessions were provided by the Swedish Univ. of Agricultural Sciences (SLU). In total, 23 accessions were analyzed.

Twenty seeds of each accession were planted in pots in a greenhouse in Alnarp, southern Sweden. Fresh young leaves were collected individually from 10 randomly chosen 8–12 days old plants for each accession. DNA was isolated using the CTAB method as described by Keneni et al. (2012). DNA concentration was estimated with a Nanodrop ND-1000 spectrophotometer (Saveen Werner, Sweden). DNA quality was also checked by electrophoresis in a 1.2% agarose gel containing ethidium bromide.

SSR analysis

The nine SSR markers were selected based on the level of their polymorphism. Eight markers, TA2, TA14, TA28, TA64, TA113, TA116, TA117 and TA200 were selected from the set published by Winter et al. (1999) and one marker, CaSTMS2 was selected from a set published by Hüttel et al. (1999). Forward primers of each primer pair were fluorescently labeled with FAM and HEX (Sigma-Aldrich) and used for PCR analysis. The PCR reactions were performed in a total volume of 20 µl containing 20 ng genomic DNA, 0.4 mM dNTPs, 0.3 mM forward and reverse primers, 1 mM $MgCl_2$, 0.8 units of Taq polymerase (Dream Tag, Fermentas) and $1\times$PCR buffer (Dream Taq buffer, Fermentas). The PCR was performed on a C1000 Thermal Cycler (BioRad, USA) using the following temperature profiles: initial denaturation at 95°C for 3 min, followed by 35 cycles at 94°C for 1 min, variable annealing temperatures for 1 min and 72°C for 1.5 min. Finally, extension at 72°C for 10 min. Annealing temperature was 56°C for primer TA14, 58°C for primers TA2, TA113, CaSTMS2, 60°C for primers TA64, TA116, TA117, TA200 and 62°C for primer TA28. Successful amplifications were confirmed by electrophoresis of PCR products in a 1.2% agarose gel containing ethidium bromide and fragment visualization in UV. The PCR products were multiplexed into four panels. The multiplexed PCR products were analyzed using an ABI PRISM 3730

DNA analyzer (Applied Biosystems) at Univ. of Copenhagen, Denmark.

Nitrogen (N) and protein contents

Nitrogen content analysis was carried out with the Dumas combustion method (Dumas 1831) using the automated N and C solid sample dynamic flash combustion technique with a Thermo Scientific Flash 2000 Elemental Analyzer (Thermo Fisher Scientific, UK). Seed samples from each chickpea accession were freeze-dried and ground using a mechanical grinder (IKA-WERKE, Germany). Samples were accurately weighed (3 to 6 mg $\pm$ 0.01 mg) in triplicate in tin capsules. The samples were placed in the combustion reactor by the autosampler. After combustion in the presence of oxygen, released gases were carried out by a helium flow passing through a special column that absorbed carbon dioxide and the water vapor. Thereafter the nitrogen (N) was separated in the GC column and the quantity was measured with a thermal conductivity detector (TCD). The equipment was previously calibrated with a pure compound of known N percentage content (acetanilide) to calculate the N percentage in the unknown samples. Protein content in the seeds was calculated from N percentage by multiplying with a nitrogen/protein factor (6.25) (AOAC 1990).

Mineral analysis

The freeze dried and ground samples were weighed (500 mg) in duplicate. To each sample, 10 ml of nitric acid was added for total combustion in a special microwave oven, MARS 5 (CEM Corporation, US). The samples were cooled and the volume adjusted to 100 ml with Millipore water. Two tubes with 10 to 12 ml of each sample were kept at 4°C until analysis. The analyses were made by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES; Perkin-Elmer, OPTIMA 3000 DV). These analyses were performed at the ICP Laboratory, Dept of Ecology, Lund University.

