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Morphological and molecular characterization of ‘Saragolla’ wheats (*Triticum turgidum* subsp. *durum* from Abruzzo, Italy)

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Abstract: A morphological and genetic characterization of autochthonous ‘Saragolla’ wheats, currently cultivated in Abruzzo Region (Italy), was carried out. Using 15 simple sequence repeat (SSR) markers and 24 UPOV morphological traits we compared: (a) 13 ‘Saragolla’ genotypes with traits of the *italicum/apulicum* botanical varieties (Saragolla (Sar.) *italicum*), (b) 26 ‘Saragolla’ genotypes with traits of *leucurum/affine* botanical varieties (Sar. *leucurum*), (c) 8 breeding varieties (pure lines), and (d) 5 Italian autochthonous wheats and 1 *turanicum* line (old wheats). One hundred twenty-six (126) alleles were identified. The number of alleles per locus spanned from 4 to 15 and the number of alleles per genotype varied between 12 and 21. Values of gene diversity (Nei) across the 53 genotypes was 0.17. The groups of Sar. *leucurum* and Sar. *italicum* genotypes were morphologically distinguishable from the groups of old wheats and pure lines. Likewise, the analysis of molecular data using the discriminant analysis revealed that genotypes with the Sar. *italicum* phenotype displayed distinct genetic differences from Sar. *leucurum,* pure lines and old wheats. These results make Sar. *italicum* genotypes distinguishable and eligible as a conservation variety. Ward’s clustering analysis of the 53-genotype pool showed that the ‘Saragolla’ landrace is a valuable repository of genetic diversity.

Keywords: Abruzzo Region, ’Saragolla’ landrace, durum wheat diversity, genetic characterization

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Introduction

Traditional crops are generally viewed by consumers as more natural and healthier options (Rascio *et al*, 2015, 2016). Their reintroduction into cultivation and reinforcing their value chain can play a vital role in bolstering conservation efforts and elevating the value of their cultivation environment. This, in turn, can substantially increase the economic benefits for rural communities (Pallante *et al*, 2016). Before the onset of the breeding era in Italy, initiated by Strampelli in the early twentieth century (Scarascia-Mugnozza, 2005), cultivated wheat primarily consisted of autochthonous varieties. Although there is not a worldwide consensus on this definition, these wheats are often referred to as old/ancient wheats, or landraces (Negri *et al*, 2009).

Autochthonous wheats are regarded as valuable sources of alleles for breeding programmes (Terzi *et al*, 2005). Indeed, over time, environmental conditions and, to a certain extent, purposeful farmer selection have resulted in the cultivation of plants exhibiting high adaptability and superior performance within their respective cultivation regions (Zeven, 1998). For

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these reasons, the EU (EU, 2009) defines landraces or varieties which are naturally adapted to local and regional conditions as conservation varieties (Spataro and Negri, 2013). They are frequently composed of mixed genotypes (populations) and exhibit a high degree of genetic diversity (Zeven, 1998), in contrast to modern breeding varieties (cultivars or pure lines).

Following the ratification of the International Treaty on Plant Genetic Resources for Food and Agriculture by the UN Food and Agriculture Organization (FAO, 2009) in 2001, most Italian regions implemented laws aimed at protecting and promoting indigenous crops. They also provided funding for projects dedicated to studying the conservation varieties' distinctive characteristics. Their registration involves a formal process where these varieties are officially recognized, documented, and often included in seed catalogues or databases. Once registered, conservation varieties are often conserved in community seedbanks or similar facilities where they can be accessed by farmers. Enhancing access to these varieties is important to support agricultural biodiversity, improve resilience in the face of environmental challenges, and contribute to the long-term sustainability of food production systems. Some countries, by officially recognizing and documenting these varieties, provide legal protection to conservation varieties to prevent unauthorized use or commercial exploitation (EU, 2009). This protection is designed to encourage the continued conservation and sustainable use of these valuable genetic resources.

In Southern Italy, the cultivation of a wheat type known as 'Saragollo forte' which is particularly well-suited for pasta production, has been extensively documented in various commercial agreements dating back to the seventeenth century (Fiore, 2013). As introduced by old botanists (Draghetti, 1927; De Cillis, 1927), the use of the plural noun 'Saragolle,' highlights the presence of multiple forms of 'Saragolla' wheat, all falling under the Saragolla (Sar.) *leucurum* botanical variety, which was one of the 22 botanical varieties of *Triticum turgidum* documented at the start of the 1900s (Percival, 1921). In 2004, the 'Produttori Sementi Bologna' company registered a variety also named 'Saragolla'. This variety resulted from crosses between the 'Iride' cultivar and the '0114' elite line. Therefore, this 'Saragolla' pure line is an enhanced variety and not a local one.

