RESEARCH ARTICLE

Genetic variation of a global germplasm collection of chickpea (*Cicer arietinum* L.) including Italian accessions at risk of genetic erosion

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Received: 19 July 2016/Revised: 18 October 2016/Accepted: 18 November 2016/Published online: 19 December 2016
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Abstract Chickpea (*Cicer arietinum* L.) is one of the most important legumes worldwide. We addressed this study to the genetic characterization of a germplasm collection from main chickpea growing countries. Several Italian traditional landraces at risk of genetic erosion were included in the analysis. Twenty-two simple sequence repeat (SSR) markers, widely used to explore genetic variation in plants, were selected and yielded 218 different alleles. Structure analysis and hierarchical clustering indicated that a model with three distinct subpopulations best fits the data. The composition of two subpopulations, named K1 and K2, broadly reflects the commercial classification of chickpea in the two types *desi* and *kabuli*, respectively. The third subpopulation (K3) is composed by both *desi* and *kabuli* genotypes. Italian accessions group both in K2 and K3. Interestingly, this study highlights genetic distance between *desi* genotypes cultivated in Asia and Ethiopia, which respectively represent the chickpea primary and the secondary centres of diversity. Moreover, European *desi* are closer to the Ethiopian gene pool. Overall, this study will be of importance for chickpea conservation genetics and breeding, which is limited by the poor characterization of germplasm collection.

Keywords Chickpea · Genetic diversity · SSR · Population structure

Introduction

Chickpea (*Cicer arietinum* L.) is a self-pollinated legume species ($2n = 2x = 16$). It is the third most cultivated legume in the world (FAOSTAT 2013) and is typically grown in the cold season of semi-arid regions. Vavilov (1926) supported the idea of Southwest Asia being the primary centre of origin, with Ethiopia as the secondary centre (Van Der Maesen 1987).

Two commercial types of chickpea, differing for seed morphological traits, are grown globally: *desi* and *kabuli*. *Desi* seeds, angular and dark-coloured, are mainly produced in the Asia and Ethiopia, and represent about 75% of world chickpea production. *Kabuli* seeds are owl-shaped, beige coloured and are generally grown in the Mediterranean region and Mexico (Kassie et al. 2009).

In Italy, about 8000 ha are cultivated with chickpea (Italian National Institute of Statistics 2014, www.istat.it/it/files/2014/11/C13.pdf) and in all the Mediterranean countries chickpea is often used in traditional recipes like hummus, panelle, farinata and falafel. Several landraces, that usually owe their name from the cultivation area, are still cultivated in marginal territories and appreciated for their quality. However, modern cultivars, selected for improved agronomic and commercial traits, are gradually replacing this gene pool, which is thus seriously exposed to the risks of genetic erosion (Negri 2003; Patanè 2006).

The description of genetic diversity is of great importance for the efficient safeguard of plant germplasm and its utilization for breeding purposes. Several molecular tools, based on DNA polymorphisms, are available to assess

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plant genetic diversity (Weising et al. 2005; Nybom et al. 2014). Simple Sequence Repeat (SSR) markers are widely used due to their high reproducibility, multi-allelic nature and co-dominant inheritance (Tautz 1989). Several SSR markers were developed and used in chickpea (Hüttel et al. 1999; Udupa and Baum 2001; Lichtenzveig et al. 2005; Sethy et al. 2006; Choudhary et al. 2006; Saxena et al. 2014; Parida et al. 2015; Khajuria et al. 2015). Most of them have a known genomic localization, following linkage analysis (Winter et al. 2000; Nayak et al. 2010; Jamalabadi et al. 2013; Khajuria et al. 2015).

The present study was addressed to the genetic characterization of a germplasm collection including accessions from main chickpea growing countries. A large set of Italian landraces was included in the analysis, resulting in useful information on a threatened gene pool that was still awaiting genetic characterization.

