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RESEARCH ARTICLE

Characterization of Italian lentil (*Lens culinaris* Medik.) germplasm by agronomic traits, biochemical and molecular markers

Massimo Zaccardelli · Francesco Lupo · Angela Rosa Piergiovanni · Gaetano Laghetti · Gabriella Sonnante · Maria Gloria Daminati · Francesca Sparvoli · Lucia Lioi

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Abstract Genetic relationships, agronomic, nutritional and technological traits of ten Italian landraces, two improved lines and two cultivars of lentil (*Lens culinaris* Medik.) were investigated using a multidisciplinary approach. Seed storage proteins, used as biochemical markers, were able to detect polymorphisms with variability mainly related to the polypeptide abundance. Microsatellite (SSR) molecular markers provided very useful information on genetic variation and relationships among landraces, with polymorphic fragments able to discriminate all the accessions. Lentil landraces were grouped in different clusters and sub-clusters principally on the basis of their geographical origin. The highest levels of genetic diversity were observed for lentils from 'Castelluccio di Norcia', 'Colliano' and 'Villalba'. Field trials, performed in two locations of Southern Italy, revealed a high influence of location on yield. Comparing performances at both tested locations, the best landraces were 'Linosa' and 'Valle di Nevola' suggesting that these have the highest adaptability. Technological and nutritional data together with the agronomic ones evidenced that 'Linosa' lentil is the best landrace, however also 'San Gerardo' deserves some attention.

Keywords Cooking test · Hydration index · Landraces · *Lens culinaris* · Seed storage proteins · SSR markers

M. Zaccardelli · F. Lupo
CRA-Centro di Ricerca per l' Orticoltura,
Via dei Cavalleggeri 25, 84098 Pontecagnano, SA, Italy

A. R. Piergiovanni · G. Laghetti · G. Sonnante · L. Lioi
CNR-Istituto di Genetica Vegetale, Via Amendola 165/A,
70126 Bari, Italy

M. G. Daminati · F. Sparvoli
CNR-Istituto di Biologia e Biotecnologia Agraria,
Via Bassini 15, 20133 Milan, Italy

Present Address:
M. Zaccardelli (✉)
CRA-Centro di Ricerca per l' Orticoltura,
Azienda Sperimentale di Battipaglia,
SS 18 204, 84091 Battipaglia, SA, Italy
e-mail: massimo.zaccardelli@entecra.it

Introduction

Lentil (*Lens culinaris* Medik.) is a grain legume originated in the Near East (Zohary 1972; Sonnante et al. 2009) widely cultivated in the world because largely appreciated by consumers. This legume crop is well suited for low input cultivation in marginal areas and produces seeds with a high protein content (up to 26–27%); nevertheless, its value is often compromised by low levels of grain yield (Avola et al. 2001). For example in Italy, in the period 2006–2009, lentil cultivation had a medium yield of about 0.67 t ha^{-1} while in the same period chickpea and common bean yields were about 1.32 and 1.71 t ha^{-1}, respectively (ISTAT 2006–2009).

At present, the surface devoted to lentil cultivation in Italy, is much lower than that of the last century. The progressive reduction registered in the last 60 years is attributable to various reasons, such as low yield and yield stability in different environmental conditions, low market price, poor mechanization of cultural steps, etc. The evolution of Italian agriculture, which occurred during the past 60 years, has transformed Italy from a producer to an importer of this grain legume (Piergiovanni 2000). At present, the cultivated area is 1,813 ha, for a total production of 1,337.5 t, not sufficient to satisfy Italian consumes (ISTAT 2008).

In the last decades some breeding programs started giving major attention to lentil. Objectives of these programs are the constitution of improved varieties with higher potential productivity, higher yield and good productive stability, high seed quality, resistance to abiotic and biotic stress; plant structure adapted to mechanization, resistance to lodging, ability to fit to soil and climatic conditions of cultivation area (Chisci and Tallarico 1994).

Lentil cultivation in Italy is mainly based on landraces, genetic material empirically selected by farmers over time and well adapted to the agro-environments in which they have been cultivated for long time (Laghetti et al. 2008). They usually take their name from the area where they are traditionally cultivated (Foti 1982). The most famous landraces are 'Castelluccio di Norcia' and 'Colfiorito' (Umbria Region), 'Fucino' and 'Santo Stefano in Sessanio' (Abruzzo), 'Leonessa', 'Onano' and 'Ventotene' (Lazio), 'Altamura', mainly the *macrosperma* type (Apulia); 'Mormanno' (Calabria); 'Villalba', 'Ustica', 'Pantelleria', 'Linosa' (Sicily). Some of these landraces are much appreciated as niche or speciality products and survive on farm, in marginal areas being exposed to a strong risk of genetic erosion and/or extinction (Piergiovanni 2000). Nowadays only lentil from 'Castelluccio di Norcia', which obtained the Protected Geographic Indication (PGI) by the European Community (EC Reg. no. 1065/97), has a consolidate market position.