Today, a significant number of farmers in Central and Southern Italy are cultivating the old 'Saragolla' wheat, either for personal consumption or to establish short food supply chains of autochthonous wheat. The resurgence of interest in 'Saragolla' can be attributed to its adaptability to low-fertility soils and its suitability for cultivation with minimal input methods, making it especially attractive for agriculturally marginal regions. Moreover, this revived interest in 'Saragolla' has been magnified by online sources (Eccellenze D'abruzzo, 2024), which suggest that it can be an Italian alternative to Khorasan wheat, marketed under the name 'Kamut' (Piergiovanni, 2013). Kamut is a registered variety belonging to the tetraploid species *Triticum turanicum* Jacubz, initially described by Percival (1921) as *T. orientale*. This hypothesis is reinforced by the morphological resemblance between the elongated seeds of 'Saragolla' cultivated in the Abruzzo Region and the seeds of 'Kamut' *T. orientale* Percival.

Considering the complexity of the situation, in the years 2018–2020, the local authorities of the Italian Region Abruzzo funded the SARAB project, 'Characterization of local Saragolla durum wheat populations', to characterize the 'Saragolla' wheat currently cultivated. Through intensive cataloguing based on morphology, the project focused on the main species and botanical varieties of 'Saragolla' cultivated in 11 different sites within the Abruzzo Region (Rascio *et al*, 2021). The results revealed a heterogeneous botanical composition both within and among these sites (Rascio *et al*, 2022). Nine botanical varieties of durum wheat were observed, the majority belonging to the *italicum* or *apulicum* botanical varieties, primarily differing in glume pigmentation intensity. There was also a smaller number of genotypes falling into the *leucurum* or *affine* botanical varities (referred to here as Saragolla (Sar.) *leucurum*), which exhibited variations in seed pigmentation intensity.

The main goals of this study were to conduct genetic and morphological characterizations of representative plants belonging to the most prevalent botanical varieties collected in 11 farms that participated in the SARAB project. Additionally, these varieties were compared with a collection of both pure lines (modern) and traditional Italian (old) wheat varieties. The results here shown indicate that, in contrast to the Sar. *leucurum*, the *italicum* genotypes shared a close genetic similarity among themselves. They are widespread in the Abruzzo region and genetically distinct from the groups of modern and old genotypes examined in this study, hence they are eligible for registration as a conservation variety. The study also explores the degree of diversity of the four groups of genotypes.

Material and methods

Plant material

Eleven samples of 'Saragolla' wheats from 11 locations in Abruzzo (shown in Supplemental Table 1) were grown and characterized at the Council for Agricultural Research and Economics - Research Centre for Cereal and Industrial Crops (CREA-CI) in Foggia (Rascio *et al*, 2022). About 1,000 individual plants of these heterogeneous wheats were morphologically examined and 434 durum wheat plants were found. A total of 39 representative spikes of this subset, selected to be indicative of the prevailing botanical varieties *italicum/apulicum* and *leucurum/affine* were used for further genetic and morphological characterization in this study. For comparison, eight modern varieties/durum cultivars and six samples of old autochthonous durum wheat samples belonging to the CREA-CI working collection were also included (Supplemental Table 2). They comprised four genotype groups:

1) **Sar. *italicum***: 13 ‘head to row’ of ‘Saragolla’ genotypes exhibiting at least four out of five traits of the *italicum/apulicum* botanical varieties

2) **Sar. *leucurum***: 22 ‘head to row’ of ‘Saragolla’ genotypes from Abruzzo and four from Puglia displaying at least four out of five traits of the *leucurum/affine* botanical varieties

3) **Modern pure lines :** Eight seed samples of durum wheat cultivars: ‘Ciccio’, ‘Cappelli’, ‘Capeiti’, ‘Colosseo’, ‘Duilio’, ‘Simeto’, ‘Svevo’ and ‘Saragolla’

4) **Old wheats:** Six seed samples of autochthonous durum wheats, primarily sourced from Sicily (Fiore *et al*, 2019) and belonging to the CREA-CI working collection. These include: ‘Realforte’, ‘Russello’, ‘Sammartinara’, ‘Scorsonera’, ‘Vallelunga pubescent’, and the *T. turanicum* pure line (PI166959), selected at CREA-CI.

Phenotypic assessment

In 2021, a total of 53 rows, each 1m in length and spaced 30cm apart, were sown according to the usual agronomic practices (Rascio *et al*, 2016). Throughout the growth stage, each row was carefully examined to ensure its purity, and one plant was selected for DNA extraction and morphological characterization. The assessment was performed on 24 traits with value scales employed for evaluation in part adhering to the guidelines outlined by the International Union for the Protection of New Varieties of Plants (UPOV, 2012) (Table 1).

