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Genetic and agronomic characterization of chickpea landraces for resistance to *Fusarium oxysporum* f. sp. *ciceris*

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Summary. Chickpea, with annual grain production of approx. 15 million tons, is the third largest world pulse crop, which is an important source of protein for human and animal diets. In Italy, chickpea production is mainly based on landraces cultivated on small farms. However, the attention that consumers give to local products stimulates farmers to extend the use of chickpea landraces by reintroducing them in crop rotations. Production of chickpea using landraces can be adversely affected by agronomic factors and, particularly, plant diseases such as Fusarium wilt, caused by the widespread fungal pathogen *Fusarium oxysporum* f. sp. *ciceris* (*Foc*). Therefore, studies of agronomic adaptation of landraces and on disease resistance are needed. The most important agronomic traits and levels of resistance to *Foc* were evaluated for 18 chickpea landraces collected from Central Italy. These landraces were also characterized for their genetic traits in comparison to some of the main Spanish cultivars, and to two reference cultivars with worldwide distribution. Molecular characterization showed variability in genetic and phenotypic traits among the Italian landraces. In particular, landrace 203 locally known as "Longano" was resistant to *Foc* and could be considered in chickpea breeding programmes. Comparative analyses based on molecular markers showed, with some exceptions, that the Italian landraces are genetically different compared to the main Spanish cultivars analyzed in this study.

Keywords. Fusarium wilt, chickpea biodiversity, germplasm conservation, molecular markers, disease resistance improvement.

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INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the only cultivated species of the genus *Cicer*, and originated in East Turkey (van der Maesen, 1987). It is a self-pollinated diploid annual plant species (2n = 2x = 16) with a small genome (740

Mbp) (Arumuganathan and Earle, 1991). Plant breeders have distinguished two chickpea types: desi and kabuli. The seeds of the desi type are normally small, angular and dark, and are produced by plants with purple flowers, while seeds of the kabuli type are large, round, white to cream, and are produced by plants with white flowers (Taylor and Ford, 2007).

Chickpea, is the third largest pulse crop in the world after dry bean and pea, with annual production of approx. 15 million tons. It is mainly cultivated on the Indian subcontinent. In Europe, the major chickpea producing country is Spain, followed by Italy, Portugal and the Balkan Countries. In Italy, chickpea is the third largest cultivated dry legume, grown on 20,025 ha, with grain production of 33,541 t (FAO, 2017). The crop is mainly grown on small farms, and landraces are particularly appreciated for local markets. The landrace varieties usually take their names from the location where they have been traditionally cultivated (Negri, 2003).

Although Italy is the second largest European producer of chickpea, cultivation is still restricted because of adverse environmental and/or agronomic factors responsible for variability in yields (Rossini, 2008). Nationally produced and imported chickpeas are mainly for human food use. The introduction of chickpea as animal feed could represent a viable alternative to soybean, enlarging its use in crop rotations, especially in arid and non-irrigated areas (Crinò and Saccardo, 2008). The use of landraces could have an important economic role, as well as social and cultural significance. Chickpea cultivation is sometimes connected to ethnic preferences of particular linguistic minorities, and also enhances plant biodiversity preservation programmes implemented by germplasm banks aimed at avoiding loss of crop plant ecotypes (Laghetti *et al.,* 2011). Cultivated landraces can be affected by a wide range of pathogens, in particular *Fusarium oxysporum* f. sp. *ciceris* (*Foc*), *Ascochyta rabiei* (Pande *et al.,* 2005; Jiménez-Díaz *et al.,* 2015), and other emerging pathogens (De Curtis *et al.,* 2014), which can cause significant yield losses.

Selection of landraces is mainly based on commercial characteristics of the grain while disease resistance is not often taken into account (Zaccardelli *et al.,* 2012).