Statistical analyses

The size of amplified alleles were scored using the GeneMarker ver. 2.2.0 software (SoftGenetics, LLS, State College, Pennsylvania). Observed number of alleles (Na), observed heterozygosity (Ho) and expected heterozygosity (He) and the Shannon's information index (I) were calculated with the program POPGENE ver. 32 (Yeh and Boyle 1997). The matrix of allele frequencies generated with POPGENE was used for UPGMA cluster analysis with Rogers-W genetic distance coefficient. The cluster analysis was performed with NTSYS-pc software (Rohlf 2005). Bootstrap analyses with 1000 resamplings were conducted using Free Tree–Freeware software (Pavlicek et al. 1999). The GenAlex ver. 6.4 (Peakall and Smouse 2006) was used to perform principal coordinate analysis (PCoA) and to produce a two-dimensional plot. The analysis of molecular variance (AMOVA) was conducted to partition the genetic variation in within- and between-accession components among all chickpea accessions. Furthermore, the analysis was performed in groups, Kyrgyz landraces and ICARDA breeding lines as well as for ICARDA's breeding lines and Kyrgyz landraces separately. The AMOVA analysis was performed with Arlequin 3.5 software (Excoffier and Lischer 2010). Analyses of variance for protein and mineral content (Tukey test at $P < 0.001$) were performed with Minitab ver. 16 (Minitab Inc., State College, PA, USA).

RESULTS AND DISCUSSION

SSR-estimated genetic diversity

The nine loci used in this study were chosen based on previous information about their ability to produce unambiguously scored fragments and the level of polymorphism (Hüttel et al. 1999; Winter et al. 1999; Sefera et al. 2011; Naghavi et al. 2012). All primer pairs generated clear fragments for all 23 chickpea accessions and were polymorphic in our material (Table 1). The nine SSR markers revealed in total 122 alleles. The simplest diversity parameter is number of alleles at each locus (Kalinowski 2004). In our study, the number of alleles per locus was high and ranged from 9 (TA116) to 20 (TA64) with an average of 13.5 alleles per locus. Polymorphic information content (PIC) and Shannon's information index (I) estimate the level of informativeness of a locus. Averaged over the nine loci, PIC was 0.83 and I was 2.1 which can be considered as high. The most informative locus was TA117 (PIC = 0.9; I = 2.53), while the least informative was TA116 (PIC = 0.71; I = 1.5). All loci detected heterozygotes and observed heterozygosity (Ho) ranged from 0.05 (TA28) to 0.43 (TA113), which averaged to 0.13 over all loci.

Since we analyzed a relatively low number of loci, we selected those which previously had proven to have a high level of polymorphism. Also Sefera et al. (2011) used a set of selected highly polymorphic markers from their earlier study. Application of highly polymorphic markers may lead to a slight overestimation of the diversity compared to randomly chosen SSR loci. On the other hand, even a relatively low number of highly polymorphic markers distributed over the genome allowed efficient characterization of the chickpea germplasm in our study.

Genetic diversity estimators, revealed in our study, were generally comparable to those of other studies. In Imtiaz et al. (2008), 48 landraces, wild species, cultivars, and breeding lines were characterized with 21 SSR

Table 1. *Genetic diversity parameters of nine polymorphic SSR loci. Number of alleles (NA), observed heterozygosity (Ho), expected heterozygosity (He), Shanon's information index (I), polymorphic information content (PIC).*

SSR locus	Linkage group	Repeat motif	NA	Ho	He	I	PIC
CaSTMS2	6	$(TAT)_{25}$	14	0.12	0.80	2.01	0.80
TA2	4	$TAA_{16}TGA(TAA)_{19}$	15	0.06	0.86	2.20	0.86
TA14	6	$(TAA)_{22}$	12	0.06	0.85	2.10	0.85
TA28	7	$TAA_{37}CAA(TAA)_{30}$	13	0.05	0.87	2.19	0.86
TA64	3	$(TAA)_{39}$	20	0.10	0.83	2.31	0.83
TA113	1	$(TAA)_{26}$	11	0.43	0.86	2.12	0.86
TA116	unmapped	$(TAA)_5TT(A)_3(TAA)_{20}$	9	0.21	0.71	1.50	0.71
TA117	7	$(ATT)_{52}$	18	0.11	0.90	2.53	0.89
TA200	2	$(TTA)_{37}$	10	0.06	0.82	1.95	0.82
		Mean	13.5	0.13	0.83	2.10	0.83
		Standard deviation	3.6	0.12	0.05	0.28	0.05

markers. They found from 8 to 40 alleles per locus which averaged to 17.6, which is higher compared to our study. On the other hand, the mean PIC value was 0.82, which is comparable with our data. Udupa et al. (1999), analyzing 78 chickpea accessions with 12 SSR markers, reported a PIC value of 0.86 and an average number of alleles per locus of 14.1. The genetic diversity estimators were slightly higher than our estimates. This may be explained by differences in used plant material: the vast majority of the accessions were landraces (71), while the remaining were cultivars (5) and wild species (2).