Materials and methods

Plant materials

The chickpea germplasm collection used in this study was made up of 103 accessions (Table 1). Sixty-nine accessions were selected from the USDA genebank collection to represent main chickpea growing countries, including those located in the diversity centres of Asia and Ethiopia. Genotypes were assigned to the commercial types *desi* and *kabuli* according to the information available at the Genesys global database on plant genetic resources (www.genesys-pgr.org). Thirty-four Italian genotypes were provided from the Institute of Biosciences and Bio-Resources (IBBR-CNR) of Bari (Italy), including 32 landraces and 2 commercial cultivars, Sultano and Califfo. Italian accessions have a clear *kabuli* phenotype, except for the accessions 110694, 110229 and DEL-01, which have a black seed resembling *desi* chickpeas.

Genomic DNA extraction

The first true leaf of a single plant for each accession was collected for DNA extraction, placed in liquid nitrogen and stored at -80°C until use. Genomic DNA was extracted using the DNeasy 96 Plant Mini Kit (Qiagen, Germany) following the manufacturer's instructions. DNA concentration and quality were measured by means of a NanoDrop 1000 Spectrophotometer (Thermo Scientific, Waltham, MA) and 1% agarose gel.

Microsatellite assay

A set of 157 SSR primer pairs was tested in a preliminary assay on 3% agarose gel, using a panel of eight chickpea

accessions chosen from the collection. This allowed the pre-selection of polymorphic SSR loci. PCR was carried out in a final volume of 12.5 μL containing 1 $\times$ Buffer, 0.2 mM of each dNTPs, 0.25 μM of primer pairs, 20 ng of genomic DNA and 0.5 units of DreamTaq DNA polymerase (Thermo Scientific, Waltham, MA). PCR reactions were performed in a C1000 thermal cycler (Bio-Rad, Hercules, CA) with the following conditions: initial denaturation of 5 min at 94°C ; 10 touch-down PCR cycles of 30 s at 94°C , 45 s at the appropriate annealing temperature and 60 s at 72°C ; 25 PCR cycles of 30 s at 94°C , 45 s at $50/48^{\circ}\text{C}$ and 60 s at 72°C ; final elongation of 5 min at 72°C . Twenty-two polymorphic primer pairs were tested on the whole germplasm collection using capillary electrophoresis. In detail, forward primers were tagged by adding a 5' tail with the universal primer M13. The reaction mixture contained 20 ng of genomic DNA, 1 $\times$ PCR buffer, 0.032 μM forward primer, 0.16 μM reverse primer, 0.08 μM of a primer complementary to the M13 tail and labelled with Fam, Vic, Pet, or Ned fluorescent dyes (Thermo Scientific, Waltham, MA), 0.2 mM dNTPs, 2.5 mM MgCl_2 , and 0.5 U of DreamTaq DNA polymerase (Thermo Scientific, Waltham, MA) in a final volume of 12.5 μL . PCR products were separated by the ABI PRISM 3130 Avant Genetic Analyzer (Thermo Scientific, Waltham, MA) capillary electrophoresis system, using a mix containing 2 μL PCR reaction, 12 μL Hi-Di Formamide (Thermo Scientific, Waltham, MA) and 0.5 μL GeneScan 600 LIZ Size Standard (Thermo Scientific, Waltham, MA). The allele size was assigned using GeneMapper 5.0 (Applied Biosystem).

Data analysis

The amplified fragments were used to get a binary matrix, in which amplicons were marked with 1 or 0 for presence and absence, respectively. A dendrogram of genetic similarity among the accessions was generated based on the Jaccard's similarity coefficient and the Unweighted Pair Group Method using Arithmetic Averages (UPGMA) implemented in NTSYS-PC v. 2.0 (Rohlf 1998). Population structure was determined using the Bayesian model-based clustering approach implemented in STRUCTURE v. 2.3.4 (Pritchard et al. 2000), which assigns individuals to K subpopulations according to a membership coefficient (qi). For each K (from 1 to 10), ten runs were performed. A Markov chain Monte Carlo (MCMC) of 100000 burn-in phases followed by 100000 iterations was run to generate the membership coefficient (qi) matrix. The optimal K value was determined using the ad-hoc statistic ΔK (Evanno et al. 2005), which was estimated with the software Structure Harvester (Earl and vonHoldt 2012). Individual samples were assigned to each subpopulation when