Molecular marker analysis

For each genotype, the extraction of DNA was performed according to the protocol used by Marone *et al* (2009). Twenty-eight microsatellite single sequence repeat (SSR) markers were selected based on published map data (Marone *et al*, 2009, 2012), according to the following criteria: locus-specific amplification, high level of polymorphism, and good genome coverage (one marker per chromosome arm). The sequences of the SSR are available in the GrainGenes database (http://wheat.pw.usda.gov). The PCR reactions were performed in 25μl volume in Applied Biosystems 2720 Thermal Cyclers. The reaction mixture contained 60ng of template DNA, 0.2mM of dNTPs, 1X Buffer (10mM Tris–HCl—pH 8.3, 50mM KCl, 1.5mM MgCl2), 0.4μM labelled reverse primer (FAM or HEX or NED or TET), 0.4μM unlabelled forward primer and 0.2U of Taq DNA polymerase (5U/μl) (Kapa). Thermal cycling conditions were as follows: 94°C for 3min, followed by 45 cycles of 94°C for 30s, the specific annealing temperature (Ta) for each primer for 30s, 72°C for 30s, with a final extension at 72°C for 2min. The amplification products were analyzed by means of capillary electrophoresis (ABI3130), multiplexing different fluorescent dyes. Electropherograms were analyzed with GeneMapper version 4.0. The internal molecular weight standard was 500-ROX (Life Technologies).

Statistical analysis

Genotypic characterization was performed with 15 SSR markers which gave a clear electrophoretic pattern. To this aim, the genotypic data were transformed into a binomial matrix as present (1) or absent (0) for each marker and this matrix was used to construct Ward’s dendrogram tree to assess genetic diversity.

Nei’s gene diversity, percentage of polymorphic loci and Shannon’s information index were determined using the PopGen 1.31 software (Yeh *et al*, 1999).

For assessing marker polymorphism and informativeness, the average polymorphic information content (PIC) was calculated using the following formula introduced by Anderson *et al* (1993):

$\text{PIC} = 1\text{-}\Sigma(\text{P}_i)^2$

where P_i is the number of polymorphic loci/all the number loci.

The distances among the four groups of genotypes (Sar. *leucurum*, Sar. *italicum*, pure lines and old wheats) were examined by multivariate discriminant analysis and cluster analysis, using the STATISTICA (StatSoft Inc.) software.

Results

Morphological characterization

As shown in Figure 1 and in Supplemental Table 3, the Sar. *italicum/apulicum*-like genotypes have rather compact, hairy glumes, lightly pigmented spikes, long red or brown-red awns, yellow-amber and elongated grains. The Sar. *leucurum/affine* genotypes have white and glabrous glumes, elongated, compact spikes, and white or red seeds.

The mean values of each morphological and phenological trait for genotypes belonging to the four groups (Sar. *leucurum*, Sar. *italicum*, modern cultivars or old wheats) show that the Sar. *italicum/apulicum* genotypes registered the highest values for glume hairiness and 1,000 seed weight (Table 2). In contrast, the Sar. *leucurum/affine* genotypes exhibited the highest values for the shape of the lower glume beak. Modern varieties displayed smaller height and earlier heading dates, a result of the extensive breeding efforts they underwent.

Results of stepwise discriminant analysis performed using the four groups of genotypes as classification categories and visualized through the biplot of canonical variables (Figure 2) showed that the model had a high discriminatory power (Lambda Wilks: 0,0075245; approx. F (48,90) = 7,8312; $p < 0,0000$), with two discriminant functions that accounted for 92.9% of the explained variance (Table 3).

Based on the absolute values of standardized coefficients of the canonical variables (Table 3) the main traits that horizontally contributed to the 4-group separation were the glume hairiness, which was absent in Sar. *leucurum* and the upper neck glaucosity, which

Table 1. The 24 traits used for the morphological characterization of wheat genotypes, and the value scale employed for evaluation (UPOV, 2012).

	Trait	Measure units/score	Assessment scale
1	1,000 seed weight	g	
2	Curvature of lower glume beak	1–7	1 = absent, 7 = strong
3	Lower glume length of beak	1–9	1 = very short, 7 = long
4	Lower glume hairiness	1–9	1 = absent, 9 = very present
5	Straw: pith in cross section	1–7	1 = thin, 7 = thick
6	Grain shape	3–7	3 = slightly, 7 = strongly elongated
7	Grain: length of brush hair	3–7	3 = short, 7 = long
8	Grain weight/plant	g	
9	Awn colour	1–5	1 = white, 3 = medium dark, 5 = very dark
10	Spike colour	1–5	1 = white, 3 = medium dark, 5 = very dark
11	Awn tip/ear length ratio	1–3	1 = low ratio, 3 = high ratio
12	Spike length	cm	
13	Spike shape in profile	1–5	1 = tapering, 2 = parallel sided, 3 = slightly clavate, 4 = strongly clavate, 5 = fusiform
14	Ear glaucosity	1–9	1 = absent, 9 = very strong
15	Awn divergence	1–2	1 = adherent, 2 = divergent
16	Spike density	3–7	3 = lax, 5 = medium, 7 = dense
17	Plant height	cm	
18	Growth habit	1–9	1 = erect, 9 = prostrate (score 9)
19	Recurved flag leaves	1–9	1 = absent, 9 = very high
20	Heading date from April 1st	days	
21	Flag leaf: glaucosity of sheath	1–9	1 = absent, 9 = very strong
22	Flag leaf lower side glaucosity	1–9	1 = absent, 9 = very strong
23	Upper node hairiness	1–9	1 = absent, 9 = very strong
24	Upper neck glaucosity	1–9	1 = absent, 9 = very strong