Fusarium oxysporum f. sp. *ciceris* is the causal agent of chickpea Fusarium wilt with worldwide distribution. This Ascomycete fungus has been characterized into eight races (0, 1A, 1B/C, 2, 3, 4, 5 and 6), which are differentiated by genetic compatibility with the host and by geographical distribution. In addition to the pathogenic variability of the fungus, two distinct types, referred to as yellowing and wilting syndromes, have been distinguished on the basis of symptoms on infected plants (Trapero-Casas and Jiménez-Díaz, 1985; del Mar Jiménez-Gasco *et al.*, 2004). The pathogen is soil-borne, and can survive in the soil 6 or more years, even in the absence of the host plants, because of its durable survival structures. The infection of host plants can occur at different phenological stages with greatest incidence during the pod-forming phase, mainly when crops are subjected to water stress and sudden temperature increases. Infections occurring during vegetative and reproductive stages can result in complete yield losses. For these reasons, disease management is critical for chickpea production (Arunodhayam *et al.,* 2014).

Chickpea resistance towards the different races of *Foc* is of the "gene-for-gene" type, and different molecular markers associated with resistance are available. A gene cluster, located on Linkage Group (LG) 2 of the chickpea genetic map, confers resistance against races 0, 1, 2, 3, 4, 5 (Sharma and Muehlbauer, 2007). A second gene conferring resistance against race 0 is located on LG5 (Halila *et al.,* 2008). Molecular markers tightly linked to *Foc* resistance genes are useful tools for characterisation and genotype selection. The microsatellite TA59, located on LG2, is the marker most closely associated with resistance to *Foc* race 5 (*Foc*5) (Jendoubi *et al.,* 2017).

Flowering time is another agronomic trait defining adaptation and included in this study. Classical genetic analyses and conventional mapping studies have resulted in the identification of Quantitative Trait Loci (QTLs) on different LGs showing that genes governing this trait are distributed throughout the chickpea genome (Mallikarjuna *et al.,* 2017). Two conserved major QTLs have been identified, QTL_{DF3} on LG3 (Cobos *et al.,* 2009; Aryamanesh *et al.,* 2010) and QTL_{DF1} on LG4 (Cobos *et al.,* 2007; Varshney *et al.,* 2014).

In the present study, we focused on race *Foc*5, which is important in the Mediterranean basin (Jiménez-Díaz *et al.,* 2015). We characterised 18 landraces of chickpea from Central Italy for resistance to *Foc*5 and for some agronomic traits. Molecular markers, distributed along the chickpea genome, were used to study genetic variability among the landraces. Flowering time, an agronomic trait defining ecotype adaptation, was also investigated.

MATERIALS AND METHODS

Plant material

Eighteen Italian chickpea landraces previously collected by *Agenzia Regionale per lo Sviluppo Agricolo*

Rurale e della Pesca, Campobasso, Italy, during its institutional activity aimed at collecting and preserving traditional crops from different regional locations for a germplasm bank, were examined in the present study (Table 1). All landraces were the kabuli type, except accession number 184 which was the desi type. Forty-eight Spanish cultivars and two undomesticated *Cicer* species used in a previous study by Castro *et al.* (2010a), were also included as reference cultivars for comparative analyses, using the same molecular markers adopted for the Italian landraces.

Morphological and agronomic assessments

For each landrace, ten seeds were sown in each of three replicate plots in an experimental field at *Instituto de Investigación y Formación Agraria y Pesquera de Andalucía*, Córdoba, Spain, on January 2015. The following morphological traits were recorded: percentage of emerged plants 15 d after sowing, flowering time (assessed as 50% full opened flowers), growth habit and seed size (determined as 100-seed weights). Phenotypic evaluation of growth habit was assessed on three replicates, each of ten adult plants, for each landrace seeded in the field, based on the scale: 1 = semi erect; 2 = erect (Ali *et al.,* 2015).