Generally plant and seed morphological traits are not sufficient to characterize lentil landraces, therefore molecular and biochemical markers are also used for a better description. Electrophoretic polymorphisms of seed storage proteins, especially 11S legumin and 7S vicilin, have been used in many legume species for germplasm description and identification (Gepts et al. 1986). These storage proteins have also been reported as good markers for polymorphism detection among different genotypes (Gepts and Bliss,1986; March et al. 1987; Staswick et al. 1983). Polymorphisms of seed storage proteins within and among Italian lentil populations have been investigated by SDS–PAGE evidencing a high genetic variation within this germplasm (Piergiovanni and Taranto 2005). This genetic variation is observed mainly in polypeptides with a molecular weight around 97 kDa and in the range 55–45 kDa and is due to differences in polypeptide number and/or intensity. Moreover, Piergiovanni and Taranto (2005) showed that small-seeded types were more polymorphic than the large ones. These data were confirmed by a very recent work in which Scippa et al. (2010) analysed the seed proteome of different lentil landraces and showed that most of the 24 protein species essential for population discrimination were major storage proteins, namely 7S (vicilins and convicilins) and 11S (legumins) globulins.

Previous studies on molecular characterization performed on a lentil collection from Mediterranean countries using ISSR (Inter Simple Sequence Repeat) markers also showed a large genetic variation within this grain legume (Sonnante and Pignone 2001). More recently, ISSRs have been used to assess molecular diversity and possible origin of Italian lentil landraces (Sonnante and Pignone 2007; Fiocchetti et al. 2009). Among molecular markers, SSRs (Simple Sequence Repeats) or microsatellites, have been shown to produce polymorphism from a different number of repetitive core motifs present at one *locus*, and are a valid tool for landrace fingerprinting (Hamwieh et al. 2009). Five highly polymorphic SSR markers have been successfully used to fingerprint and assess genetic diversity in a number of cultivated lentil from Central Asia and Caucasian countries (Babayeva et al. 2009). A few number of SSRs were able to provide significant insights on genetic diversity in 25 lentil accessions from different areas (Bacchi et al. 2010).

In this study, genetic diversity and relationships among ten Italian lentil landraces, two improved lines and two cultivars (cvs) were investigated using seed storage protein patterns and SSR markers. Moreover, agronomic, nutritional and technological traits were evaluated to identify the material better performing in southern Italian environments.

Materials and methods

Plant materials

Analyses were performed on fourteen lentil accessions belonging to *microsperma* morphotype, with the exception of 'Villalba' lentil, a *macrosperma* type. In particular, ten were landraces collected in Southern and Central Italy (Fig. 1); two were selected lines (L 13VT and L 16VT) obtained at "Università della Tuscia", Viterbo (Italy); two were cvs registered in Italy (Gaia and Itaca).

Field trials and agronomic characterization

Agronomic characterization was performed growing the 14 lentil accessions in two experimental fields located in Southern Italy (Fig. 1). The first one was at the experimental farm of CRA-ORT at Battipaglia (Sele Valley, Campania Region), about 65 m above the sea level. This is an intensive horticultural area, 7 km far from the Tirreno sea coast, characterized by an annual mean rainfall of 947 mm and an annual mean temperature of 16.6°C (30-year average data). The second field was located at the experimental farm of Basilicata University, at Guardia Perticara (Agri Valley, Basilicata Region), about 720 m above the sea level, in an internal hillside and marginal environment, characterized by an annual mean rainfall of 646 mm and an annual mean temperature of 13.7°C (thirty-year average data). The climatic parameters of the trials were in the range recorded in the last 30-years. The 14 lentil accessions were characterized by a complete randomised block design with 4 repetitions (plots of 3.2 sq m); density was 143 seeds sq m^{-1}, obtained adopting distances of 20 cm among the rows and 3.5 cm on the rows. Sowing was performed on January 10th at Battipaglia, and on April 5th 2006 at Guardia Perticara. No manure was supplied in both locations. Harvestings were performed in the third decade of June at Battipaglia and in first decade of July at Guardia Perticara. For both locations, the following bio-agronomic traits were recorded at flowering time: number of lower branches (NLB), number of flowers (NF) and number of pods (NP); while at harvest time they were: plant height (PH), distance of the first pod from ground (DFP), number of seeds/pod (NSP), weight of 1000 seeds (WS) and seed yield (YLD).

Fig. 1 Italy and its regions. Regions of provenance and names of the lentil landraces used in this study are indicated. Experimental field: *B* Battipaglia; *G* Guardia Perticara

Statistical analyses

For all bio-agronomic traits, descriptive statistics were calculated, together with Pearson correlation coefficient and the analysis of variance (ANOVA) adopting the GLM procedure of the statistical package SAS 9.1 (2002–2003). To compare averages of single accessions, the test on multiple comparisons by Student–Newman–Keuls (SNK) was used. Principal component analysis (PRINCOM) on the average standardized values was also carried out to study the structure of variation of the studied landraces. A cluster analysis was performed with the CLUSTER procedure using the Ward's minimum variance hierarchical method.