Figure 1. Comparison of distinguishing characteristics of glume (A), and spike and seed (B) of Saragolla botanical types Sar. *italicum* and Sar. *apulicum*: hairy with red glumes and red awns; Sar. *leucurum* and Sar. *affine*: glabrous with white glumes and white awns.

Table 2. Mean values of morphological traits of the four groups of durum wheat genotypes. The qualitative traits, for which no specific unit of measurement is provided, were assessed using the value scale established by the International Union for the Protection of New Varieties of Plants (UPOV, 2012).

Trait		**Sar. *leucurum***	**Sar . *italicum***	**Modern cultivars**	**Old wheats**
Shape of lower glume beak	Mean	6,9	2,0	2,7	2,1
	SD	0,6	1,3	2,0	1,6
Glume length of beak	Mean	4,0	4,9	4,3	3,6
	SD	1,8	1,6	3,0	1,9
Glume hairiness	Mean	1,3	8,2	1,3	1,0
	SD	1,5	1,7	0,8	0,0
Straw: pith in cross section	Mean	5,5	6,7	6,7	6,4
	SD	1,9	0,6	0,8	1,0
Ear length (cm)	Mean	9,2	9,4	8,5	8,4
	SD	1,2	1,0	2,3	2,2
Grain shape	Mean	4,6	6,8	3,7	3,6
	SD	1,7	0,6	1,0	1,5
Grain: length of hair	Mean	3,3	4,1	3,1	3,0
	SD	0,9	1,3	0,2	0,0
Awn colour	Mean	1,8	2,3	2,2	2,6
	SD	1,1	0,9	1,6	1,4
Glume colour	Mean	1,7	2,0	1,5	2,0
	SD	0,8	0,9	0,5	1,5
Awn tip/ear length ratio	Mean	1,6	2,7	1,7	1,6
	SD	0,7	0,6	0,8	0,8
Ear shape	Mean	2,0	2,2	3,0	2,0
	SD	0,9	1,2	1,3	0,8
Awn compactness	Mean	1,4	1,5	1,5	1,9
	SD	0,6	0,8	0,5	1,5
Ear density	Mean	5,2	4,7	4,7	6,1
	SD	1,9	1,4	1,5	1,1
Plant height (cm)	Mean	108,7	120,0	79,2	101,4
	SD	16,2	7,9	17,7	21,7
Grain weight/plant (g)	Mean	19,7	15,2	19,0	15,3
	SD	6,3	4,2	1,9	3,4
1,000 seed weight (g)	Mean	46,5	73,3	48,7	46,4
	SD	8,5	3,6	5,3	7,0
Growth habit	Mean	3,7	4,4	2,3	4,4
	SD	1,4	1,0	2,1	1,0
% recurved flag leaves	Mean	6,1	6,7	2,7	5,0
	SD	1,6	0,8	1,5	2,0
Heading date	Mean	28,3	29,4	18,1	20,7
(days from April 1st)	SD	4,1	1,6	6,6	6,3
Flag leaf glaucosity	Mean	5,1	5,8	4,7	4,4
	SD	1,5	1,3	2,0	2,2
Flag leaf lower glaucosity	Mean	3,4	3,0	3,7	3,3
	SD	1,4	0,0	1,0	1,4
Upper node hairiness	Mean	3,3	3,0	2,3	3,0
	SD	1,3	0,0	1,0	1,2
Upper neck glaucosity	Mean	5,1	3,3	5,0	4,4
	SD	1,5	0,8	1,3	1,5
Spike glaucosity	Mean	4,8	6,7	5,7	5,3
	SD	1,4	1,1	1,0	1,4