Evaluation of resistance to Fusarium oxysporum *f. sp.* ciceris *Race 5*

The landraces were evaluated for wilt reaction to race 5 of *Foc* under controlled condition. The pathogenicity test was conducted in a growth chamber (daily cycle of 12 h light at 25 ± 2°C and 12 h dark at 22 ± 2°C). Lines ILC3279 and WR315 from the International Centre for Agricultural Research in the Dry Areas, which are, respectively, susceptible and resistant to *Foc*5, were included as experimental controls. Ten seeds of each landrace and control line were sown into plastic trays (60×40×10 cm - five lines per tray) that were filled with perlite. Three replicates were sown of each seedline. The trays were irrigated with nutrient solution. Conidia of *Foc*5 were obtained by growing the fungus in potato dextrose broth at 25°C and 100 rpm for at least 7 d. After incubation, fungal mycelium was removed, conidia were collected by centrifugation and their concentration adjusted to 1×10^6 conidia mL^{-1}. When plants were approx. 9 cm height they were removed from perlite and their roots were cut to approx. 5 cm lengths, and the plants were then dipped in conidial suspension for 5 min. The plants were then replanted in the same trays from which they were previously removed. Disease incidence, as percentage of dead plants, was recorded, commencing with appearance of the first symptoms on the susceptible

Table 1. Geographical origins and agronomic traits of Italian chickpea landraces assessed in this study.

Landraces	Town/Province[a]	Geographical coordinates	Type[b]	Germination (%)	Growth habit[c]	100 seeds weight (g)	Flowering time (days)
62	Cercemaggiore/CB	41° 28′ N/14° 43′ E	K	90	1	27.1	87
64	Cercemaggiore/CB	41° 28′ N/14° 43 ′E	K	80	1	36.7	87
73	Salcito/CB	41° 45′ N/14° 31′ E	K	90	1	37.3	86
76	S. Elia a Pianisi/CB	41° 37′ N/14° 53′ E	K	100	1	38.4	87
83	Casacalenda/CB	41° 44′ N/14° 51′ E	K	90	1	36.2	88
97	S. Angelo del Pesco/IS	41° 53′ N/14° 15′ E	K	100	1	35.3	86
99	Venafro/IS	41° 29′ N/14° 02′ E	K	90	1	31.7	84
111	Ripabottoni/CB	41° 41′ N/14° 49′ E	K	100	1	52.4	84
125	Morrone del Sannio/CB	41° 43′ N/14° 47′ E	K	60	1	48.6	84
147	Riccia/CB	41° 29′ N/14° 50′ E	K	80	1	42.5	84
148	Filignano/IS	41° 32′ N/14° 03′ E	K	100	1	30.5	84
160	Miranda/IS	41° 39′ N/14° 15′ E	K	90	1	35.8	87
184	Cercemaggiore/CB	41° 28′ N/14° 43′ E	D	100	1	21.9	87
203	Longano/IS	41° 31′ N/14° 15′ E	K	90	1	47.9	67
228	Riccia/CB	41° 29′ N/14° 50′ E	K	100	1	35.4	84
237	Montagano/CB	41° 39′ N/14° 40′ E	K	100	1	34.6	79
241	Riccia/CB	41° 29′ N/14° 50′ E	K	100	1	31.2	83
245	Capracotta/IS	41° 50′ N/14° 16′ E	K	90	2	27.2	87

[a] CB= Campobasso (IT), IS= Isernia (IT); [b] K=Kabuli, D=Desi; [c] 1= semi-erect, 2 = erect.

control ILC3279. Disease severity on each plant of all landraces was assessed each week for 3 weeks.

Foc symptoms and pathogen resistance were evaluated by using the following empirical disease scale: 0–10% of plants wilting = high resistance (R), 11–89% of plants wilting = intermediate resistance (I), >90% of plants wilting = high susceptibility (S) (Sharma *et al.*, 2005).

Molecular marker analyses

DNA extraction was carried out on young leaflets from five different plants of each landrace. Approximately 0.1 g of the mixed tissues was frozen in liquid nitrogen and stored at -80°C. DNA was isolated using the Plant DNAzol® Reagent (Invitrogen™). DNA was quantified by Nanodrop and used in PCR reactions to amplify the 12 microsatellite markers listed in Table 2. These molecular markers have been selected for their distribution across different linkage groups of the chickpea genome (Winter *et al.,* 2000; Millan *et al.,* 2010). Among the used markers, nine (those marked with TA prefix) were reported by Winter et al. (2000), two (CaGM14822 and CaGM07922) were reported by CAGM (http://cegresources.icrisat.org/CicArMiSatDB/, and one (H2I20) was reported by Lichtenzveig et al. (2005).