Seed quality

About 100 g of dry seeds of each accession were taken from the bulk harvested in the experimental fields. Seeds were ground in a Cyclotec 1093 mill Tecator (Sweden) to give a fine meal used for the measurements. Moisture was determined by loss of weight after meal drying in an oven according to the method 930.15 (AOAC 1970); protein contents (N × 6.25) were determined by the Kjeldahl method 979.09 (AOAC 1970). Technological traits evaluated on whole dry seeds were: coat percentage, hydration index, swelling index and cooking time. Coat percentage, calculated in relation to the whole seed weight, was measured from 30 soaked seeds by

separating manually the coats and keeping them in a lyophilisator overnight. Hydration index at time *t*, expressed as percentage, was measured at room temperature according to Onayemi et al. (1986). All samples were analysed in duplicate and the average result is assumed as moisture at each tested time. Swelling index was calculated as the ratio of the volume difference of soaked and unsoaked seeds and the unsoaked seed volume. Volume of 30 seeds was measured by displacement of 96% (v/v) ethyl alcohol, at beginning and after 24 h of soaking in water at room temperature. Six grams of dry seeds were used for cooking test. After 20 min of cooking, softness of ten seeds was checked. The test was repeated every 2 min until complete cooking.

Protein extraction and SDS/PAGE

Total seed proteins were extracted from flour, obtained grinding a bulk of 10 seeds from each accession, using 20 volumes of 20 mM borate buffer (pH 9) for 2 h at 4°C and recovered in the supernatant of a 20 min centrifugation at 10.000*g*. Protein extracts were heat denatured in reducing conditions (20 mM Tris–HCl, pH 8.6, containing 1% SDS, 8.3% glycerol and 0.5% β-mercaptoethanol) and then separated on 15% SDS/PAGE (Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis), as described by Bollini and Chrispeels (1978). Gels were stained with Comassie Brilliant blue.

DNA extraction and SSR markers

Plants were grown in a greenhouse and DNA was extracted from young leaves of ten single plants *per* accession using the CTAB method described by Paz and Veilleux (1997). DNA concentration was assessed by agarose gel electrophoresis in comparison with a quantitative reference marker.

Sixteen primer pairs, reported to amplify microsatellite regions in lentil (Hamwieh et al. 2005), were used (Table 1). One primer for each pair was fluorescently labelled (Sigma–Aldrich, USA), so that amplified fragments could be visualized on an automated sequencer (CEQ 8800, Beckman-Coulter, Fullerton, CA, USA) and allelic variants at each *locus* studied determined.

PCR was carried out in a total reaction volume of 25 µl containing 1.25 U Taq DNA polymerase (5 PRIME, Germany), 10 mM Tris–HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.25 mM of each dNTPs (5 PRIME, Germany), 0.05 µM of each primer and 20 ng of template DNA. PCR was carried out using a PE 9700 Thermo Cycler (PerkinElmer, USA). Amplifications were programmed for an initial step at 95°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at the required Tm for 30 s and elongation at 72°C for 45 s, followed by a final elongation step at 72°C for 5 min.

Table 1 List of 16 microsatellite *loci* for lentil, temperature used (*Tm*), fragment sizes, and number of alleles detected

Locus name	Core motif	*Tm*[a]	Fragment size range (allele n.)
SSR19	$(TG)_{14}$	56	263–277 (6)
SSR33	$(CA)_{21}(GA)_{25}$	54	243–304 (17)
SSR48	$(TG)_{13}$	56	164–189 (10)
SSR80	$(TC)_{14}(AC)_{12}(AT)_2$	54	135–159 (10)
SSR96	$(TG)_{10}$	49	207–213 (2)
SSR99	$(TG)_8TC(TG)_2$	56	155 (1)
SSR113	$(AC)_{17}(AT)_{13}$	51	213–279 (22)
SSR119	$(TA)_4TT(TA)_{11}(TG)_{19}$	49	246–288 (16)
SSR124	$(TGC)_3 + (GT)_9TA(TG)_2$	51	174–176 (2)
SSR130	$(GT)_9$	54	195–197 (2)
SSR156	$(TC)_2(TG)_{13}$	52	168–195 (10)
SSR167	$(TA)_{16}(TG)_{21}$	52	109–174 (19)
SSR184	$(GT)_{10}(AT)_{15}(GT)_{19}$	53	248–282 (12)
SSR204	$(TG)_4\cdots(AC)_{7\ \text{imperfect}}$	51	188–198 (5)
SSR212-1	$(AT)_2(TC)_{26}(AC)_8$	49	162–211 (17)
SSR323	$(AT)_{22}(CA)_4$	51	212–329 (17)

Primer design is as reported by Hamwieh et al. (2005)

[a] The calculated *Tm* (°C) values are based on a salt concentration of 50 mM

SSR data analysis

The average number of alleles observed per locus (n_o), the effective number of alleles (n_e) and the percent of polymorphic loci (5% criterion) were computed. The genetic diversity computed as $H = 1 - \Sigma p_i^2$, where p_i is the frequency of the *i*th allele at each *locus*, is equivalent to the expected heterozygosity (H_e) (Nei 1978). This value provides an estimation of the probability that two individuals, taken at random from a panmictic population, will have different alleles. Based on allelic frequencies,

Table 2 Grain yield (g/sq m) recorded at Battipaglia and Guardia Perticara for the tested lentil accessions

Accession	Yield (g/sq m)	
	Battipaglia	Guardia Perticara
San Gerardo	280.4 *a*	21.2 *ab*
Gaia	275.4 *a*	30.0 *ab*
Linosa	251.2 *ab*	50.1 *a*
Valle di Nevola	241.6 *abc*	33.7 *ab*
L 13VT	197.8 *abcd*	37.1 *ab*
Altamura	171.4 *bcde*	20.8 *ab*
Villalba	162.4 *cde*	43.3 *ab*
Miccula	149.2 *de*	27.2 *ab*
L 16VT	146.4 *de*	7.5 *b*
Castelluccio di Norcia	143.5 *de*	43.6 *ab*
Itaca	140.0 *de*	19.8 *ab*
Colfiorito	129.6 *de*	37.2 *ab*
Mormanno	124.0 *de*	21.8 *ab*
Colliano	81.4 *e*	14.3 *ab*
Mean	178.0	29.1

Values followed by the same letter within a column are not significantly different ($P > 0.05$)

Nei's distance matrix was used to construct a UPGMA dendrogram by means of the software POPGENE version 1.32 (Yeh et al. 1999).