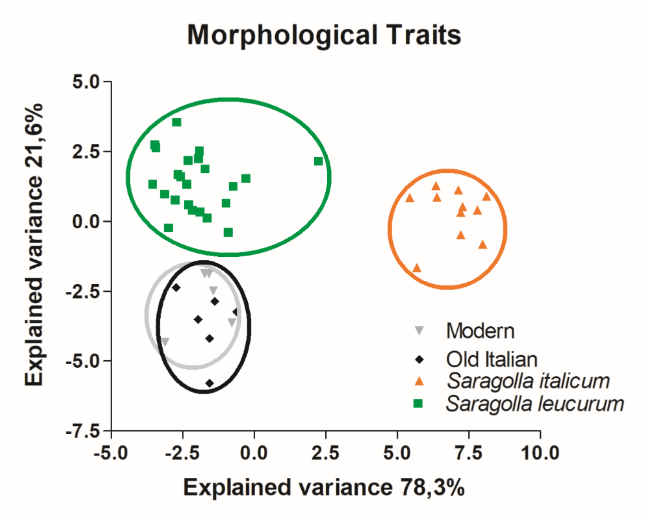

Figure 2. The biplot of canonical variables obtained using a stepwise discriminant analysis based on 24 UPOV morphological descriptors and four groups of genotypes. The percentages of explained variance by the two roots are shown.

was lacking in Sar. *italicum* (Table 2). The differences in heading date along with the upper neck green glaucosity degree (Table 2) mainly contributed to the vertical separation of Sar. *leucurum* and *italicum* from the pool of new and old genotypes.

The Mahalanobis distances between groups were all highly significant (Table 4), except between pure lines and old wheats; Sar. *leucurum* and Sar. *italicum* were the two most distant and hence morphologically different groups.

SSR patterns

The molecular analysis utilized a set of 15 SSR markers out of initially 28 tested, each characterized by a distinct electrophoretic pattern for all genotypes. In total, 126 alleles were identified, with the number of alleles per locus spanning from 4 to 15. The Polymorphic Information Content (PIC) values of the SSR markers ranged from 0.52 (for gwm60) to 0.91 (for wmc606 and gwm459), resulting in an average PIC value of 0.77 per locus (Table 5).

Excluding from the analysis the alleles that occurred at a low frequency ($p < 0.05$), the number of alleles per genotype varied between 12 and 21, with the most frequent value being 16 (Table 6). This occurrence was four times higher than what was observed in a study where 104 Ethiopian durum wheat genotypes, representing 13 populations, three regions, and four altitudinal classes, were analyzed using 14 SSR markers (Dagnaw *et al*, 2023).

Diversity

The diversity analysis for all cultivars based on SSR markers (Table 7) yielded low mean values (0.28±0.20) of Shannon’s index. The values (0.176±0.5) of Nei’s gene diversity were lower than the minimum observed in 40 winter wheat genotypes (Petrović *et al*, 2017) coming from European countries (Croatia, Austria, France, Italy, and Russia) and lower than that (0.56) resulting for 124 Ethiopian genotypes (Dagnaw *et al*, 2023).

The among-groups comparison indicated that old wheats and Sar. *leucurum* exhibited the highest percentage of polymorphic loci, followed by pure lines and old wheats. The measurement of gene diversity, estimated by both Nei’s gene diversity and Shannon’s information index, yielded similar values for Sar. *leucurum*, breeding lines, and old wheats, and the lowest values for Sar. *italicum*.

In the case of Sar. *leucurum*, Sar. *italicum*, old wheats and pure lines, the highest average number of amplified alleles per locus was observed in 1B (long arm), 6B (short arm) and 6B (long arm), respectively, with average values of 9.0, 6.5, 2.5 and 2.3, respectively. It’s worth noting that old wheats and modern pure lines exhibited the highest percentage of polymorphisms detected by SSR markers in the B genome (Table 8), likely originating from a species, or several species closely related to *Aegilops speltoides* Tausch, a cross-pollinating species; while the Sar. *leucurum* and Sar. *italicum* sets had the highest percentage of polymorphisms in the A genome, which can be traced back to diploids like *T. urartu* Thumanjan ex Gandilyan (Wang *et al*, 2007).

A similar clustering pattern was observed when analyzing both the morphological traits (Figure 2) and molecular marker profiles (Figure 3) of all 53 genotypes. This analysis employed a hierarchical grouping method, without missing data in the dataset. The resulting dendrogram revealed four major clusters (Figure 3). Cluster 1A comprised all 13 Sar. *italicum* genotypes, 9 out of 22 Sar. *leucurum* genotypes of Abruzzo and 1 Sar. *leucurum* genotype from Puglia. The second major cluster, 1B, could be further subdivided into two subclusters: 1B1 and 1B2. The 1B1 cluster included two subgroups: the first subgroup contained four closely related breeding lines (‘Colosseo’, ‘Simeto’, ‘Ciccio’ and ‘Capeiti’), two Sar. *leucurum* genotypes, and the old wheat ‘Vallelunga pubescent’; the second subgroup was larger, consisting of modern varieties (‘Duilio’, ‘Realforte’, ‘Svevo’ and the ‘Saragolla’ pure line), some old wheats (‘Russello’, ‘Scorsonera’, ‘Sammartinara’, ‘Cappelli’ and the *T. turanicum* line), along with eight Sar. *leucurum* genotypes from Puglia or Abruzzo. Cluster 1B2 included six strongly related Sar. *leucurum* genotypes: one was from Puglia and five from Abruzzo.