In the present study a high number of markers for LG2 (TA27, TA59, CaGM07922) was included, to target the resistance gene associated to *Foc*5 (Castro *et al.,* 2010b). The microsatellite H2I20 located on LG5 has been associated with a gene conferring resistance to *Foc*0 (Jendoubi *et al.,* 2016). The markers CaGM14822 associated with QTL_{DF1} on LG4 and TA142 linked to QTL_{DF3} on LG3 were used for their associations with flowering time (Ali, 2015).

Microsatellite alleles were visualized by electrophoresis in 2.5% (w/v) agarose, and polyacrylamide (10%, C2, 67%) gels, or with capillary electrophoresis using an automatic capillary sequencer (ABI 3130 Genetic Analyzer Applied Biosystems /HITACHI, Madrid, Spain) at the Central Research Support Service, University of Córdoba, Spain. Data from Fragment Analysis were analyzed using the GeneMapper and the Peak Studio V2.2 software packages (McCafferty *et al.,* 2012).

Statistical analyses

Assessment of Fusarium wilt resistance

In the pathogenicity tests, the number of plants showing; i) no symptoms (healthy), ii) light symptoms (yellowing and/or loss of leaf turgidity), iii) heavy symptoms (withering), or iv) dead plant, were periodically assessed. Disease symptom data were used to calculate the Area Under the Disease Progress Curve (AUDPC) with the following equation:

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{Y_i + Y_{i+1}}{2}\right)(t_{i+1} - t_i)$$

where Y_i is Fusarium wilt severity at the i^{th} observation, t_i is time (d) at the i^{th} observation, and n is the total number of observations (Campbell and Madden, 1990).

The AUDPC data from the different assessments were subjected to ANOVA using the SPSS statistics software v.25. Means were separated by Tukey's tests.

Analyses with molecular markers

Allele frequencies of data obtained in the diversity analyses were calculated and used to determine; ì) size range, ìì) number of alleles, and ììì) Polymorphism Information Content (PIC) of each marker (Shete *et al.*, 2000). The alleles were scored as present (1) or absent (0) to create a binary data matrix. This matrix was used to calculate the degree of genetic similarity between all pairwise combinations, using the Dice coefficient of similarity. Clustering of the genotypes was determined using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Statistical analyses was performed using the NTSYS-pc 2.02j software.

RESULTS

Agronomic traits

In the field assessments, seed germination was greater than 80% for all the chickpea lines except accession 125, for which 60% of seeds germinated. Only accession 245 had an erect growth habit (value 2), whereas the other landraces had semi-erect growth habits (value 1). Landraces 111, 125, 147 and 203 had large seed sizes (100-seed weights from 42.5 to 52.4 g) while landraces 62, 245 and 184 had smaller seed sizes (100-seed weights, respectively, 27.1, 27.2, and 21.9 g). Flowering began 83 d after sowing in all landraces except for landraces 237 and 203, which flowered at, respectively, 79 and 67 d (Table 1).

Evaluation of Fusarium wilt resistance

Data collected from the pathogenicity tests were used to calculate the AUDPC index, which allows disease

progression to be compared, and reveals the presence of susceptible and resistant phenotypes. Resistance in control genotype WR315 was confirmed by the absence of *Foc* wilt symptoms (AUDPC = 0). In contrast, the *Foc* susceptible control ILC3279 showed wilt symptoms at the first assessment date, and had a final average AUDPC = 14. Among all the tested landraces, accession 203 showed complete resistance to the pathogen, and disease symptoms were absent at all the assessment dates. All the other landraces developed symptoms and were susceptible to the pathogen, with AUDPC values from 10 to 15 (Figure 1).