In contrast, Keneni et al. (2012) only found 111 alleles when assessing the diversity of 155 chickpea accessions with 33 SSR markers. The average number of alleles per locus was 3.36 and the average PIC value was 0.412. This is even more surprising since 139 accessions were Ethiopian chickpeas collected from diverse production areas in this country, eight nationally released cultivars and eight introduced breeding lines from ICARDA and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).

Differences in genetic diversity levels among the studies could be explained by the use of different primers targeting different genomic regions, thus displaying different levels of polymorphism.

However, five loci used in our study, TA14, TA28, TA64, TA113 and TA116 were in common with the studies of Naghavi et al. (2012) and Sefera et al. (2011). We calculated total number of alleles, average number of alleles per locus and average PIC values for these common loci which resulted in 65, 13 and 0.82 (our study); 110, 22 and 0.84 for Naghavi et al. (2012); 57, 11.4 and 0.81 for Sefera et al. (2011). Genetic diversity estimators are also influenced by the type of analyzed germplasm and by the number of accessions analyzed. The genetic diversity residing within our 23 accessions represented by landraces, breeding lines and cultivars was lower compared to that of the extensive set of Iranian landraces, 307 in Naghavi et al. (2012) and higher compared to the set of 48 chickpea cultivars used by Sefera et al. (2011).

The analysis of genetic diversity for each separate accession is presented in Table 2. The number of alleles (Na) varied from 1.22 ('Raphat') to 4.67 (12-6KG and Orestes) with a mean value of 3.3. Expected heterozygosity (He) ranged from 0.06 ('Raphat') to 0.73 (Orestes) and averaged 0.53. The Shannon's information index (I) ranged from 0.09 ('Raphat') to 1.36 (Orestes) and averaged 0.9. Nei's gene diversity (Nei) varied from 0.06 ('Raphat') to 0.69 (Orestes) and averaged 0.5. The most diverse accessions were Orestes and 12-6KG. As expected, the Kyrgyz cultivars 'Saira' and 'Raphat' had the lowest genetic diversity values. These two cultivars are listed in the national cultivar register in Kyrgyzstan and thus it was important to confirm their homogeneity with molecular markers.

We revealed 13 private alleles, i.e. alleles which are only found in a single population among a range of populations (Szpiech and Rosenberg 2011). In this study we considered private alleles as those which were present in two or more individuals of the same population but not in any other population (Table 2). Different loci amplified different numbers of private alleles. Thus, locus CaSTMS2 amplified four, locus TA64 – three, locus TA116 – two and loci TA2, TA14, TA28 and TA117 – one private allele, respectively.

We also detected eight unique alleles (Table 2), here defined as alleles present in a single individual among all the 230 studied individuals. The unique alleles were amplified by all but two primers, TA64 and TA117. Two unique alleles were found in locus TA2, while the other loci amplified one unique allele each. The highest level of unique alleles, three, were found in the Spanish population Orestes. The accessions 12-7KG, 12-9KG, 12-13KG, 12-18 and TK1 had one unique allele each. Although rare

Table 2. *Genetic diversity within 23 chickpea accessions. Number of alleles (NA), private alleles (Pa), unique alleles (Ua), expected hetozygosity (He), Shanon's information index (I), Nei's gene diversity (Nei), observed heterozygosity (Ho). *KG indicates Kyrgyz landraces. **Genetic diversity parameters were calculated for ten individuals in each accession and averaged over nine loci. StDev standard deviation.*

