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Evolution of durum wheat from Sicilian landraces to improved varieties

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Abstract. In the early twentieth century a large number of durum wheat landraces were grown in Sicily. They were characterized by high adaptability to Mediterranean environments and were composed of a mixture of different genotypes. In recent decades the genetic improvement has led to the constitution of modern durum wheat cultivars made up of individual pure lines, highly uniform and productive. Today, the old tetraploid landraces are a precious source of biodiversity and can be successfully used in breeding programs aimed to improving technological quality and resistance to biotic and abiotic stresses. Some of these landraces are still cultivated and used for the production of typical local breads. In the present work twelve Sicilian old landraces have been compared with sixteen durum wheat improved cultivars. For all genotypes morphological parameters were detected according to UPOV and IPGRI descriptors; biochemical characterization was performed by SDS – PAGE of low molecular weight and high molecular weight gluten subunits; molecular analyses were carried out using SSR with thirteen primer pairs. The joint analysis of the results allowed an assessment of genetic diversity between the two groups of genotypes.

Keywords. *Triticum turgidum* subsp. *durum* – Genetic development – Local populations – Cultivar – SSR*.*

Evolution des variétés locales siciliennes de blé dur pour améliorer les variétés

Résumé. *Au début du XXe siècle, bon nombre de variétés locales de blé dur étaient cultivées en Sicile. Elles étaient caractérisées par une grande capacité d'adaptation aux conditions de milieu méditerranéennes et étaient composées par un mélange de différents génotypes. Au cours des dernières décennies, l'amélioration génétique a conduit à l'obtention de cultivars de blé dur modernes, incluant des lignées pures individuelles, très homogènes et productives. Aujourd'hui, les anciennes variétés locales tétraploïdes sont une précieuse source de biodiversité et peuvent être exploitées avec succès dans les programmes de sélection visant à améliorer la qualité technologique et la résistance aux stress biotiques et abiotiques. Certaines de ces variétés locales sont encore cultivées et utilisées pour la production de pains typiques de la région. Dans le présent travail, douze anciennes variétés locales siciliennes ont été comparées avec seize cultivars améliorés de blé dur. Pour tous les génotypes, les paramètres morphologiques ont été détectés en fonction des descripteurs de l'UPOV et de l'IPGRI ; la caractérisation biochimique a été effectuée par SDS - PAGE des sous-unités gluténines de bas poids moléculaire et de haut poids moléculaire ; les analyses moléculaires ont été réalisées à l'aide de SSR avec treize paires d'amorces. L'analyse conjointe des résultats a permis d'évaluer la diversité génétique entre les deux groupes de génotypes.*

Mots-clés. *Triticum turgidum ssp. durum – Développement génétique – Populations locales – Ccultivar – SSR.*

I – Introduction

In the first half of the last century, many durum wheat landraces were grown in Sicily. The large number of these cultivars were grown due to their specific characteristics and their adaptability to Mediterranean environmental conditions (De Cillis, 1942). These ecotypes are characterized by tall plants, good quality characteristics of the grain and long biological cycle (Boggini *et al*., 1989). Because of their high adaptability and their particular qualitative characteristics some landraces are still cultivated in small areas of Sicily. In recent decades the genetic improvement has led to the composition of modern durum wheat cultivars made up of individual pure lines, highly uniform and productive. Today, the old tetraploid landraces are a precious source of biodiversity that must be preserved and can be successfully used in breeding programs aimed to improve technological quality and resistance to biotic and abiotic stresses.

Many studies aimed to characterize and identify accessions in wheat species are based on morphological characterization according to UPOV and IPGRI descriptors (Zarkti *et al*., 2010). Morphological traits have been studied for the estimation of genetic diversity and as selection criteria for wheat breeding (Schut *et al*., 1997; Marti *et al*., 2007), but are influenced by environmental factors. Conversely, biochemical characterization and molecular analysis, based on DNA polymorphism, are independent of environmental conditions.

In this study, old Sicilian durum wheat landraces have been compared with modern improved cultivars, taking into account morphological traits, biochemical parameters and molecular markers.

II – Material and methods

A set of 28 accessions of durum wheat (*Triticum turgidum* L. ssp. *durum*) was studied: 12 old Sicilian landraces and 16 improved varieties as shown in table 1.

Table 1. Durum wheat accessions used in the experiment.

Improved varieties	Constitution Year	Sicilian landraces
Cappelli	1930	Biancuccia
Grifoni 235	1955	Bivona
Capeiti	1955	Castiglione
Trinakria	1970	Ciciredda
Appulo	1973	Cotrone
Creso	1974	Duro Lucano
Valnova	1975	Farro Lungo
Produra	1980	Gioia
Valforte	1980	Regina
Primadur	1984	Ruscia
Lira	1985	Sammartinara
Simeto	1988	Timilia
Colosseo	1995	
Bronte	1996	
Sant’Agata	2004	
Ciclope	2006	

1. Morphological characterization

An agronomical trial was carried out in location Libertinia (Catania), with cultural techniques typical of the area. The genotypes tested were grown in 10 m^2 plots, with 3 replications. The morphological characters measured were plant height, culm solid/hollow, ear shape, awn length, rachis length and caryopsis shape.