Results

Grain yield and other bio-agronomic traits

Grain yield of all lentil accessions (Table 2) was strongly influenced by environment and sowing data; it was higher at Battipaglia (mean 178 g sq m^{-1}) and lower at Guardia Perticara (mean 29.1 g sq m^{-1}). Strong yield differences were observed among lentil accessions for both environments (Table 2). Accessions that showed the highest grain yield were 'San Gerardo', 'Gaia' and 'Linosa' at Battipaglia; 'Linosa', 'Castelluccio di Norcia' and 'Villalba' at Guardia Perticara. The accessions with the lowest grain yield were 'Colliano' at Battipaglia and L 16VT line at Guardia Perticara.

If average data of the two locations are considered, 'Villalba' had the highest weight of 1,000 seeds (WS) (65 ± 2 g) and 'Mormanno' the lowest (15 ± 5); 'Colliano' showed the tallest plants (PH) (39 ± 2 cm) and 'Linosa' the shortest (25 ± 1 cm); 'Miccula' had the highest number of flowers (NF) (54 ± 5) while 'Linosa' the lowest (13 ± 1); 'San Gerardo' was characterized by the highest number of pods (NP) (55 ± 6) and L 16VT and 'Colliano' by the lowest (16 ± 2 and 17 ± 4 respectively); for 'Colliano', the best distance of the first pod from ground (DFP) (26 ± 1 cm) was recorded (data not shown).

According to ANOVA analysis (Table 3), accessions, locations and 'accessions *X* locations' interaction were different for yield and all bio-agronomic traits analysed, except for number of lower branches (NLB), that was different for accessions only. In particular, the effect of the 'location' was predominant on the other sources of variation for the traits NF, NP and seed yield (YLD), as well as the 'accessions *X* locations' interaction was predominant on the other sources of variation for PH and DFP.

Table 3 Analysis of variance for tests of significance of differences among accessions, locations and their interaction, for six traits in a collection of 14 Italian lentil accessions

Source of variation	Error *df*	Mean square					
		NLB	NF	NP	PH	DFP	YLD
Accessions (A)	13	2.5***	12,105***	10,025***	1,059***	1,138***	8,718***
Locations (L)	1	0.1	217,945***	499,673***	128,630***	32,794***	610,510***
A × L	13	0.3	6,500***	9,560***	261***	400***	6,901***
Error	1,079[a]						

NLB number of lower branches, *NF* number of flowers, *NP* number of pods, *PH* plant height, *DFP* distance of the first pod from ground, *YLD* seed yield

*, **, *** Significant at $P = 0.05$, 0.01 and 0.001, respectively

[a] 888 for PH, 864 for DFP, 82 for YLD

Table 4 Pearson's correlation coefficients among seven traits in a collection of 14 Italian lentil accessions

	NF	NP	PH	DFP	YLD	WS
NLB	0.26***	0.13***	0.09**	0.09**	0.21*	−0.17
NF	–	0.47***	0.42***	0.38***	0.18	0.14
NP		–	0.42***	0.24***	0.48***	0.31*
PH			–	0.79***	0.61***	0.27*
DFP				–	0.36***	0.28*
YLD					–	0.25

NLB number of lower branches, *NF* number of flowers, *NP* number of pods, *PH* plant height, *DFP* distance of the first pod from ground, *YLD* seed yield, *WS* weight of 1,000 seeds

*, **, *** Significant at $P = 0.05$, 0.01 and 0.001, respectively

Analysis of correlations

Table 4 shows Pearson correlation coefficients among bio-agronomic traits; NLB, NF, NP, PH, DFP and YLD were all positively and significantly correlated to each another, except for YLD/NF. Weight of 1000 seeds (WS) was positively and significantly correlated only with NP, PH and DFP, while it was positively, but not significantly, correlated with NF and YLD and negatively, but not significantly, with NLB.

Multivariate analysis

Table 5 shows relative and per cent proportions of the total variance for each of the first three principal components, the calculated eigenvalues and the coefficient of correlations between the principal components (PRIN1, PRIN2 and PRIN3) and the original variables; these coefficients indicate the contribution of each trait to the formation of PRIN1, PRIN2 and PRIN3.