Molecular marker analyses

The analyses were performed using twelve microsatellite markers (Table 2). All markers revealed high levels of polymorphisms, displaying a total of 100 different alleles with fragment sizes ranging from 150 to 350 bp. The number of alleles per locus varied from two to 15, with an average value of 8.33. STMS TA142 and TA78 amplified, respectively, the minimum (two) and maximum (15) number of alleles (Table 2). Eighteen out of the 100 alleles detected in the chickpea landraces were classified as 'rare' because of their low frequency (<0.03), 69 as 'common' (0.03-0.20) and 13 were considered the 'most frequent' alleles (>0.20). Only common alleles were detected at all the 12 STMS loci studied. Rare alleles per locus ranged from one to five in TA27, TA59, TA11, TA14, TA78 and TA144. The number of common alleles per locus ranged from one (CaGM07922 and TA142) to 12 (TA11 and TA78). For the most frequent alleles, zero, one or two such alleles were detected in the majority of the STMS loci, except for TA135 in which three alleles were detected (Table 2). Based on PIC values obtained, most STMS, except for CaGM07922, TA135, TA142, CaGM14822 and H2I20, were considered informative markers (PIC >0.63). The most polymorphic marker was TA78 with a PIC value of 0.88 and 15 alleles (Table 2).

All landraces were genotyped with two markers previously associated with flowering time (CaGM14822 and TA142). CaGM14822 had three alleles of 300, 320 and 350 bp, and TA142 two alleles of 150 and 160 bp. All these alleles showed clear association with early/late flowering time. The control line WR315 (early flowering) had alleles 380 and 133 for both markers, whereas the second control line ILC3279 (late flowering) had alleles 350 and 144 for both markers (Table 3).

For analyses of the *Foc*5 resistance genes located on LG2, the markers TA27, TA59 and CaGM07922 were considered. The resistant landrace 203 showed a 230/233 bp allele for TA27, a 225 bp allele for TA59 and a 350 bp

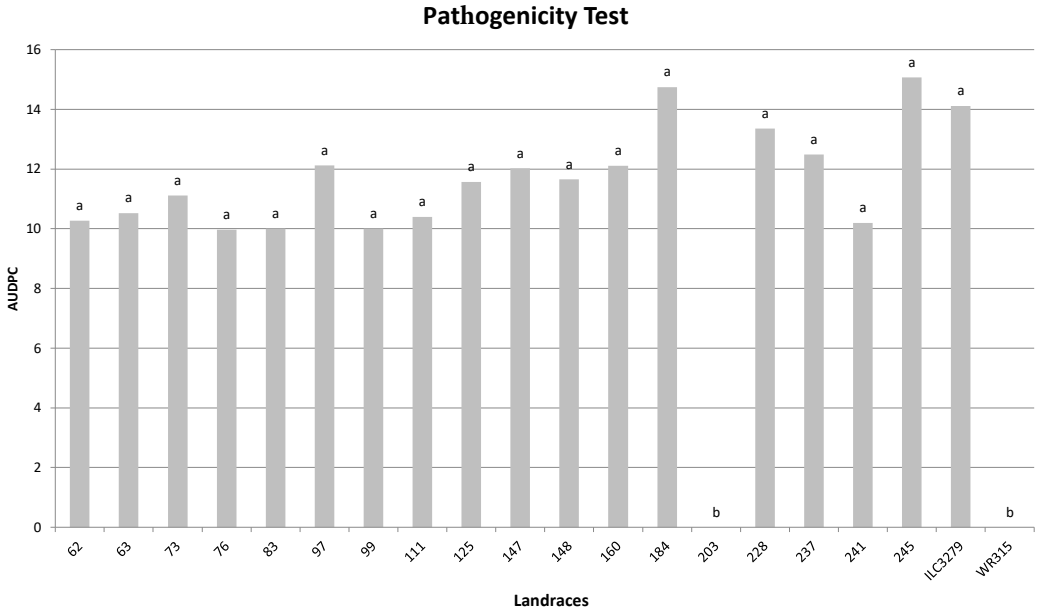

Figure 1. Average Area Under the Disease Progress Curve (AUDPC) obtained from pathogenicity tests carried out in controlled conditions on Italian chickpea landraces inoculated with *Fusarium oxysporum* f. sp. *ciceris* race 5 (*Foc*5). *Foc*5-susceptible ILC3279 and *Foc*5-resistant WR315 were included as controls. Accessions with letters in common above the columns are not significantly different ($P = 0.05$), according to Tukey's Test.

Table 2. Size ranges, numbers and frequencies of alleles and Polymorphism Information Content (PIC) observed in 18 Italian chickpea landraces, studied with 12 microsatellite markers.