Table 1 List of the 103 *Cicer arietinum* accessions genotyped in this study

Accession number	Origin	Seed type	Biological status	Structure
PI 115448	India	<i>Desi</i>	Traditional cultivar/landrace	K1
PI 359054	India	<i>Desi</i>	Traditional cultivar/landrace	K1
PI 450561	India	<i>Desi</i>	Traditional cultivar/landrace	Adm
PI 214043	India	<i>Desi</i>	Traditional cultivar/landrace	K1
PI 358927	India	<i>Desi</i>	Traditional cultivar/landrace	K1
PI 214312	India	<i>Desi</i>	Traditional cultivar/landrace	K1
PI 315806	India	<i>Desi</i>	Traditional cultivar/landrace	K1
PI 297264	India	<i>Desi</i>	Traditional cultivar/landrace	K1
PI 359014	India	<i>Desi</i>	Traditional cultivar/landrace	K1
PI 212890	India	<i>Desi</i>	Traditional cultivar/landrace	K1
PI 359018	India	<i>Desi</i>	Traditional cultivar/landrace	K1
PI 216026	India	<i>Desi</i>	Traditional cultivar/landrace	K1
PI 512301	India	<i>Desi</i>	Advanced/improved cultivar	K1
PI 358914	India	<i>Desi</i>	Traditional cultivar/landrace	K1
PI 315798	India	<i>Desi</i>	Traditional cultivar/landrace	K3
PI 251514	Iran	<i>Desi</i>	Traditional cultivar/landrace	K3
W6 16882	Iran	<i>Kabuli</i>	Traditional cultivar/landrace	K3
PI 250143	Pakistan	<i>Desi</i>	Traditional cultivar/landrace	K3
PI 359002	Myanmar	<i>Desi</i>	Traditional cultivar/landrace	K1
PI 220649	Afghanistan	<i>Desi</i>	Traditional cultivar/landrace	K1
PI 426565	Pakistan	<i>Desi</i>	Traditional cultivar/landrace	Adm
PI 513144	Pakistan	<i>Desi</i>	Traditional cultivar/landrace	K1
PI 140293	Iran	<i>Desi</i>	Traditional cultivar/landrace	K1
PI 222774	Iran	<i>Desi</i>	Traditional cultivar/landrace	K1
PI 358934	Iran	<i>Desi</i>	Traditional cultivar/landrace	K1
PI 583753	Bangladesh	<i>Desi</i>	Advanced/improved cultivar	K1
110219	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K3
110398	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K3
110213	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K2
110227	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K3
SANT-01	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K3
110490	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K3
110493	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	Adm
110410	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K3
110467	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K3
110412	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K3
110222	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K3
110226	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K3
110229	Italy	<i>Desi</i>	Traditional cultivar/landrace	K3
MAT-02	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K3
MAT-03	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K3
DEL-01	Italy	<i>Desi</i>	Traditional cultivar/landrace	K3
110216	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	Adm
110694	Italy	<i>Desi</i>	Traditional cultivar/landrace	K3
110696	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K3
110400	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K3
110402	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K3
110228	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K3
110231	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K3
110407	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K2