Discussion

The morphological and genetic characterization of autochthonous wheats serves the dual purpose of safeguarding the economic interests of farmers and increasing consumers’ trust in the origin and quality of food products entering the market (Terzi *et al*, 2005).

A recent morphological analysis conducted by the SARAB project on wheat crops in 11 farms across

Table 3. Values of the standardized coefficients for the canonical variables included in the discriminant functions, obtained using the four groups of genotypes (Sar. *leucurum*, Sar. *italicum*, pure lines and old wheats) as classification categories.

	root 1	**root 2**
Glume hairiness	-0,99	-0,26
Heading date (days from 1/4)	0,05	-1,39
Upper neck glaucosity	0,73	-0,98
Growth habit	-0,48	0,64
Grain shape	-0,06	-0,50
1,000 seed weight	0,03	0,44
Spike glaucosity	-0,43	-0,05
Spike shape	-0,27	-0,08
Glume colour	0,18	-0,57
Awn colour	-0,40	0,38
Plant height	0,48	-0,17
Flag leaf lower side glaucosity	0,19	-0,49
Grain weight/plant	0,09	-0,36
Straw: pith in cross section	-0,23	0,20
Lower glume: length of beak	-0,36	-0,29
Spike density	0,21	0,31
Eigenvalue	12.4	3.4
Explained cumulative variance (%)	72.6	92.9

Table 4. Pairwise square Mahalanobis distances (plain text) and probability values (italics) for the contrasts between the four groups of genotypes.

	Sar. *leucurum*	**Sar. *italicum***	**Pure lines**	**Old wheats**
Sar. *leucurum*		*0,0000*	*0,00036*	*0,00003*
Sar. *italicum*	69,48		*0,00000*	*0,00000*
Pure lines	23,76	78,44		*0,01962ns*
Old wheats	27,19	68,77	20,95	

Table 5. List of SSR markers used for molecular analysis, number of alleles and Polymorphic Information Content (PIC) obtained for each marker in the 39 Saragolla wheat lines. A, A genome; B, B genome; L, long arm; S, short arm.

	Marker	**Chromosome**	**No. of alleles**	**PIC**
1	gwm311	2A(L)	11	0,83
2	gwm1042	3A(L)	6	0,55
3	gwm299	3B(L)	7	0,74
4	barc45	3A(S)	4	0,77
5	gwm495	4B(S)	8	0,82
6	gwm1093	4A(S)	14	0,83
7	gwm1084	4B(L)	8	0,75
8	gwm865	5A(L)	8	0,80
9	gwm154	5A(S)	9	0,73
10	gwm499	5B(L)	11	0,80
11	gwm1017	6A(L)	6	0,80
12	gwm459	6A(S)	12	0,91
13	gwm193	6B(L)	4	0,76
14	gwm60	7A(S)	7	0,52
15	wmc606	7B(S)	11	0,91

Table 6. Average number of SSR alleles per genotype. Only alleles occurring with a frequency higher than 11 ($p < 0.05$) are included. The codes from S1 to S11 indicate the cultivation sites (see Supplemental Table 1) of Saragolla wheats from Abruzzo and S12 indicates the cultivation site in Puglia. The extra letters and numbers differentiate the genetically characterized plants within each site. Old wheats and pure lines are described in Supplemental Table 2.

Group	Genotypes	Average allele no.	Group	Genotypes	Average allele no.
Sar. *leucurum*	S1F3	13	Sar. *italicum*	S1H3	16
	S2E	13		S2P2A	15
	S2F	14		S3P27F	20
	S3P27A	16		S4P23B	14
	S3P3B	15		S5P49B	21
	S3P3I	16		S6P6A	16
	S4P4B	16		S7P43C	16
	S4P4A	16		S8P8D	16
	S4Z	15		S8P9A	16
	S5P5C	16		S10P50C	15
	S5P31D	16		S11P11A	17
	S6P21D	15		S7A	16
	S6P41C	15		S7C	14
	S8A1	14	Old wheats	'Sammartinara'	15
	S8R1	14		'Realforte'	12
	S8	15		'Scorsonera'	17
	S9P22D	15		'Russello'	16
	S9P9D	23		'Vallelunga Pubescent'	13
	S9P22A	15		'Turanicum'	17
	S10P32A	14	Modern pure lines	'Capeiti'	14
	S11P24A	15		'Ciccio'	15
	S12P12A	14		'Simeto'	15
	S12P54B	16		'Colosseo'	15
	S12P35A	14		'Duilio'	16
	S12P54A	14		'Saragolla'	15
	S1E4	15		'Cappelli'	13
				'Svevo'	16

Table 7. Genetic diversity indices over 15 SSR loci for all 53 Italian genotypes tested in the study, as well as for the four groups categorized by 'Saragolla' botanical variety or control group (Means±SD). PIC, Polymorphic Information Content.

