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Traceability of Sicilian Durum Wheat Landraces and Historical Varieties by High Molecular Weight Glutenins Footprint

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Abstract: Over the last new decade, there has been a strong interest in landraces and historical wheat varieties from farmers, manufacturers and consumers. They are agronomically and nutritionally interesting but the supply chain (from seed to end-product) is not solid and traceable. High molecular weight glutenins (HMW-GS) can act as markers to trace the varietal correspondence and to verify the genetic purity of the grain and consequently of the flours, marketed and labeled as mono-varietal. In the present work, HMW-GS of different durum wheat Sicilian landraces (Timilia, Russello, Perciasacchi) and one historical variety Margherito were analyzed. At first, specific protein profiles were assigned to each Sicilian landrace by SDS-PAGE and MALDI-TOF/MS analyses, thanks to the availability of pure seeds. Analysis of the protein profiles were then carried out from random samples of seed batches of the same landraces grown on a farm in South-East Sicily. The results highlighted the presence of different protein bands within the individual seed batches, which are reflected in complex profiles in the corresponding commercial flours labelled as mono-varietal. The bread wheat landrace Maiorca cultivated in the same farm was also found as a contaminant at different percentages in the durum wheat batches. The results of this study offer opportunities to improve the supply chain of the different Sicilian landraces or historical varieties cultivated, underlining the need for accurate controls from the field to the transformation process to be labelled as mono-varietal products.

Keywords: Sicilian wheat landraces; germplasm conservation; molecular markers; SDS-PAGE; MALDI/TOF-MS; traceability

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1. Introduction

Genetic improvement programs of durum wheat, launched in Italy in the early 1900s, have had a significant impact on productivity and technological grain quality as a result of the needs of the processing industry and consumers' demands [1,2]. Nevertheless, the domestication process of wheat and polyploidy speciation has caused a reduction of genetic variability and confined the cultivation of the "old landraces" to niche areas, with their maintenance being entrusted to public research institutions and custodian farmers [3]. Over the last new decade, there has been a strong interest in landraces and historical wheat varieties from farmers, manufacturers and consumers [3–5]. Indeed, a higher tolerance of landraces against biotic and abiotic stresses, compared to the modern varieties, has been demonstrated [6,7]. Moreover, durum wheat landraces have a higher content of phenols, known for their healthy properties, than modern varieties [4,8], as well as a higher concentration of minerals [9]. From a technological point of view, some old wheat varieties are also well suitable to pasta production, such as Cappelli [10], and for bread-making, such as Andriolo [11].

Sicily, a wide region of Southern Italy, is particularly rich in landraces of many agricultural species, especially durum wheat. Because of their adaptability to low agronomic input and sustainability for organic farming, several durum wheat landraces were recently re-discovered and re-employed [3].

Thanks to the conservation work of the seeds and the agricultural cultural heritage of the CREA (Research Centre for Cereal and Industrial Crops of Acireale (CT)), it has been possible to repopulate the countryside with the traditional grains that are grown in organic or natural environments, thanks to their characteristics of rusticity and tolerance to biotic and abiotic stresses. Today, the flours of Sicilian landraces such as Timilia, Russello, Perciasacchi and Maiorca, or of historical varieties such as Margherito and Cappelli, are commercial available to increase the supply of native varieties.

Since 2009, the Ministry of Agriculture, Food, Forestry and Tourism Policies of Italy established the National Register of the varieties of agricultural plant species (Legislative Decree n. 149) [12] for the registration of landraces (syn. local varieties) naturally adapted to local conditions and threatened by genetic erosion. Unfortunately, only some landraces appear to be registered and the seeds are produced and maintained among farmers. This implies that it is difficult to classify different landraces that can be mixed during harvesting by mechanical means or during storage of the grain, or to a much lesser extent, to any cross-pollination between different genotypes during farming.

In order to preserve these landraces and derived-products, already present in the market, the discovery of markers for their identification is necessary. While in the past wheat genetic diversity has been mostly evaluated using morphological and phenological descriptors, with the development of PCR, DNA-based markers have been abundantly utilized because they are easy to use and can be also applied to flour and end products. In wheat, single nucleotide polymorphisms (SNPs) are the most employed molecular markers [13–17].