Table 1 continued

Accession number	Origin	Seed type	Biological status	Structure
110408	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K2
110397	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K2
110215	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K2
110693	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K2
106900	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K2
106872	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K2
106867	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K2
106941	Italy	<i>Kabuli</i>	Traditional cultivar/landrace	K3
Califfo	Italy	<i>Kabuli</i>	Advanced/improved cultivar	K3
Sultano	Italy	<i>Kabuli</i>	Advanced/improved cultivar	K3
PI 502999	Mexico	<i>Kabuli</i>	Advanced/improved cultivar	K2
W6 17597	Mexico	<i>Kabuli</i>	Breeding/research material	Adm
PI 507020	Mexico	n.a	Traditional cultivar/landrace	K2
PI 339154	Turkey	<i>Kabuli</i>	Traditional cultivar/landrace	K2
PI 339181	Turkey	<i>Kabuli</i>	Traditional cultivar/landrace	K2
PI 509299	Turkey	<i>Kabuli</i>	Traditional cultivar/landrace	K2
PI 509207	Turkey	<i>Kabuli</i>	Traditional cultivar/landrace	K2
PI 509147	Turkey	<i>Kabuli</i>	Traditional cultivar/landrace	K1
PI 509163	Turkey	<i>Kabuli</i>	Traditional cultivar/landrace	K1
PI 509236	Turkey	<i>Kabuli</i>	Traditional cultivar/landrace	K2
PI 642853	Australia	<i>Kabuli</i>	Advanced/improved cultivar	K3
PI 518255	Australia	<i>Desi</i>	Traditional cultivar/landrace	K1
W6 2973	n.a	<i>Kabuli</i>	Traditional cultivar/landrace	K1
PI 543921	USA	<i>Desi</i>	Advanced/improved cultivar	K1
W6 10046	Bulgaria	<i>Desi</i>	Traditional cultivar/landrace	K3
PI 193487	Ethiopia	<i>Desi</i>	Traditional cultivar/landrace	K3
PI 193481	Ethiopia	<i>Desi</i>	Traditional cultivar/landrace	K3
PI 196840	Ethiopia	<i>Desi</i>	Traditional cultivar/landrace	K3
PI 193479	Ethiopia	<i>Desi</i>	Traditional cultivar/landrace	K2
PI 193480	Ethiopia	<i>Desi</i>	Traditional cultivar/landrace	K2
PI 257586	Ethiopia	<i>Desi</i>	Traditional cultivar/landrace	K3
W6 10451	Russia	<i>Desi</i>	Traditional cultivar/landrace	K3
PI 343014	Russia	<i>Kabuli</i>	Advanced/improved cultivar	K3
PI 343019	Russia	<i>Desi</i>	Advanced/improved cultivar	K3
PI 343016	Russia	<i>Kabuli</i>	Advanced/improved cultivar	K3
PI 503025	USA	<i>Kabuli</i>	Traditional cultivar/landrace	Adm
PI 503001	USA	<i>Kabuli</i>	Advanced/improved cultivar	K2
PI 533683	Spain	<i>Desi</i>	Advanced/improved cultivar	K3
PI 533676	Spain	<i>Desi</i>	Advanced/improved cultivar	K3
PI 543010	Spain	<i>Desi</i>	Advanced/improved cultivar	K3
PI 533681	Spain	<i>Kabuli</i>	Advanced/improved cultivar	K2
PI 533673	Spain	<i>Kabuli</i>	Advanced/improved cultivar	K2
PI 572411	Spain	<i>Desi</i>	Traditional cultivar/landrace	K3
PI 509196	Turkey	<i>Kabuli</i>	Traditional cultivar/landrace	K1
W6 11071	Hungary	<i>Kabuli</i>	Advanced/improved cultivar	K3
PI 508096	USA	<i>Desi</i>	Advanced/improved cultivar	K3
PI 512300	USA	<i>Kabuli</i>	Advanced/improved cultivar	K3
PI 503006	Chile	<i>Kabuli</i>	Traditional cultivar/landrace	K2
W6 2938	n.a.	<i>Kabuli</i>	Traditional cultivar/landrace	K2

Table 1 continued

Accession number	Origin	Seed type	Biological status	Structure
PI 292006	Jordan	<i>Kabuli</i>	Traditional cultivar/landrace	Adm
PI 513151	Pakistan	<i>Kabuli</i>	Traditional cultivar/landrace	K2
PI 357651	Serbia-Montenegro	<i>Kabuli</i>	Advanced/improved cultivar	K3
PI 357648	Serbia-Montenegro	<i>Kabuli</i>	Advanced/improved cultivar	K2

For each genotype, geographical origin, seed type, biological status and subpopulation (K) resulting from STRUCTURE analysis are reported. Adm indicates genotypes of admixed ancestry

the value of the corresponding q_i was ≥ 0.6 . Genotypes with the highest $q_i < 0.6$ were considered of admixed ancestry.

To evaluate the allelic and genetic diversity for each locus, number of observed alleles (N_o), expected (H_e) and observed heterozygosity (H_o), fixation index and Shannon's information index (I) were calculated using POPGENE v1.32 (https://sites.ualberta.ca/~fyeh/popgene_download.html). The informativeness of each marker was also inferred by the calculation of the Resolving Power (Rp) and Discriminating Power (PD) parameters, as indicated by Prevost and Wilkinson (1999) and Kloosterman et al. (1993).