	Shannon's information index	Percentage of polymorphic loci	Nei's gene diversity	PIC
Sar. *leucurum*	0.19±0.19	71.4	0.10±0.13	0.902±0.08
Sar. *italicum*	0.08±0.18	22.2	0.05±0.13	0.097±0.08
Old wheats	0.17±0.22	39.7	0.10±0.14	0.929±0.10
Modern pure lines	0.19±0.19	61.1	0.11±0.12	0.764±0.11
All genotypes	0.28±0.20	97.63	0.17±0.15	0.673±0.35

Table 8. Percentage of polymorphism detected by SSR in A and B genomes of four groups of genotypes

	'Saragolla' *leucurum*	'Saragolla' *italicum*	Old Italian wheats	Modern pure lines
Genome A	54,1	54,5	46,2	48,5
Genome B	45,9	45,5	53,8	51,5

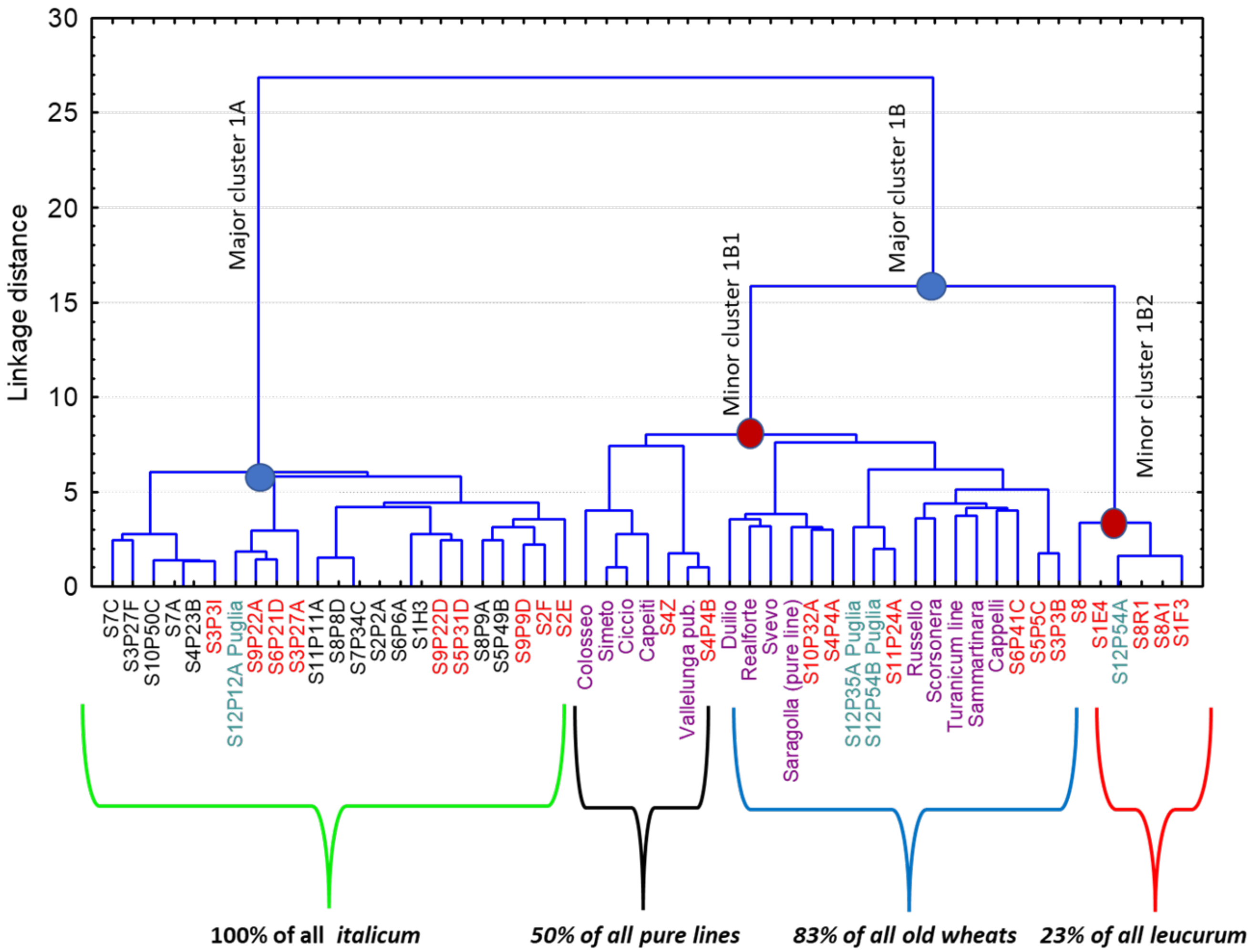

Figure 3. SSR-based genetic distances among the 53-genotype pool, through Ward's clustering. The percentage of the prevalent type of genotypes included in each cluster is indicated. Characters: red = Sar. *leucurum/affine* genotypes from Abruzzo; blue = Sar. *leucurum* from Puglia; black = Sar. *italicum/apulicum* genotypes from Abruzzo; violet = modern and old varieties. Codes are as in Table 6.

the Abruzzo Region revealed significant morphological diversity within the cultivated 'Saragolla' variety (Rascio *et al*, 2022). This diversity poses a challenge in accurately defining their distinct traits.