Marker	Linkage Group	Size range (bp)	Nº of alleles	Rare alleles (<0.03)	Common alleles (0.03-0.2)	Most frequent alleles (> 0.2)	PIC
TA113	1	169-217	11	0	10	1	0.81
TA27	2	218-248	11	4	7	0	0.84
TA59	2	222-273	12	5	6	1	0.80
CaGM07922	2	300-350	3	0	1	2	0.34
TA142	3	150-160	2	0	1	1	0.21
TA135	3	187-199	5	0	2	3	0.59
CaGM14822	4	300-350	3	0	2	1	0.35
H2I20	5	180-230	5	0	3	2	0.39
TA11	5	220-262	14	2	12	0	0.83
TA14	6	242-278	11	3	8	0	0.82
TA78	7	191-236	15	3	12	0	0.88
TA144	8	230-254	8	1	5	2	0.73
Total			100	18	69	13	
Mean			8.33	1.5	5.75	1.08	0.63

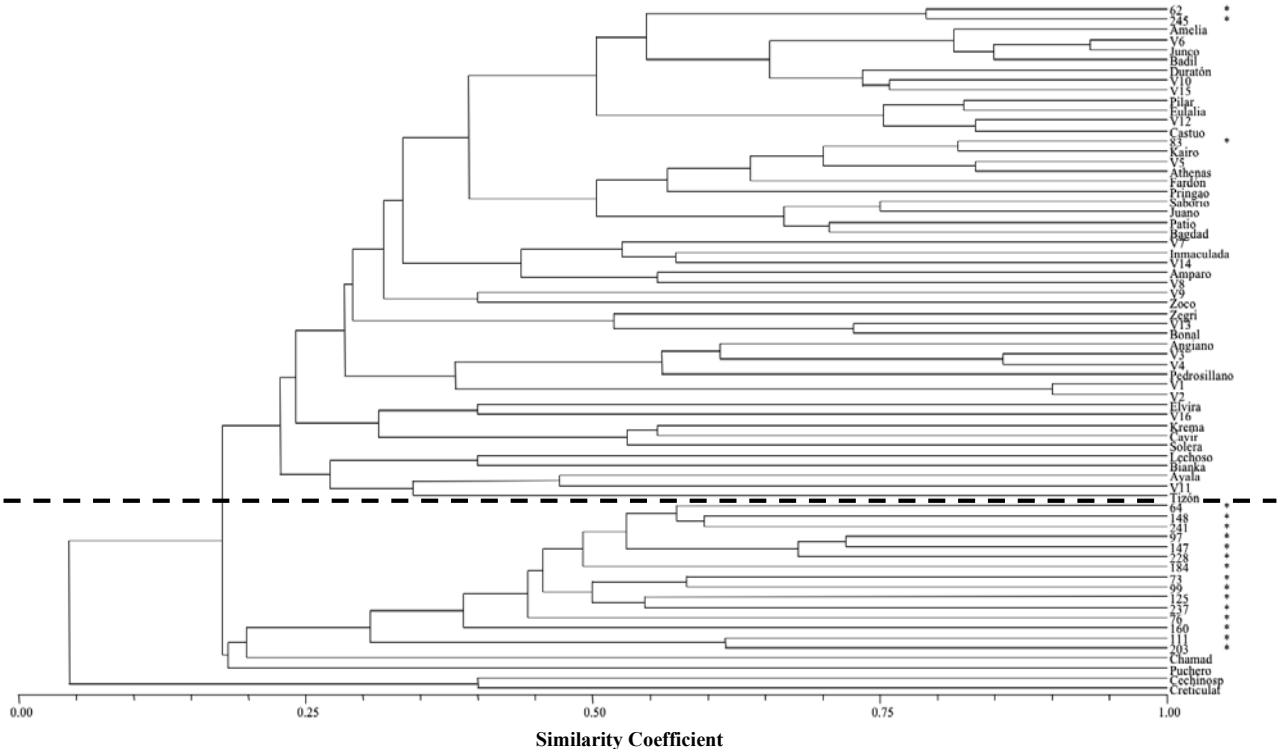

Figure 2. UPGMA dendrogram obtained from cluster analyses of 18 Italian chickpea landraces (marked with asterisks) and 48 Spanish cultivars, based on Dice coefficients of similarity, using 12 microsatellite markers selected for their Polymorphism Information Content (PIC) values. The dotted line separates two Subgroups, which mainly include Italian (Sub-group 1) and Spanish (Sub-group 2) chickpea lines. *Cicer reticulatum* and *Cicer echicnospermum* were included as outgroup controls.