Results

SSR markers

One-hundred and fifty-seven SSR markers were selected among those reported from Khajuria et al. (2015) (69), Sethy et al. (2006) (17) and from Nayak et al. (2010) (88). Preliminary tests on agarose gel electrophoresis and a subset of eight accessions allowed the identification of 22 polymorphic SSR loci, which were further scored on capillary gel electrophoresis, enabling the discrimination of up to 2 bp length polymorphisms. Information and statistics on each marker are reported in Table 2. In total, 218 different alleles were detected in a germplasm collection of 103 accessions. Marker size ranged from 131 bp (for NCPGR-21) to 344 bp (for ICCM-242) (Table 2). The highest number of alleles (26) was scored for the marker CaGMS-13, whereas the lowest (2) was associated with the markers CaGMS-1235 and NCPGR-76. The average expected heterozygosity (H_e) was considerably higher (0.70) than observed heterozygosity (H_o) (0.26). Extreme values were 0.30 (CaGMS-561) and 0.94 (CaGMS-1 and CaGMS13) for H_e and 0.06 (CaGMS-1250 and NCPGR-53) and 0.93 (NCPGR-76) for H_o . In accordance with these data, inbreeding coefficients were positive for most of the markers. The Shannon's Information Index (I) was on

average 1.63 and ranged from 0.66 (CaGMS-1250 and CaGMS-561) to 2.96 (CaGMS13), thus reflecting the effectiveness of the SSR loci in revealing genetic variation. The number of distinguished cultivars (NDC) ranged from 0 (CaGMS-1235, NCPGR-53 and NCPGR-76) to 20 (ICCM-0068). The resolving power (Rp) ranged from 0.17 (NCPGR-76) to 3.6 (ICCM-69). The extreme values of Discrimination power (PD) were associated with NCPGR-76 (0.14) and CaGMS-1 (0.96). Correlation with NDC was highly significant ($p < 0.001$) for both the PD ($r = 0.71$) and for the Rp parameter ($r = 0.57$).

Genetic structure

The software STRUCTURE was used to estimate the number of hypothetical subpopulations (K) present in our collection and the probability of each genotype to fall into one of the designated subpopulations. An admixture-based clustering model was used to infer the genetic structure of 103 chickpea accessions. Using the ΔK approach of Evanno et al. (2005), we found that a model with 3 subpopulations (K1; K2; K3) best fits the data with 93.2% of ancestry derived from one of the subpopulations and 6.8% of admixed ancestry (highest membership coefficient $q < 0.6$) (Fig. 1; Table 1). The subpopulations could be discriminated to a great extent on the basis of the seed commercial type and the geographical origin. In more detail, the subpopulation K1 is formed by 22 *desi* accessions, all from the Asian continent except for PI543921 (from U.S.) and PI518255 (from Australia), and four *kabuli* accessions (PI509196, PI509147, PI509163 and W62973); the subpopulation K2 includes 22 *kabuli* accessions from different geographical origin and two Ethiopian *desi* accessions (PI193479 and PI193480); the subpopulation K3 groups 27 *kabuli* and 18 *desi* accessions. The vast majority of *desi* K3 accessions originate from Ethiopia and European countries. Of the 34 Italian accessions genotyped in this study, 23 group in the K3 subpopulation, including the commercial cultivars Califfo and Sultano and the black-seeded genotypes 110694, 110229 and DEL-01, 9 group in the K2 subpopulation and 2 are of admixed ancestry.

Table 2 Allelic variation of 22 simple sequence repeat (SSR) loci in 103 chickpea genotypes