The data matrix was then standardized and elaborated with “R” software for the calculation of principal components (R Development Core Team, 2008).

A principal component analysis (PCA) of standardized data was applied to observe the main defining characteristics. A Genotype x Trait biplot (GT biplot) was created to recognize the genetic variability and the relationships among durum wheat genotypes. The first two PC (PC1 and PC2) were used to generate the GT biplot. For the assessment of the distance of similarity a dendrogram has been developed by the Ward’s method was applied.

2. Biochemical characterization

The grain storage proteins were characterized by SDS-PAGE electrophoretic patterns of low molecular weight (LMW) and high molecular weight (HMW) glutenin subunits, according to the method described by Payne *et al*. (1980). As Sicilian populations are heterogeneous, 26 seeds were analysed for each landrace and the prevalent composition was detected, also recording different electrophoretic patterns. Three seeds of each improved cultivar were analysed.

3. Molecular characterization

DNA was extracted from fresh tissue of plants germinated in Petri dishes. Genomic DNA was isolated using 100 mg of fresh tissue by DNeasy Plant Mini Kit (Quiagen). All extracted samples were estimated by spectrophotometer readings and by electrophoresis. Thirteen primer pairs Xgwm localized on chromosomes of the A and B genomes were used (Table. 2). Amplification products derived from fluorescently labeled primers were resolved by capillary electrophoresis on the ABI Prism 3130 Genetic Analyzer (Applied Biosystems).

III – Results

1. Morphological evaluation

The distribution of the accessions in the dendrogram suggested a cut at a height of 8, resulted in three clusters. The clusters were then reported in a biplot of the first two principal components, PC1 and PC2, which explained more than 50% of the total variation (PC1=29%; PC2=24%).

The ear profile, awn length, rachis length and plant height had long vectors, suggesting that there was relatively large variation among accessions (Fig. 2).

In contrast, the fullness of the culm had short vectors, suggesting that there was little or no variation among accessions. The cosine of the angle between the vectors of two traits measures the similarity or the correlation between them relative to their variation among genotypes. An angle of zero indicates a correlation of +1, an angle <90° suggests a positive correlation, an angle of 90° indicates no correlation, implying independence, an angle >90° indicates negative correlation, and an angle of 180° represents a correlation of -1.

Thus, rachis length, culm fullness, awns length had acute (<90°) angles between them, indicating that their variations are similar. On the contrary, these morphological traits had obtuse (>90°) angles with ear profile and caryopsis shape, indicating that their variations are negatively correlated. In particular, awn length had a near-right angle with height or ear profile against caryopsis shape and rachis length, indicating that their variations are more or less independent of these traits.

The genotypes studied, grouped into 3 groups as represented in the biplot (Fig. 2) could be described for traits in common. So the biplot shows that cluster 1 is characterized by units in which the overriding factor is the shape the caryopsis. The accessions of this cluster are also distinguished by having short awns. It consists of quite old varieties except Biancuccia which is an ancient Sicilian population.

The second cluster is characterized by accessions discriminated for plant height that are also distinguished, in part, to the fullness of culm and for the length of rachis. This cluster consists exclusively of Sicilian populations.

The third cluster is characterized by accessions determined by the profile of ear more predominant than the other observed factors. Its accessions present small stature and it groups together almost entirely varieties except the landrace Timilia.

Table 2. List of primers, with their forward and reverse primers, repeat motif and annealing temperature (AT).

Locus	Forward primer	Reverse primer	Repeat	An. Temp.
Xgwm 6-4B	CGTATCACCTCCTAGCTAAACTAG	AGCCTTATCATGACCCTACCTT	(G A)40	55°
Xgwm 46-7B	GCACGTGAATGGATTGGAC	TGACCCAATAGTGGTGGTCA	(GA) 2GC (GA) 33	60°
Xgwm 67-5B	ACCACACAAACAAGGTAAGCG	CAACCCTCTTAATTTTGTTGGG	(CA) 10	60°
Xgwm 95-2A	GATCAAACACACACCCCTCC	AATGCAAAGTGA AAA ACCCG	(AC) 16	60°
Xgwm 107-4B	ATTAATACCTGAGGGAGGTGC	GGTCTCAGGAGCAAGAACAC	(CT) 21	60°
Xgwm 124-1B	GCCATGGCTATCACCCAG	ACTGTTCGGTGCAATTTGAG	(CT) 27 (GT) 18imp	60°
Xgwm 131-1B	AATCCCCACCGATTCTTCTC	AGTTCGTGGGTCTCTGATGG	(CT)22	60°
Xgwm 153-1B	GA TCTCGTCAC CCGGAATTC	TGGTAGAGAAGGACGGAGAG	(GA) 18	60°
Xgwm 155-3A	CAATCATTTCCCCCTCCC	AATCATTGGAAATCCATATGCC	(CT) 19	60°
Xgwm 408-5B	TCGATTTATTTGGGCCACTG	GTATAATTCGTTCACAGCACGC	(CA) > 22 (TA) (CA) 7 (TA) 9	55°
Xgwm 413-1B	TGCTTGTCT AGA TTGCTTGGG	GATC GTCTCGTCCTTGGCA	(GA) 18	60°
Xgwm 5I3-4B	ATCCGTAGCACCTACTGGTCA	GGTCTGTTCATGCCACATTG	(CA) 12	60°