Table 3. Associations between phenotypic data for flowering and resistance to *Foc*5 with microsatellite marker alleles found in the 18 Italian chickpea landraces. Microsatellite markers associated with flowering time were CaGM14822 and TA142, and with resistance to *Foc* were TA27, TA59 and CaGM07922.

Landraces	Flowering time[a]	Flowering time (days)		Resistance to *Foc*5[b]	Foc		
		CaGM14822	TA142		TA27	TA59	CaGM07922
62	T	300	150	S	236-239	246-255-273	300
64	T	300	150-160	S	221-233-242	225-234	300
73	T	300	150	S	221-227-239	231-234-237	350
76	T	300	150	S	227-230	228-234	300
83	T	350	160	S	221-236-239	243-246	350
97	T	300	150	S	221-239-242	231-234	350
99	T	300-350	150-160	S	221-227	231-234-237	300
111	T	320	150	S	221-227-230	225	300
125	T	300	150	S	221-233-236	234	350
147	T	300	150	S	239-242	222-225-234	350
148	T	300	150	S	221-227-233-236	228	300
160	T	300	150	S	221-224-230	237-240	300
184	T	300-350	150	S	221-236-239-242	246	300
203	P	320	150	R	230-233	225	350
228	T	300	150	S	221-239-242-245-248	234	320
237	T	300	150-160	S	221-230-233-236	234-237	300-350
241	T	300	150	S	218-221-227-239	225-234-237	300
245	T	300	150	S	236	252-255	300
WR315	P	380	133	R	221	225	270
ILC3279	T	350	144	S	216	234	300

[a] T = late, P = early; [b] R = high resistance, S = high susceptibility.

allele for CaGM07922. The allele displayed for TA59 in landrace 203 was also present in the resistant reference line WR315 (Table 3).

Genetic diversity analyses

The comparison between Italian landraces and Spanish cultivars revealed the presence of two genetic subgroups (Figure 2). Although with a low level of similarity of close to 0.20, subgroup 1 included 15 Italian landraces and the Spanish cultivars 'Chamad' (of unknown origin) and 'Puchero' (originating from mass selection of Spanish germplasm). Subgroup 2 included all the other Spanish cultivars and the Italians landraces 62, 83 and 245. Landraces 62 and 245, with a similarity coefficient of 0.79, were similar to the Spanish cultivars 'Amelia', 'Badil', 'Junco' and 'Duraton', with which they are grouped with a similarity coefficient of 0.55. Landrace 83 and the Spanish variety "Kairo", with a similarity coefficient of 0.83, were similar to each other, and both were grouped with the Spanish varieties "Athenas" and V5 (similarity coefficient = 0.70), that were subsequently grouped with 'Fardón', 'Pringao', 'Juano', 'Saborio', 'Bagdad' and 'Patio' (Figure 2).

DISCUSSION

Chickpea landraces could be valuable resources for improving sustainability of production on small farms of southern Italy (Negri, 2003; Crinò and Saccardo, 2008). The most important environmental and agronomic factors affecting yields are the length of cultural cycles and growth habits. Currently, the short cycle with spring sowing of chickpea crops is adopted in Italy. Although the long cultural cycle with winter sowing is potentially more productive, crop yields are often affected by adverse factors such as low seed germination rates, high disease incidence and early appearance of weeds (Rossini, 2008).