SSR	LG	Range	N _o	H _o	H _e	F	I	NDC	Rp	PD	Reference
NCPGR-53	LG1	197–205	4	0.06	0.54	0.89	0.86	0	1.96	0.58	Sethy et al. (2006)
NCPGR-21	LG2	131–175	16	0.15	0.87	0.83	2.25	15	2.28	0.89	Sethy et al. (2006)
CaGMS-24	LG3	228–252	4	0.09	0.51	0.82	0.78	1	1.76	0.60	Khajuria et al. (2015)
CaGMS-1270	LG3	214–228	6	0.17	0.75	0.77	1.47	6	2.42	0.81	Khajuria et al. (2015)
CaGMS-13	LG3	258–327	26	0.11	0.94	0.89	2.96	15	2.33	0.94	Khajuria et al. 2015
ICCM-178	LG3	281–299	7	0.17	0.80	0.79	1.69	5	2.57	0.85	Nayak et al. (2010)
CaGMS-33	LG4	153–185	11	0.14	0.75	0.81	1.73	12	2.29	0.79	Khajuria et al. (2015)
ICCM-0068	LG4	196–268	18	0.28	0.90	0.68	2.47	20	2.73	0.94	Nayak et al. (2010)
CaGMS-1154	LG4	227–235	4	0.16	0.61	0.74	1.13	1	1.72	0.69	Khajuria et al. (2015)
ICCM-0069	LG4	243–284	14	0.80	0.90	0.11	2.39	4	3.60	0.90	Nayak et al. (2010)
CaGMS-18	LG5	212–185	4	0.13	0.63	0.79	1.08	2	2.06	0.69	Khajuria et al. (2015)
CaGMS-992	LG5	173–213	8	0.20	0.76	0.74	1.59	7	2.40	0.83	Khajuria et al. (2015)
ICCM-0076	LG5	265–312	17	0.31	0.89	0.65	2.43	16	2.65	0.91	Nayak et al. (2010)
ICCM-242	LG6	282–344	14	0.17	0.90	0.81	2.41	10	2.34	0.93	Nayak et al. 2010
NCPGR-50	LG6	189–234	17	0.16	0.88	0.81	2.34	14	2.33	0.90	Sethy et al. (2006)
CaGMS-561	LG6	140–156	6	0.20	0.30	0.33	0.66	6	1.05	0.37	Khajuria et al. (2015)
CaGMS-742	LG7	239–263	7	0.14	0.53	0.74	1.09	4	1.48	0.62	Khajuria et al. (2015)
CaGMS-1235	LG8	298–310	2	0.12	0.50	0.75	0.69	0	1.76	0.59	Khajuria et al. (2015)
CaGMS-1250	LG8	242–246	3	0.06	0.44	0.86	0.66	1	1.30	0.49	Khajuria et al. (2015)
CaGMS-1	LG8	237–331	24	0.26	0.94	0.72	2.93	19	2.80	0.96	Khajuria et al. (2015)
NCPGR-86	n.a.	210–216	4	0.85	0.52	−0.64	0.82	1	0.50	0.28	Sethy et al. (2006)
NCPGR-76	n.a.	244–248	2	0.93	0.50	−0.86	0.69	0	0.17	0.14	Sethy et al. (2006)
Mean	–	–	9.91	0.26	0.70	0.59	1.63	7.23	2.02	0.72	–
Min	–	–	2.00	0.06	0.30	−0.86	0.66	0	0.17	0.14	–
Max	–	–	26	0.93	0.94	0.89	2.96	20	3.60	0.96	–

For each marker, linkage group (LG) localization, number of alleles (N_o), observed and expected heterozygosity (H_o and H_e), Wright's fixation index (F), Shannon's information index (I), number of distinguished cultivars (NDC), discrimination power (PD) and resolving power (Rp) are reported

Genetic relationships among individuals

The UPGMA dendrogram based on the Jaccard's similarity supports to a great extent the results obtained with structure analysis. In more detail, two main clusters included most accessions of the subpopulations K1 and K3 (Fig. 2). The rest of the dendrogram includes *kabuli* accessions from K1, K2 and K3, with the exception of the *desi* accessions PI358943, PI193479, PI193480 and PI115448.

Discussion

The genetic basis of cultivated chickpea is narrow, as a consequence of several bottlenecks occurring during its evolutionary history (Abbo et al. 2003). This represents a major limitation for chickpea breeding (Amin and Melkamu 2014). Therefore, the collection and study of chickpea

germplasm is of utmost importance to prevent further genetic erosion and efficiently address breeding efforts. A main objective of this work was the genetic characterization of a global chickpea germplasm collection, including several Italian landraces threatened by the advent of modern cultivars and potentially useful as a source of genes for adaptation and qualitative traits. In total, 218 alleles were scored by the analysis of 103 accessions with 22 SSR primer combinations, corresponding to an average of 9.9 alleles per locus. This confirms the value of SSR markers as a tool to detect intraspecific polymorphism in chickpea (Hüttel et al. 1999; Singh et al. 2003; Upadhyaya et al. 2008; Hajibarat et al. 2015).