2. Biochemical characterization

The results of protein characterization pointed out large biodiversity among Sicilian landraces. Regarding the HMW glutenin subunits encoded by the locus Glu-B1 in the long arm of chromosome 1B, six landraces had subunit “20” as prevalent composition, two had the subunit pair “13+16”, three had the subunit pair “6+8” and one the “7+8” (Table 3).

The landraces Bivona and Timilia showed polymorphism within the landrace populations, with prevalent composition “6+8” and presence of subunit “20”. All the tested landraces were “Null” for HMW subunits encoded by the locus Glu-A1 in the long arm of the chromosome 1A. Concerning the LMW glutenin subunits, all landraces presented the subunit type “2” in all seeds analysed.

The study of 16 improved varieties showed the HMW glutenin subunits “6+8” and “7+8” for the most of new cultivars. Four genotypes, the oldest varieties, showed the subunit “20”. Only one cultivar, Colosseo, had “13+16” subunit.

3. Microsatellite marker analysis

Thirteen Xgwm microsatellite markers localized on chromosomes of A and B genomes were used to test polymorphism between accessions of durum wheat (Table 2).

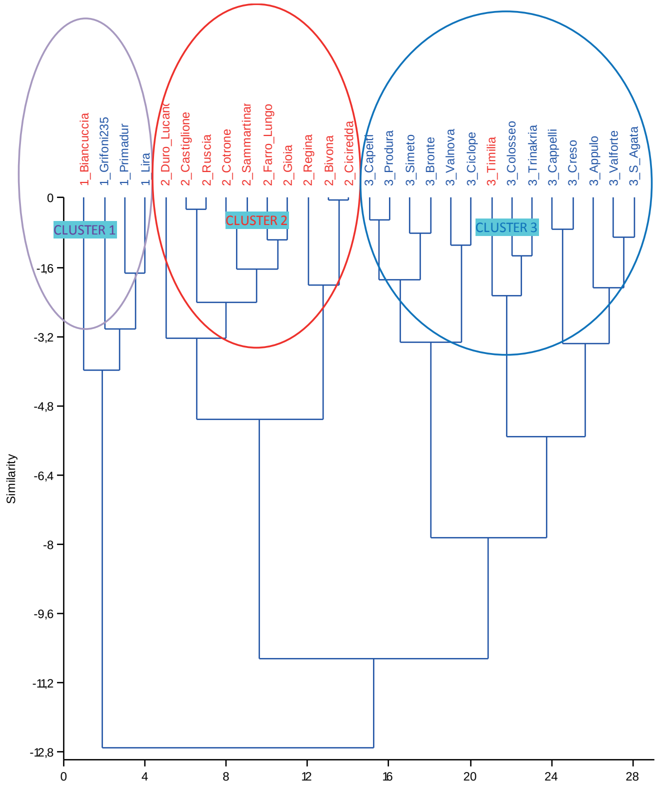

Figure 1. Dendrogram for the assessment of similarity distance of the durum wheat tested accessions (Ward’s method).