Accession	Origin	Na**	Pa	Ua	He	I	Nei	Ho
12–02	ICARDA	2.11	1	–	0.44	0.61	0.41	0.13
12–29	ICARDA	2.33	–	–	0.48	0.69	0.45	0.11
12–18	ICARDA	2.44	1	1	0.48	0.72	0.46	0.22
12–30	ICARDA	3.78	2	–	0.61	1.06	0.58	0.11
12–15	ICARDA	3.44	3	–	0.62	1.04	0.59	0.19
12–1KG*	Jalal-Abad	2.89	–	–	0.40	0.68	0.38	0.10
12–22KG	Jalal-Abad	3.89	–	–	0.67	1.15	0.63	0.15
12–3KG	Jalal-Abad	2.67	–	–	0.36	0.61	0.34	0.09
12–4KG	Jalal-Abad	3.67	–	–	0.59	1.02	0.56	0.09
12–5KG	Jalal-Abad	3.22	–	–	0.38	0.68	0.36	0.16
12–6KG	Jalal-Abad	4.67	–	–	0.71	1.32	0.68	0.24
12–7KG	Osh	3.22	1	1	0.65	0.99	0.59	0.17
12–8KG	Osh	4.00	–	–	0.61	1.08	0.58	0.16
12–9KG	Osh	4.11	–	1	0.57	1.05	0.54	0.20
12–10KG	Osh	4.44	–	–	0.70	1.28	0.67	0.10
12–11KG	Kemin	3.44	–	–	0.58	0.97	0.55	0.14
12–12KG	Kemin	3.78	–	–	0.65	1.11	0.62	0.07
12–13KG	Osh	3.56	–	1	0.50	0.87	0.48	0.16
'Raphat'	Kyrgyzstan, cultivar	1.22	–	–	0.06	0.09	0.06	0.08
'Saira'	Kyrgyzstan, cultivar	1.44	–	–	0.17	0.24	0.16	0.09
Orestes	Spain	4.67	2	3	0.73	1.36	0.69	0.13
SN1	Spain	3.89	2	–	0.71	1.21	0.67	0.08
TK1	Turkey	3.11	1	1	0.49	0.82	0.46	0.07
Mean		3.30	1.63	1.33	0.53	0.90	0.50	0.13
StDev		0.93	0.74	0.82	0.17	0.32	0.16	0.05

marker alleles may be considered as peripheral in the population, they still may be preserved in germplasm collections representing individuals that carry rare genes (Garkava-Gustavsson et al. 2005). This is important if the plant material has not yet been sufficiently well characterized for important agronomic and quality traits.

Chickpea is a self-pollinated crop, fertilization occurs before flowering and outcrossing is reported to be rare, less than 2% (Tayyar et al. 1996). However, observed heterozygosity (Ho) ranged from 0.07 (12-12KG, TK1) to 0.24 (12-6KG) with a mean of 0.13 (Table 2). Udupa and Baum (2001) reported high mutational rates and high mutational bias in chickpea. Mutations in SSR loci result in allelic heterozygosity (Saeed et al. 2011). Furthermore, heat stress can lead to pollen sterility, preventing self-pollination (Devasirvatham et al. 2012) and may have enforced outcrossing which resulted in allelic heterozygosity.

Multivariate analyses of genetic diversity

The first component of principal coordinate analysis (PCoA) accounted for 34.6% whereas the second and third components accounted for 22.5% and 14.8% of the total variation, respectively. The PCoA bi-plot illustrates the differentiation among accessions (Fig. 1). One grouping was clearly dominated by the Kyrgyz landraces and the other included breeding lines from ICARDA and Turkey, placed loosely together. The Kyrgyz cultivars were found apart from these groups, especially 'Raphat'. The UPGMA cluster analysis, based on Rogers-W genetic distance coefficient, generally confirmed the results of PCoA and clearly separated the two Kyrgyz cultivars from the subcluster formed by Kyrgyz landraces and the subclusters formed by breeding lines from ICARDA along with landraces from Turkey and Spain (Fig. 2). In the dendrogram and on the PCoA bi-plots, chickpea genotypes were adjoined according to their geographic origin. The Turkish accession TK1 was found very close to accession 12-02 from ICARDA. The Spanish accessions Orestes and SN1 were placed between the Kyrgyz landraces and the breeding lines from ICARDA in the PCoA plot. In the dendrogram, these accessions were found close to ICARDA accessions. The Kyrgyz landraces were collected in three regions: Jalal-Abad and Osh are situated in the southern and