Other markers utilized for the classification of wheat are the gluten proteins. Gluten is a protein complex, typical of cereals, made up of hundreds of protein components structured in the form of monomers or polymers. In general, gluten proteins are distinguished in polymeric alcohol-insoluble glutenins and monomeric alcohol-soluble gliadins. They form complexes among themselves through the formation of intra-chain or inter-chain disulfide bridges, hydrogen bonds and hydrophobic interactions, giving rise to a protein network that plays an important role in determining the quality of bread-making and pasta-making processes [18,19]. Glutenin can be divided into two categories: high MW subunits (HMW-GS, 70–140 kDa) and low MW subunits (LMW-GS, 30–50 kDa). Durum wheat HMW-GS is coded by the loci *Glu-A1* and *Glu-B1*, which are positioned respectively on the long arm of chromosomes 1A and 1B. On each locus there are two genes closely associated with each other that code for a subunit of type x (x-type) and one of type y (y-type), which have a different electrophoretic mobility; the x subunits have a lower mobility with respect to the subunits y. Each HMW-GS protein variant determines different quality characteristics of the wheat flour, so once the allelic structure has been identified, it is also possible to assign to each profile a different quality level; for example, the allelic variation in the *Glu-B1* locus coding for the Bx7 + By8 subunits is associated with a good quality of durum wheat; while the Bx20 profile is associated with a lower quality [20–22]. In this regard, HMW-GS are quality markers that are used in breeding programs, to select wheat cultivars that have advantageous technological characteristics [23].

The most used technique for identifying the composition of HMW-GS is SDS-PAGE (sodium dodecyl sulfate-poly-acrylamide gel electrophoresis) [24], but mass spectrometry (MS) methods are also currently used [25–27]. MS differs to the more conventional methods in terms of resolution, sensitivity, accuracy, throughput and precision in quantification of proteins.

The aim of this work is the application of SDS-PAGE and MALDI/TOF-MS to assign the allelic composition of HMW-GS of three durum wheat Sicilian landraces (Timilia, Russello, Perciasacchi) and one historical variety (Margherito) in order to trace the varietal

correspondence and the genetic pureness of grains and flours labeled as mono-varietal, produced by a Sicilian organic management farm. The Maiorca Sicilian landrace was also used to verify the presence of possible hexaploid wheat contamination.

2. Materials and Methods

2.1. Materials

In this work, seed batches and whole flours labelled as mono-varietal of three Sicilian durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) landraces, including Timilia, Russello, Perciasacchi, one historical variety, Margherito, and one bread wheat (*Triticum aestivum* L. subsp. *aestivum*) Sicilian landrace, Maiorca, were supplied by a farm in the province of Ragusa (Sicily) that grows and grinds wheat.

Pure seeds of the same landraces/historical variety, in particular Timilia (syn. Tumminia), Russello (syn. Priziusa), Ruscìa (syn. Russello ibleo), Perciasacchi (syn. Strazzavisazzi), Margherito (syn. Bidì) and Maiorca propagated in large carefully purified plots derived from the collection at the CREA (Research Centre for Cereal and Industrial Crops of Acireale, CT). The pure seeds of commercial durum wheat reference varieties Aureo (Bx6 + By8), Biensur (Bx7 + By8) and Cappelli (Bx20 + By20) supplied by CREA were used for the comparison of landraces and historical variety protein assets.