In cultivated chickpea varieties, bushy growth habit similar to that of the wild relative *Cicer reticulatum* is typical of varieties or landraces with a low amounts of selection (Cobos *et al.,* 2009). As expected, most of the tested landraces showed semi-erect growth habits (Table

1). Most of these landraces were selected in mountainous or hilly areas, where chickpeas are usually produced in marginal and minimally mechanized cropping systems. As a consequence, growers primarily selected chickpea seeds based on seed size and yield. In modern agriculture, growth habit is important for mechanical harvesting (Cobos *et al.,* 2009) and to escape weeds which are common in winter production (Rossini, 2008).

Landrace 203 ('Longano') flowered 67 d after sowing and was the earliest flowering line (Table 3). Early flowering, not affected by photoperiod and typical of plants selected and developed at low latitudes, results in a biological cycle that is 1 month shorter than for other genotypes. Early flowering genotypes escape summer drought stress at high latitudes, resulting in increased yields (Cobos *et al.,* 2009). Based on the knowledge acquired so far, there are no other Italian ecotypes with a flowering trait as early as 67 d. Flowering time is an important trait to increase profitability from chickpea crops, and early flowering allows the plants to escape biotic and abiotic stresses (Semere Mallu *et al.,* 2014). Furthermore, in the Mediterranean basin which is characterized by frequent water stress in the summer, the early flowering phenotypes can allow farmers to obtain increased yields (Siddique and Loss, 2003; Rubio *et al.,* 2004).

Morphological analyses carried out in the present study showed the potential improving chickpea landraces for traits needed to increase yields, particularly growth habit and flowering time. These improvements could be pursued by crossing different landraces to introduce required characteristics without losing the unique genetic characteristics of these ecotypes.

Most of the evaluated landraces were very susceptible to *Foc*5. The exception was landrace 203 ('Longano'), which was highly resistant to the pathogen (AUDPC = 0), and similar to the resistant ecotype WR315 used as control (Table 3 and Figure 1). Genetic resistance to *Foc*5 is conferred by a single gene located on LG2 of the chickpea map (Castro *et al.,* 2010a). High resistance to *Foc*5 in landrace 203 was observed previously in preliminary assays carried out in naturally infested experimental fields at two different locations in southern Spain (Córdoba and Escacena del Campo) (data not shown).

Differences in disease severity among the other landraces studied here are unlikely to be associated with different degrees of genetic resistance to *Foc*5, but are more likely to be from the effect of different responses to specific functions involved in the wilt stress. These may include uptake of iron and other nutrients, and response to water deficiency (Blum, 2017).

Among the tested landraces, accession 203 ('Longano') showed valuable agronomic traits, including large seed size, early flowering and, particularly, a high level of genetic resistance to *Foc*5, which is considered the most aggressive chickpea pathogen worldwide. Based on our findings, landrace 203 could satisfy the current high demand for local products by consumers, and could be a good candidate for large-scale and wide-spread use in agriculture.

Our results with STMS markers did not allow establishment of relationships between different alleles and phenotypic traits, such as flowering time with resistance to *Foc*5. The prediction of the resistance alleles with STMS markers is complex because they show extensive polymorphisms within species. Therefore, their use is not recommended for screening germplasm collections (Madrid *et al.,* 2014). Haplotypes with SNP markers characteristic of the main sources of disease resistance should also be used (Caballo *et al*., 2019). However, in the case of TA59, tightly linked to *Foc* resistance and used previously in breeding programmes (Castro *et al.,* 2010b), the alleles present in resistant and susceptible controls, respectively, were also present in the resistant landrace 203 and the susceptible line 228 (Table 3).

The study of similarity among Italian landraces and Spanish cultivars, based on molecular markers, showed that the two subgroups, in most of cases, correlated with geographic origins. Some exceptions were found in subgroup 1, where two Spanish cultivars joined with Italian landraces, and in subgroup 2, where three Italian landraces joined with the Spanish cultivars (Figure 2). This genetic similarity could be explained by historic germplasm exchanges during the Spanish domination of southern Italy during the 15th to 18th Centuries.

Future chickpea improvement programmes aimed at conservation and promotion of genetic resources should consider these landraces as good genetic resources for selection and/or breeding. In addition, our results highlight the potential of local varieties to be improved and exploited as productive and profitable crops even in marginal areas, stimulating the farmers of these areas to expand the cultivation of chickpea, and to provide alternative crops in the cropping systems.

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