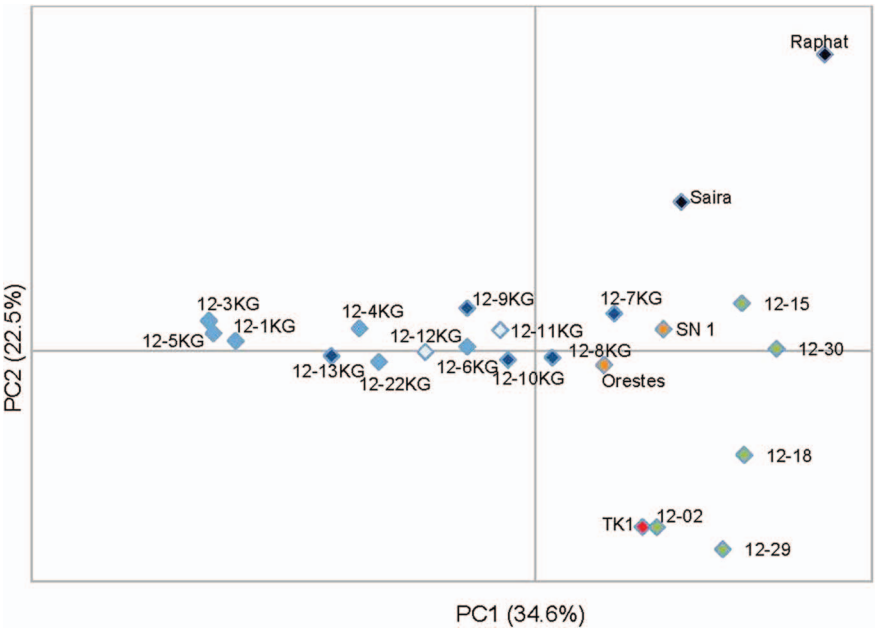

Fig. 1. A two-dimensional plot of Principal co-ordinate analysis (PCoA) of 23 chickpea accessions of different geographic origin.