Table 5 Correlation between the first three principal components (PRIN) and original variables for a collection of 14 Italian accessions of lentil

Trait	PRIN1	PRIN2	PRIN3
NLB	−0.004	0.01	0.003
NF	−0.23	0.61	0.36
NP	0.20	0.42	0.61
PH	−0.10	0.13	−0.07
DFP	−0.10	0.12	−0.11
YLD	0.93	−0.01	0.04
WS	0.13	0.65	−0.70
Eigenvalue	1,258	148	88
Proportion of total variance	0.82	0.10	0.06
Cumulative variance	0.82	0.91	0.97

NLB number of lower branches, *NF* number of flowers, *NP* number of pods, *PH* plant height, *DFP* distance of the first pod from ground, *YLD* seed yield, *WS* weight of 1,000 seeds

The first three principal components explain 97% of the total variance; in particular PRIN1 contributing with 82%, PRIN2 with 10% and PRIN3 with 6%. PRIN1 is mostly positively correlated with YLD (0.93) and NP (0.20) and negatively correlated with NF (−0.23); PRIN2 is mostly positively correlated with WS (0.65), NF (0.61), NP (0.42) and PRIN3 is correlated with WS (−0.70), NP (0.61) and NF (0.36).

PRIN1 and PRIN2 (92% of the total variance) were used to obtain the diagram of dispersion (Fig. 2) for all the fourteen lentil accessions giving the picture of the differences among the three groups of genotypes. The first group includes 'Mormanno' and 'Colliano' landraces and the L 16VT line; the second one includes 'Villalba', 'Miccula', 'Altamura', 'Castelluccio di Norcia', 'Colfiorito' landraces and the 'Itaca' cv; the third one is formed by 'San Gerardo', 'Valle di Nevola', 'Linosa' landraces, the L 13VT line and the 'Gaia' cv.

Seed quality

An amount of seeds sufficient to carry out a detailed nutritional and technological evaluation was obtained only at Battipaglia. Results of these analyses are resumed in Table 6. As expected, some seed quality traits (see 1,000 seed weight, cooking time and swelling index) recorded for 'Villalba' lentil strongly diverged from the other accessions since this is the

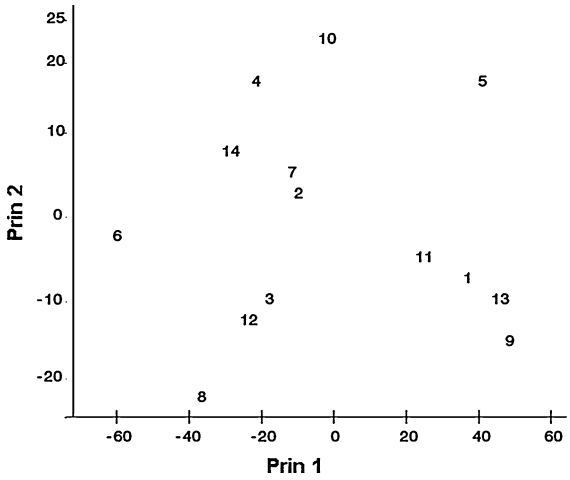

Fig. 2 Scatter diagram of the first two principal component mean values for the fourteen lentil accessions. *1* Valle di Nevola; *2* Castelluccio di Norcia; *3* Colfiorito; *4* Miccula; *5* San Gerardo; *6* Colliano; *7* Altamura; *8* Mormanno; *9* Linosa; *10* Villalba; *11* L 13VT line; *12* L 16VT line; *13* Gaia; 14: Itaca

only *macrosperma* biotype included in this study. A narrow range of variation was recorded for coat amount and cooking time among the *microsperma* tested. Conversely, protein content, hydration and swelling indices appeared to be highly variable among the tested materials. Although all accessions showed a medium–high protein content, only lentils from 'Colfiorito' and 'Colliano' showed values comparable with that of cv. 'Itaca', which in turn had a protein content significantly superior to that of cv. 'Gaia'. The very low value recorded for L 13VT makes this line poorly attractive from a nutritional point of view.

Major seed storage protein analysis

Major storage proteins in lentil seeds are represented by 7S globulins, with Mr of 45 and 50 kDa and 11S globulins which are made up by an acidic (40 kDa) and a basic (20 kDa) subunits linked by disulfide bonds (Saenz de Miera and Perez de la Vega 1998; Scippa et al. 2010). We detected most of the variation in the electrophoretic in the range between 42 and 60 kDa (circle and bar in Fig. 3), where most of 7S and 11S polypeptides migrates, and around 97 kDa (asterisk in Fig. 3). The variability we observed is mainly related to the abundance of specific polypeptides, although in some cases also slight differences in the electrophoretic mobility could be detected, confirming findings reported by other authors (Piergiovanni and Taranto 2005; Scippa et al. 2010). The most diverse landrace resulted 'San Gerardo' (Fig. 3, lane 5) in which a major polypeptide of about 60 kDa (circle in Fig. 3) is less abundant and similar in amount to a second slower migrating one detected only in this sample. Landraces

Table 6 Results of physic-chemical and nutritional seed traits evaluated on the tested landraces of lentil cultivated at Battipaglia in 2006

Accession	1,000 Seed weight (g)	Coat (g kg^{-1})	Protein (g kg^{-1})	Cooking time (min)	Hydration index (%)[a]	Swelling index (%)
Valle di Nevola	40.7	68.6	229	30	25.3	50
Castelluccio di Norcia	27.8	68.3	254	24	26.5	43
Colfiorito	28.2	63.9	271	24	28.2	80
Miccula	35.7	67.6	245	28	37.6	50
San Gerardo	31.8	67.4	241	28	42.0	38
Colliano	33.8	68.0	260	24	32.7	50
Altamura	34.1	70.0	255	28	37.5	71
Mormanno	29.2	70.3	250	26	45.4	67
Linosa	30.6	67.4	245	24	22.0	57
Villalba	67.0	72.0	250	40	45.2	112
L 13VT	32.0	69.4	218	28	45.2	83
L 16VT	31.3	71.0	245	28	37.0	100
Gaia	36.6	67.5	238	30	32.7	86
Itaca	29.2	66.4	271	26	45.4	43