For most markers, observed heterozygosity (H_o) was considerably lower than expected heterozygosity (H_e), leading to high values of inbreeding coefficients. This was expected, as chickpea is a strictly self-pollinated crop. The highest number of discriminated cultivars (NDC) was

Fig. 1 Genetic structure of 103 chickpea accessions as revealed by the SSR markers used in this study. **a** Plot of mean likelihood and standard deviation (SD) for ten independent runs for a number of subpopulations (K) ranging from 1 to 10. **b** Evanno's Delta K plot generated by structure analysis. $K = 3$ shows a peak indicating that three subpopulations sufficiently define genetic variation in the dataset of 103 accessions used in this study. **c** Bar-plot describing

population structure. Each individual is represented by a *thin horizontal line*, which is partitioned into K coloured segments whose length is proportional to the estimated membership coefficient (q) in each subpopulation ($K1$, $K2$ and $K3$). Individuals with the highest $q < 0.6$ are not assigned to any of the populations and thus considered of admixed ancestry. (Colour figure online)

associated with the marker ICCM-0068. Our work, showing a statistic correlation between NDC and either PD or Rp, supports the conclusion of previous studies reporting a linear relationship between the efficacy of a primer combination to distinguish accessions (NDC) and the two parameters Rp and PD (Prevost and Wilkinson 1999; Fernandez et al. 2002).

A common conclusion from previous studies on chickpea genetic diversity was the distinction of two cultivated gene pools, corresponding to the two main commercial types *desi* and *kabuli* (Upadhyaya et al. 2008). Accordingly, structure analysis performed in this work led to the identification of two subpopulations, K1 and K2, which were largely composed by *desi* and *kabuli* accessions, respectively. However, our study also supports the identification of a third gene pool (K3), including both *desi* and *kabuli* accessions (Fig. 1; Table 1). Moreover, we found that K1 includes four *kabuli* accessions and K3 includes two *desi* accessions. Genetic similarity between some *desi* and *kabuli* genotypes was reported by previous studies. For

example, following the SSR analysis of a chickpea composite collection, Upadhyaya et al. (2008) generated a Neighbour-Joining tree in which a small cluster of *desi* was included in major branch containing *kabuli* accessions and vice versa. In addition, the recent work of Penmetsa et al. (2016), evaluating the genetic structure of a representative set of cultivated chickpea, also questioned the strict dichotomy of a *desi* and *kabuli* genetic subdivision within cultivated chickpea.

This work highlights genetic divergence between *desi* accessions from Asia and other geographical origin, including Ethiopia, which is considered a secondary evolutionary centre (Van Der Maesen 1987). Notably, the work of Penmetsa et al. (2016), published during the revision of this manuscript, clearly distinguishes between chickpea gene pools from Ethiopia and the Indian sub-continent. Although, the 103 accessions genotyped in this study might not be representative of the whole genetic variation in cultivated chickpea, another relevant finding is that European *desi* accessions seem to be closer to the

Fig. 2 Unweighted pair group method with arithmetic mean (UPGMA) dendrogram based on Jaccard's similarity. Main nodes separating accessions of the subpopulations K1 and K2 are indicated with circles

Ethiopian gene pool than to the Asian one (Fig. 2), and might thus originate from the former. Geographical patterns cannot explain the distribution of *kabuli* germplasm in the two subpopulations K2 and K3. Italian landraces, which can be in most cases classified as *kabuli*, clustered both in K2 and K3. This might indicate the presence of a wide genetic basis in Italian chickpea germplasm. However, this conclusion needs to be confirmed by future studies on chickpea genetic diversity.

In conclusion, the results of this study provide insights on the genetic diversity of cultivated chickpea and is expected to be useful for conservation genetics and breeding. We are currently performing an extensive characterization of agronomic, nutritional and technological properties of Italian accessions characterized in this study at the DNA level, in order to detect the traits of interest for breeding programs.

Acknowledgements This work was supported by the SaVe-GraINPuglia Programme (Reg.CE n. 1698/2005 Programma di Sviluppo rurale per la Puglia 2007/2013. Misura 214—Azione 4 Sub azione a). The authors would like to acknowledge Dr. Venturino Bisignano for kindly providing some Apulian genotypes characterized in this study.

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