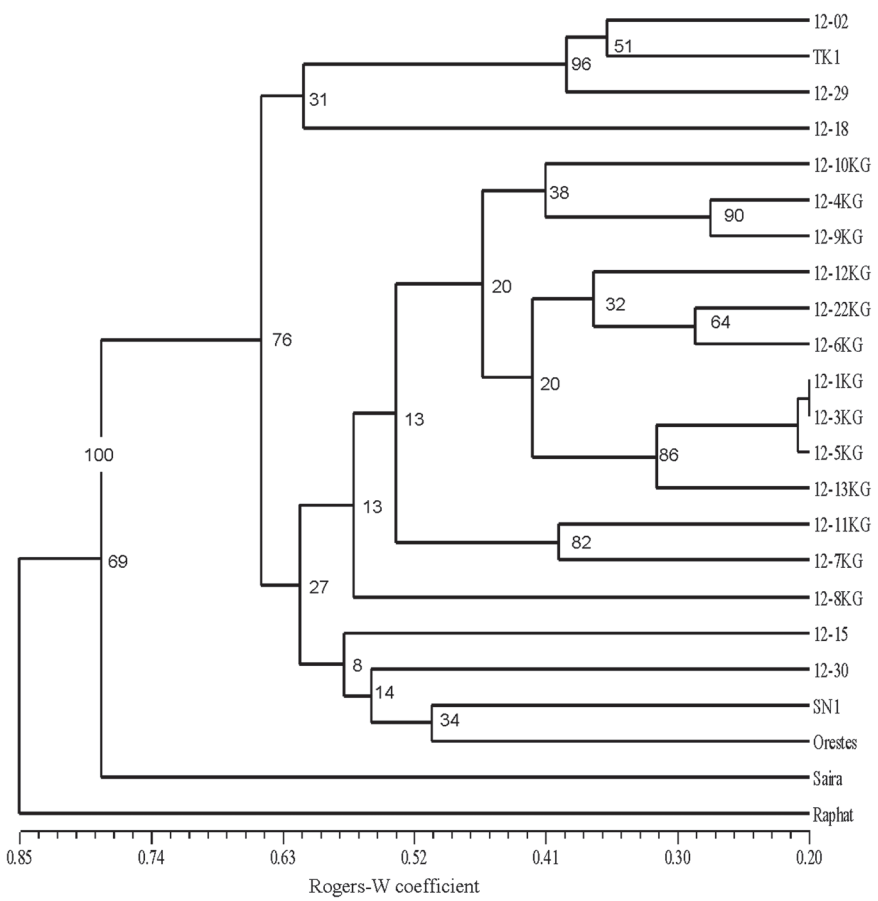

Fig. 2. A UPGMA dendrogram representing genetic relationships among 23 chickpea accessions of different geographic origin.

Kemin in the northern parts of the country. No clear grouping according to these regions was observed among the Kyrgyz landraces and the accessions from the three regions were in general intermixed in both the PCoA bi-plot (Fig. 1) and the dendrogram (Fig. 2). This indicates movement of seeds around the country. The only exception were accessions 12-1KG, 12-3KG and 12-5KG from the Jalal-Abad region which grouped close together both in the PCoA-plot and in the dendrogram. Keneni et al. (2012), analyzing 155 Ethiopian chickpea germplasm accessions with 33 polymorphic SSR markers, found more clear patterns of grouping of accessions according to their geographic origin. Our study demonstrates the separation of Kyrgyz landraces from accessions from other countries. However, to be able to reveal a more clear distribution pattern of genetic diversity among Kyrgyz landraces, more accessions collected from farmers' fields around the country must be investigated with a higher number of SSR loci.

The two cultivars 'Saira' and 'Raphat' released by Kyrgyz breeders in 2009 and 2012, respectively, were separated from Kyrgyz landraces both in the PCoA bi-plot and in the dendrogram, which was not surprising since 'Saira' and 'Raphat' were selected from ICARDA's breeding lines and thus were expected to have little in common with the Kyrgyz landraces. Surprisingly, they did not group together with the accessions from ICARDA.

Partitioning of genetic diversity

AMOVA analysis of the 23 chickpea accessions revealed significant differentiation among accessions ($p < 0.001$) (Table 3). The majority of variation, 62%, resided within accessions, while the remaining 38% resided among accessions when all populations were included in the analysis. When the analysis was restricted to Kyrgyz landraces, 75% of the variation resided within accessions and 25% between. When the analysis was performed separately on the ICARDA material, 65% of the variation was found within lines and 35% between them. When the accessions were grouped in accordance with their origin, 82% of the variation was accounted within the groups and 18% between the groups.

The results indicate that most of the genetic variation was found within populations or groups of accessions in our study. These findings may indicate that no appropriate care was taken by farmers in maintaining seed purity, thereby leading to seed mixtures or to genetic exchange among chickpea populations. Keneni et al. (2012) analyzed cultivars and breeding lines and found a similar pattern: 73% of the variation was found within populations and 27% among populations. In Kyrgyzstan, as in many other developing countries, farmers use home-saved seeds, and no proper care is given to maintaining seed purity. Furthermore, cultivation of heterogeneous accessions is advantageous for farmers since it lowers the risk for complete yield loss in case of unfavorable growing conditions, shortage of fertilizers and lack of plant protection measures. A similar situation was observed in barley accessions collected from farmers' fields in Kyrgyzstan, indicating seed mixtures (Usubaliev et al. 2013).