[a] Hydration index measured after 2 h of soaking

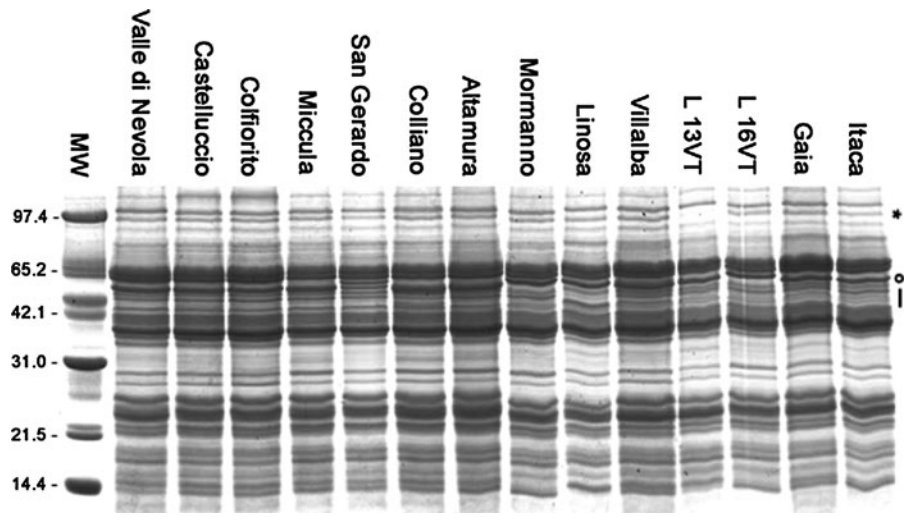

Fig. 3 SDS–PAGE analysis of total seed storage proteins of fourteen Italian lentil accessions

'Castelluccio di Norcia' and 'Colfiorito' have an almost identical electrophoretic pattern, which appears to be very similar to that of 'Valle di Nevola', 'Altamura', 'Colliano', 'Miccula' and 'Linosa' (Fig. 3, lanes 2, 3 and 1, 4, 6, 7, 9, respectively). 'Villalba' showed a pattern more similar to the two cultivated varieties 'Gaia' and 'Itaca' and the line L 13VT (Fig. 3, lanes 10, 13, 14 and 11 respectively), while 'Mormanno' was more similar to L16VT line, since in both cases the polypeptide of about 60 kDa (circle) is migrating a little bit slower than in the other samples (Fig. 3, lanes 8 and 12).

SSR markers

All the used SSR primer pairs produced an amplification fragment of the expected length. A total of 168 alleles were scored, ranging from 1 to 22 alleles *per locus*. Polymorphisms were observed for fifteen *loci*, and numbers and length range of alleles for each primer pairs are reported in Table 1. Example of electropherograms showing different alleles at the *locus* SSR204 are reported in Fig. 4. A particularly high level of polymorphism was observed at SSR113 (22 alleles) and SSR167 (19 alleles) *loci*. The

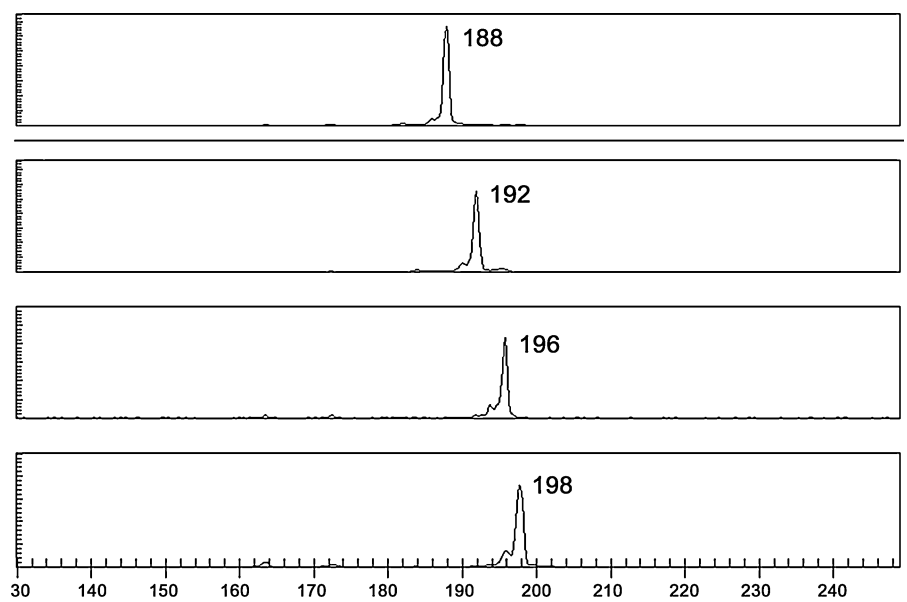

Fig. 4 Example of electropherograms showing different alleles at the *locus* SSR204