The morphotypes that are both quantitatively and widely spread, across most of the 11 wheat farms included in the SARAB project, belong to the Sar. *italicum/apulicum* or the Sar. *leucurum/affine* botanical varieties (Rascio *et al*, 2022). The genetic characterization described here aimed to validate whether the observed morphological similarity among the genotypes from Abruzzo corresponds to genetic similarity and to develop a tool to differentiate them.

The results presented here confirm the efficacy of SSR markers to characterize wheat genotypes (Wang *et al*, 2007; Dagnaw *et al*, 2023). In fact, the 15 SSR primers used in this experiment showed detectable polymorphisms in all the 53 genotypes and their mean polymorphic information content (PIC = 0.77) makes their use very informative. 'Saragolla' genotypes, belonging to the *italicum/apulicum* botanical varieties can be eligible as conservation varieties. These genotypes are widely cultivated across the Abruzzo Region, and they also exhibit a noteworthy genetic similarity, as indicated by the low values of Nei's gene diversity and Shannon's information index. Additionally, Sar. *italicum* genotypes display both a distinct phenotype and genotype in comparison to Sar. *leucurum*, older wheats and pure lines.

It is worth noting that Ward's clustering analysis of the 53-genotype pool revealed significant genetic diversity between Sar. *italicum* and Sar. *leucurum* genotypes. Out of the 26 Sar. *leucurum* genotypes examined, only 9 displayed a significant genetic resemblance to Sar. *italicum*. Six were categorized within the broader groups of pure lines and old wheats, while five formed a distinct group of genotypes very closely related genetically, but distinct from all others. The clustering analysis also revealed a stronger genetic similarity between most Sar. *leucurum* genotypes and three out of four 'Saragolla' genotypes from Puglia and a somewhat lesser degree of affinity with the oldest genotypes. Expanding on the hypothesis (Zeven, 1998), that factors such as geographic distance, environmental conditions and the selection made by farmers can shape the genetic composition of local wheats, it is plausible to infer that the migration of wheat commenced from Sicily. In fact, the *leucurum* genotypes were documented in Southern Italy as early as the beginning of the 1900s (Percival, 1921; De Cillis, 1927; Draghetti, 1927) and were likely among the oldest cultivated in Sicily (Porceddu *et al*, 1981). From Sicily, it is plausible that these wheats initially spread to the nearby region of Puglia and then reached Abruzzo where the cross with indigenous wheat occurred as well as the selection of alleles improving adaptability, productivity and quality. In terms of affinities with 'Kamut', the results suggest that the Sar. *italicum* genotypes, despite having elongated and large seeds similar to *T. turanicum*, formed a distinct cluster and showed a closer genetic relationship to Sar. *leucurum* and old durum wheats.

Conclusions

The 'Saragolla' wheat presently grown in the Abruzzo Region is characterized by its rich diversity, predominantly comprising Sar. *italicum/apulicum* and Sar. *leucurum*-like durum wheats, some of which share close morphological and genetic traits. Recently, these genotypes have been officially registered as 'Saragolla' conservation varieties from Abruzzo. The genetic distance observed among 39 'Saragolla' genotypes, representative of only two out of the nine previously identified botanical varieties of durum wheat, exceeded that found among the other 13 modern, or old durum wheats used in this study, which differ for age of cultivation and origin. Consequently, at the Maiella National Park seedbank, targeted *ex situ* conservation measures will be implemented to preserve the currently cultivated populations. A more extensive genetic characterization will enable the assessment of existing variability within the 'Saragolla' landrace, for adaptive and agronomically valuable traits, useful for breeding improved varieties.

Supplemental data

Supplemental Table 1. Geographic coordinates of the cultivation sites for the 12 Saragolla wheats.
Supplemental Table 2. Passport details of old and modern wheats used in the present work.
Supplemental Table 3. Morphological traits of Saragolla *leucurum*, *italicum*, and modern and old wheats.

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Author contributions

AR, study conception and manuscript draft; VDS, molecular analysis; LG, analysis and interpretation of results; SP, data collection; MT, field trials; GS, manuscript revision.

Conflict of interest statement

The authors declare no conflict of interest.

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