Nutritional value

The grain protein and mineral composition of chickpea landraces, cultivars and breeding lines are presented in Table 4. Protein and mineral compositions varied significantly ($p < 0.001$) among landraces, cultivars and breeding lines. The protein content varied from 14.5% ('Raphat') to 26.9% (SN1) in chickpea seeds and was generally rather high. The highest protein content was observed in Kyrgyz landraces (15.3–25.6%) and Spanish accessions 23.8% (SN1) and 26.9% (Orestes). The crude protein content

Table 3. *Analysis of molecular variance (AMOVA) for 23 chickpea accessions grouped according to their origin. AA = among accessions, WA = within accessions, AG = among groups, WG = within groups.*

Groups	Source of variation	DF	Variance components	Percentage of variation	Fixation indices	p-value
All accessions	AA	22	1.23030	37.75	$F_{st} = 0.37753$	0.000
	WA	437	2.02849	62.25		
Total		459	3.25879			
Groups to origin	AG	1	0.63010	18.37	$F_{st} = 0.18370$	0.000
	WG	358	2.79999	81.63		
Total		359	3.43009			
ICARDA's materials	AA	4	1.08028	34.66	$F_{st} = 0.34656$	0.000
	WA	95	2.03684	65.34		
Total		99	3.11712			
Kyrgyz landraces	AA	12	0.63384	25.47	$F_{st} = 0.25471$	0.000
	WA	247	1.85466	74.53		
Total		259	2.4885			

Table 4. *Average mineral and protein content in the 23 chickpea accessions analyzed in this study (mg 100 g^{-1} and % dry weight).*

	Ca	S	Mg	P	K	Fe	Mn	Cu	Zn	
Genotype	macronutrients					micronutrients				Protein (%)
12-02	139 *efgh*[c]	228 *efg*	128 *hijk*	440 *e*	1096 *ef*	4.8 *cdef*	2.5 *hi*	8.4 *fg*	5.6 *bcd*	15.5 *kl*
12–29	115 *j*	291 *a*	159 *b*	534 *a*	1327 *a*	12.1 *a*	1.9 *kl*	10.8 *b*	6.8 *a*	19.6 *h*
12–18	142 *defgh*	253 *c*	149 *c*	535 *a*	1223 *b*	10.2 *b*	2.4 *ij*	11.7 *a*	6.9 *a*	16.7 *jk*
12–30	134 *ghij*	239 *de*	128 *hijk*	461 *de*	1031 *g*	5.3 *cdef*	3.4 *c*	8.4 *efg*	4.6 *efg*	17.1 *ij*
12–15	132 *ghij*	204 *k*	141*cdef*	473 *d*	1182 *bc*	6.0 *cde*	2.9 *ef*	10.0 *c*	5.9 *b*	16.3 *jk*
12–1KG	178 *ab*	216 *hij*	139 *defj*	344 *g*	868 *ij*	5.1 *cdef*	3.7 *ab*	9.0 *d*	5.6 *bcd*	24.1 *bcde*
12–2KG	128 *hig*	241 *cd*	165 *ab*	454 *de*	1079 *f*	5.7 *cdef*	1.8 *l*	11.6 *a*	4.6 *efg*	19.4 *h*
12–3KG	160 *bcd*	266 *b*	145 *cde*	397 *f*	972 *h*	5.7 *cdef*	1.0 *m*	10.3 *c*	5.1 *cde*	25.0 *bc*
12–4KG	177 *abc*	218 *ghij*	119 *kl*	282 *i*	808 *k*	4.3 *ef*	3.0 *def*	6.9 *i*	3.6 *ij*	25.6 *ab*
12–5KG	160 *bcd*	228 *efg*	129 *hij*	328 *gh*	832 *jk*	4.4 *def*	2.0 *k*	7.2 *hi*	3.6 *ij*	22.9 *defg*
12–6KG	159 *cd*	207 *jk*	118 *l*	239 *j*	800 *k*	5.0 *cdef*	2.8 *fg*	6.4 *j*	3.5 *ij*	21.6 *g*
12–7KG	158 *de*	227 *fgh*	128 *hijkl*	334 *gh*	837 *jk*	4.9 *cdef*	2.0 *k*	8.3 *g*	4.2 *fghi*	23.7 *cdef*
12–8KG	180 *a*	223 *ghi*	134 *fghi*	315 *h*	896 *i*	6.1 *cd*	3.7 *a*	7.5 *h*	4.0 *ghij*	22.6 *efg*
12–9KG	184 *a*	212 *ijk*	126 *ijkl*	392 *f*	844 *jk*	5.4 *cdef*	2.7 *gh*	6.8 *ij*	3.4 *j*	22.8 *efg*
12–10KG	140 *efgh*	218 *ghij*	134 *fghi*	391 *f*	896 *i*	5.4 *cdef*	3.1 *de*	8.8 *def*	3.8 *hij*	24.4 *bcd*
12–11KG	135 *fghi*	211 *ijk*	120 *jkl*	343 *f*	862 *ij*	4.9 *cdef*	3.6 *abc*	6.9 *i*	3.8 *hij*	22.4 *fg*
12–12KG	133 *ghij*	218 *ghij*	148 *cd*	447 *e*	963 *h*	5.7 *cdef*	3.5 *bc*	6.8 *ij*	4.7 *ef*	23.1 *defg*
12–13KG	182 *a*	217 *hij*	132 *fghi*	471 *cd*	1075 *f*	5.2 *cdef*	2.6 *h*	9.9 *c*	5.7 *bc*	15.3 *kl*
Raphat	135 *fghi*	236 *def*	137 *efgh*	456 *de*	1125 *de*	5.2 *cdef*	2.3 *g*	8.6 *defg*	5.5 *bcd*	14.5 *l*
Saira	154 *def*	238 *def*	140 *cdefg*	502 *bc*	1063 *fg*	4.1 *f*	2.9 *fg*	9.0 *d*	4.5 *efgh*	18.3 *hi*
Orestes	136 *fgh*	266 *b*	168 *ab*	508 *b*	1096 *ef*	5.6 *cdef*	2.1 *d*	8.9 *de*	4.9 *de*	23.8 *cdef*
SN1	149 *defg*	266 *b*	172 *a*	497 *b*	1149 *cd*	5.9 *cd*	3.1 *de*	4.6 k	4.4 *efgh*	26.9 *a*
TK1	118 *ij*	207 *jk*	130 *ghi*	452 *de*	1128 *de*	6.3 *c*	3.2 *d*	8.5 *efg*	5.8 *b*	15.6 *jkl*
Mean	149	232	139	417	1007	5.8	2.7	8.5	4.8	20.7