Table 7 Statistics of genetic diversity related to SSR markers for each lentil accessions examined

Accession	n_o^a	n_e^b	P^c	H_e^d	H_o^e
Valle di Nevola	2.56	1.62	81.25	0.365	0.000
Castelluccio di Norcia	3.50	3.03	75.00	0.550	0.008
Colfiorito	2.75	2.08	56.25	0.301	0.031
Miccula	1.31	1.14	31.25	0.100	0.000
San Gerardo	1.62	1.21	62.50	0.158	0.008
Colliano	2.62	1.70	87.50	0.387	0.078
Altamura	1.31	1.19	31.25	0.125	0.000
Mormanno	2.19	1.52	68.75	0.263	0.015
Linosa	1.75	1.47	50.00	0.220	0.023
Villalba	3.12	2.62	68.75	0.422	0.008
L 13 VT	1.06	1.04	6.25	0.025	0.000
L 16 VT	1.06	1.04	6.25	0.025	0.000
Gaia	1.00	1.00	0.00	0.000	0.000
Itaca	1.00	1.00	0.00	0.000	0.000

[a] Mean number of observed alleles

[b] Mean number of effective alleles

[c] Percentage of polymorphic loci (5% criterion)

[d] Expected heterozygosity

[e] Observed heterozygosity

diversity parameters were very low or null for the selected lines and cvs. respectively. Conversely, all the landraces investigated showed quite high values for all the genetic diversity parameters (Table 7). The landraces showed a mean of 2.27 observed alleles *per locus* and a mean value of polymorphic loci (P, 5% criterion) equal to 61.25%. The mean genetic diversity or expected heterozygosity (H_e) was 0.29. The deviation of observed from expected heterozygosity (Table 7) might be due to the prevalent autogamous breeding system of lentil (Sonnante and Pignone 2001). The results indicate that the examined landraces retain a high level of genetic diversity. The highest values were registered for 'Castelluccio di Norcia', 'Colliano' and 'Villalba', while 'Miccula' and 'Altamura' resulted more uniform. Pairwise Nei's genetic distances (Table 8) were comprised between 0.036 ('Altamura' and 'Miccula') and 1.662 ('Gaia' and Itaca').

The UPGMA dendrogram (Fig. 5) based on Nei's genetic distances as defined by SSR markers, showed that some landraces were grouped on the basis of their geographical origin: this is the case of 'Castelluccio di Norcia' and 'Colfiorito' (Umbria region) on

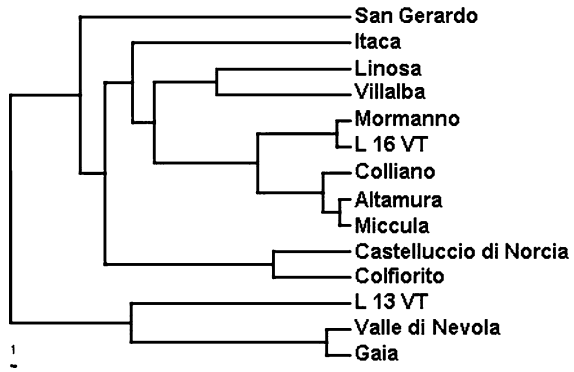

Fig. 5 UPGMA tree based on Nei's genetic distances obtained from SSR markers showing relationships among the lentil accessions examined

one side, and 'Linosa' and 'Villalba' (Sicily region) on the other side. 'Mormanno' landrace was closely related to the selected line L 16VT, suggesting that these two materials share a common genetic background, and both were grouped with 'Colliano', 'Altamura', and 'Miccula' landraces. The cv 'Gaia' was genetically closely related to 'Valle di Nevola' landrace and these last two lentils, together with the selected line L 13VT, were quite distant from the other materials analysed, thus indicating a distant genetic relationships among these groups (see Table 8).

Discussion

As in other lentil trials (Sarker et al. 2010), agronomic results showed a strong effect of the environment mainly on plant growth and production. Guardia Perticara is a hilly and marginal environment, very cold in winter and with a high oscillation in temperature in spring. This climatic conditions drastically reduced grain yield compared to Battipaglia location (Table 2). Moreover, sowing time at Guardia Perticara was about three months later than at Battipaglia, thus further affecting phenology and grain yield. Agronomic results indicated more uniform yields at Guardia Perticara than at Battipaglia, most likely due to the limiting growing conditions, while at Battipaglia large differences in grain yield were registered between 'Colliano' (lowest yield) and 'San Gerardo', 'Gaia', 'Linosa', and 'Valle di Nevola' (highest yields). Comparing performances at

both sites the best landraces were 'Linosa' and 'Valle di Nevola' suggesting that these are the genotypes with the highest adaptability.

In general, in the present field trials, landraces yields were higher than those of varieties and selected lines only in limiting conditions (averages at Guardia Perticara: 31.3 g sq m^{-1} vs. 23.6 g sq m^{-1}). On the contrary, in an optimal growing environment, varieties and selected lines gave better yields than the landraces (averages at Battipaglia: 189.9 g sq m^{-1} vs. 173.5 g sq m^{-1}). These findings are in accordance with the data of Avola et al. (2001), who showed that, in a 2-year (2000–2001) field trial carried out in Sicily, landraces gave a better agronomic performance than foreign varieties.