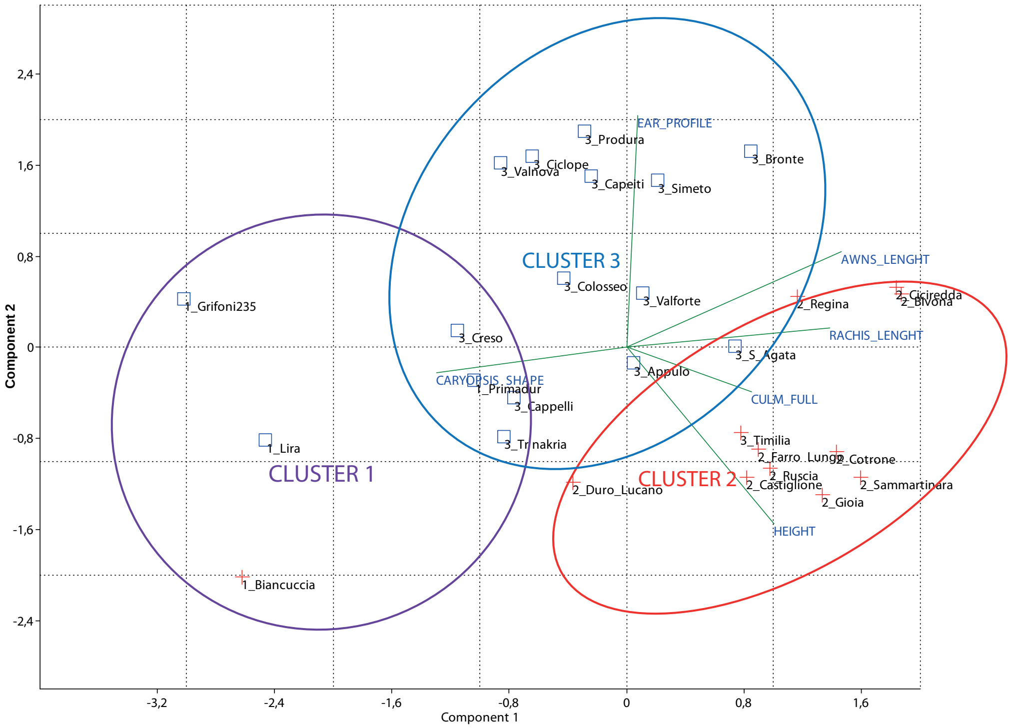

Figure 2. Biplot showing distance among 28 durum wheat accessions constructed on morphological traits.

Table 3. HMW glutenin subunits in Sicilian landraces and improved varieties.

Sicilian landraces		Improved varieties	
Biancuccia	20	Cappelli	20
Bivona	6+8/20	Griggoni 235	7+8
Castiglione	20	Cappelli	20
Ciciredda	13+16	Trinakria	20
Cotrone	20	Appulo	20
Duro Lucano	20	Creso	6+8
Farro Lungo	20	Valnova	7+8
Gioia	20	Produra	6+8
Regina	7+8	Valforte	7+8
Ruscia	6+8	Primadur	6+8
Sammartinara	13+16	Lira	6+8
Timilia	6+8/20	Simeto	7+8
		Colosseo	13+16
		Bronte	7+8
		Sant'Agata	7+8
		Ciclope	7+8

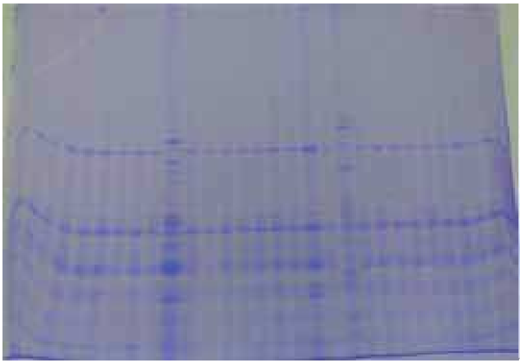

Figure 3. Sicilian landrace: Castiglione.

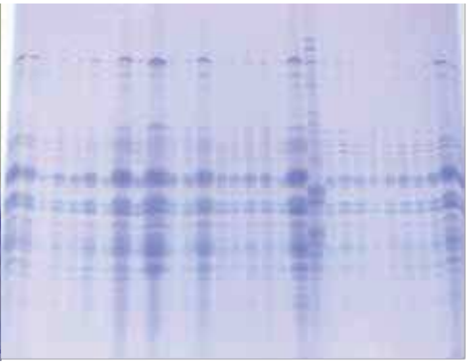

Figure 4. – Improved variety: Sant'Agata.

Preliminary results have shown amplification products between 120 and 270 bp. The most polymorphic primers were Xgwm 413-1B and Xgwm 513-4B. The Xgwm 413-1B produced fingerprinting patterns easily distinguishable for the cultivars Sant'Agata, Ciclope and Simeto, whereas Xgwm 153-1B was able to detect polymorphism intra and inter landraces. The results indicate that microsatellite loci of the B genome are highly variable.

V – Conclusions

Concerning morphological evaluation of old and new genotypes, the evolution of durum wheat varieties occurred primarily in morphological changes of plants, which led to the creation of more productive varieties. The plant height reduction and the compactness of the ear have allowed the plant to take advantage of a greater quantity of assimilated.

As regard protein composition, the results showed a progressive affirmation: the modern cultivars have the "6+8" and "7+8" HMW glutenin subunits that are suitable for the technological process.

The molecular characterization confirmed that DNA markers, which are not subject to environmental influences, are useful tools for genetic fingerprinting of old and new genotypes and to improve the efficiency in programs of gene recombination and selection.

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