[c]Means in the same mineral and protein column with different letters are significantly different at $p < 0.001$ according to the Tukey test.

varied between 18% and 31% in the investigation by Sharma et al. (2013) and was generally higher in kabuli chickpea (28–31%) cultivars than in desi (18–23%). Our accessions had values close to that of desi chickpeas. Surprisingly, the two released cultivars, 'Raphat' and 'Saira' had a relatively low protein content, 14.5% and 18.3%, respectively.

The average content of potassium (1007 mg 100 g^{-1}), calcium (149 mg 100 g^{-1}), magnesium (139 mg 100 g^{-1}), iron (5.8 mg 100 g^{-1}), and zinc (4.8 mg 100 g^{-1}) were close to those previously noted by Thavarajah and Thavarajah (2012). The concentration of copper (8.5 mg 100 g^{-1}) and phosphorus (417 mg 100 g^{-1}) were, however, higher in our investigation. In our study, manganese (2.7 mg 100 g^{-1}) was close to that reported by Ereifej et al. (2001). The average concentration of phosphorus, potassium, copper and zinc were generally higher in ICARDA breeding lines, compared to other materials. Especially valuable in this respect were two breeding lines, 12–18 and 12–29, which also had a high average concentration of iron in addition to high average concentrations of the four previously mentioned nutrients. Our results indicate that Kyrgyz chickpea landraces and ICARDA's breeding lines are variable and generally can be regarded as a valuable source of nutritional components. However, chemical composition of different chickpea genotypes can vary due to influence of climatic, environmental (e.g. soil composition) or physiological factors (Grusak and Dellapenna 1999; Abbo et al. 2000; Ereifej et al. 2001; Iqbal et al. 2006). To be able to draw a definite conclusion about the nutritional value of the studied accessions, they must be grown on the same field site and evaluated during at least two years.

This is the first study evaluating genetic diversity using microsatellites and describing nutritional value of chickpea landraces and cultivars (including breeding lines) grown in Kyrgyzstan. A relatively rich genetic diversity and good nutritional values of chickpea landraces grown in Kyrgyzstan make them an interesting source for further breeding purposes aimed on improving this crop.

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