Nutritional and technological analyses indicated that high protein content together with a short cooking time were detected in 'Colfiorito' and 'Colliano' landraces and 'Itaca' cv seeds (260–271 g/kg^{-1} and 24–26 min). Other landraces with good protein content were 'Colliano', 'Altamura', 'Castelluccio di Norcia', 'Villalba', 'Linosa' and 'Miccula' landracecs and L 16VT line (245–255 g/kg^{-1}), although only 'Colliano', 'Castelluccio di Norcia' and 'Linosa' showed short cooking time (24 min). These data together with the agronomic ones confirmed that Linosa is the best landrace, however also 'San Gerardo' deserves some attention. In fact, together with cv Gaia, it was the one with the best yield at Battipaglia, showed a medium-good protein content (241 g/kg^{-1}) and had a short cooking time (24 min). For these reasons, appropriate support actions to promote its on farm survival could be developed.

Analyses with molecular (SSR) and biochemical (seed storage proteins) markers confirmed some group clusters already found by using the agronomic traits: 'Castelluccio di Norcia' and 'Colfiorito'; Mormanno' and 'L VT16 line'; 'Altamura' and 'Miccula'; 'Valle di Nevola' and 'Gaia', always appear to retain a high level of genetic similarity. On the contrary, 'San Gerardo' landrace showed specific traits and was confirmed to be distantly related to the other materials (see Table 8).

Globally, the agronomic, nutritional and technological data presented here can help in choose the best lentil landraces/varieties for specific marginal areas in Southern Italy. The correlation data will be useful for breeders to set up the lentil ideotype and to employ among the most correlated traits, the easier

Table 8 Pairwise comparison of Nei's genetic identity (above diagonal) and genetic distance (below diagonal) between major lentil accessions examined

Accession	Linosa	Mormanno	L 13 VT	L 16 VT	Altamura	Colliano	Miccula	Castell	Nevola	Colfiorito	S Gerardo	Villalba	Gaia	Itaca
Linosa	–	0.472	0.292	0.443	0.481	0.529	0.440	0.509	0.364	0.370	0.386	0.623	0.281	0.352
Mormanno	0.750	–	0.229	0.955	0.751	0.683	0.741	0.615	0.383	0.423	0.477	0.540	0.221	0.497
L 13 VT	1.231	1.473	–	0.192	0.236	0.325	0.199	0.376	0.501	0.258	0.373	0.416	0.442	0.253
L 16 VT	0.812	0.045	1.649	–	0.780	0.664	0.751	0.523	0.351	0.319	0.412	0.537	0.189	0.506
Altamura	0.731	0.285	1.443	0.248	–	0.923	0.964	0.493	0.378	0.326	0.418	0.562	0.250	0.516
Colliano	0.635	0.381	1.123	0.409	0.079	–	0.897	0.539	0.481	0.350	0.432	0.641	0.365	0.494
Miccula	0.820	0.298	1.610	0.286	0.036	0.108	–	0.502	0.373	0.322	0.420	0.486	0.246	0.517
Castelluccio di Norcia	0.673	0.484	0.978	0.647	0.705	0.617	0.689	–	0.462	0.768	0.375	0.566	0.331	0.491
Valle di Nevola	1.008	0.959	0.690	1.045	0.972	0.731	0.986	0.770	–	0.396	0.338	0.528	0.922	0.359
Colfiorito	0.992	0.858	1.353	1.141	1.118	1.049	1.132	0.263	0.925	–	0.274	0.409	0.297	0.306
San Gerardo	0.951	0.739	0.984	0.885	0.870	0.839	0.865	0.979	1.082	1.291	–	0.394	0.271	0.403
Villalba	0.958	0.614	0.875	0.621	0.576	0.443	0.720	0.567	0.638	0.892	0.929	–	0.401	0.457
Gaia	1.266	1.506	0.814	1.661	1.386	1.006	1.399	1.104	0.080	1.214	1.303	0.913	–	0.250
Itaca	1.043	0.695	1.374	0.680	0.660	0.705	0.658	0.710	1.023	1.183	0.908	0.782	1.386	–

ones to be recorded. Moreover, SSR analysis provided useful information on genetic variation and relationship among landraces. These data can be partially integrated with some results obtained in a previous analysis, comparing eleven lentil landraces using ISSR markers (Sonnante and Pignone 2007). In that study, while 'Colfiorito' and 'Castelluccio di Norcia' were confirmed grouping together, other landraces in common with the present study ('Villalba', 'Linosa', and 'Altamura') were differently clustered. This finding is often observed when comparing analyses performed using different classes of markers (Varshney et al. 2007; Maras et al. 2008) and most likely is due to the fact that different portions of the genome are taken into account.

World-wide lentil germplasm collections, analyzed by morphological, biochemical, and molecular markers, revealed a large genetic diversity among accessions collected in distant geographic areas (Sonnante and Pignone 2001; Piergiovanni and Taranto 2003). Our study, performed on ten lentil landraces cultivated in Central/Southern Italy and on two lines and two varieties constituted in this country, are in agreement with the above findings and confirm this species as one of the legume crops with the largest genetic variation. The results presented here will provide useful information and tools to develop new lentil varieties in Italy, where local materials are represented by old landraces and registered varieties are lacking.

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