2.2. Extraction and Quantification of Gluten Proteins in Seeds and Commercial Flours

Gluten proteins, gliadins, HMW-GS subunits and LMW-GS subunits were determined in the commercial flours labelled as mono-varietal from the different genotypes using the sequential extraction procedure [28]. An amount of 30 mg of flour was subjected to extraction with 1.5 mL of 550 mL·L^{-1} propan-2-ol for 20 min with continuous mixing at 65 °C, followed by centrifugation at 10,000× *g* for 5 min. This step was repeated three times, and the supernatants were combined and dried in a vacuum centrifuge to obtain the protein gliadin fraction. The remaining pellet containing the GS fractions was suspended in a 400 µL solution of 550 mL·L^{-1} propan-2-ol, 0.08 mol·L^{-1} tris (hydroxymethyl) aminomethane hydrochloric acid (Tris–HCl, pH 8.3) and 10 g·L^{-1} 1.4-dithiothreitol (DTT, as reducing agent) and incubated for 30 min at 60 °C with continuous mixing. After centrifugation at 14,000× *g* for 5 min, the supernatant was transferred to a new tube. To precipitate HMW-GS, acetone was added to obtain a final concentration of 400 mL·L^{-1}, which was then centrifuged at 14,000× *g* for 10 min. The LMW-GS fraction was precipitated in the remaining supernatant by adding acetone to obtain a final concentration of 800 mL·L^{-1}, and this was then centrifuged at 10,000× *g* for 10 min. The GS fractions and gliadins were dissolved in 500 mL·L^{-1} acetonitrile (ACN) with 1 mL·L^{-1} trifluoroacetic acid (TFA); relative quantification was determined by colorimetric Bradford assay (Bio-Rad, Hercules, CA, USA). Five technical replicates were performed for each sample.

Single seeds of the different pure wheat landraces and commercial varieties from CREA and each single seed deriving from batches of grains labelled as mono-varietal from the farm (Ragusa-Sicily) were crushed in a mortal with a pestle until a fine powder was obtained. The procedure for extracting HMW-GS from seeds (30 mg) was the same as reported above. Gliadins and LMW-GS were discharged during the procedure while the HMW-GS pellet was air dried and left at −20 °C for SDS-PAGE and MALDI-TOF/MS protein analyses.

2.3. Proximate Composition of Commercial Wheat Flour Samples

Moisture content, total protein, total fat, total fiber, starch and ash content were determined according to AOAC [29]. All analyses were performed in triplicate for each sample and are expressed as the means.

2.4. HMW-GS Protein Separation by SDS-PAGE

SDS-PAGE was performed in a Mini-PROTEAN Tetra Cell (Bio-Rad, Hercules, CA, USA) on 80 g·L^{-1} acrylamide gels. Aliquots of dried HMW-GS were suspended in 20 µL

of loading buffer containing 20 g·L^{-1} SDS, 0.2 g·L^{-1} bromophenol blue, 1 mL·L^{-1} β-mercaptoethanol, 0.05 mol·L^{-1} Tris–HCl (pH 6.8) and 100 mL·L^{-1} glycerol and boiled at 95 °C for 5 min before loading onto the gels. A ColorBurst™ Electrophoresis Marker High Range (MW 30,000–220,000 Da) was used to detect HMW-GS. After electrophoretic separation at 40 mA, the gels were stained with brilliant blue G-colloidal solution (Sigma-Aldrich, Milan, Italy) fixed in 70 mL·L^{-1} acetic acid and 400 mL·L^{-1} methanol, and de-stained in 250 mL·L^{-1} methanol. Image Lab 4.5.1 software (Bio-Rad) was used to assess protein molecular weights (MWs) of HMW-GS single protein subunits on each gel.

2.5. MALDI-TOF/MS HMW-GS Identification

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) analysis in linear mode was carried out as previously described [30] by using a 4800 Plus MALDI-TOF/TOF™ (AB SCIEX, Framingham, MA, USA) equipment. A mass range of 10,000–100,000 Da was used, and about 400 laser shots were averaged to improve the signal-to-noise level. Calibration was performed by a ProteoMass Protein MALDI-MS Calibration kit (Sigma Aldrich, St. Louis, MO, USA). The matrix solution was prepared by dissolving sinapinic acid (SA) in 50% ACN at a concentration of 10 µg·µL^{-1} according to Liu et al. (2009) [24]. One microliter of 1:10 (v/v) sample/matrix solution mixture was spotted directly onto a stainless steel MALDI target plate, and the solution was allowed to dry at room temperature. Positively charged ions were analyzed in linear mode. Three biological replicates for each sample were performed.

2.6. Statistical Analyses

Statistical analysis of the data was performed with the Statgraphics Centurion XIV software (StatPoint Technologies, Inc., Warrenton, VA, USA) and the results compared by one-way ANOVA. Significant differences were determined by Tukey's test.

3. Results and Discussion

3.1. Landraces History

Table 1 shows the origin, historical information and local use of the Sicilian wheat landraces and commercial varieties chosen for this study [31]. All genotypes were the most cultivated landraces in Sicily until the middle of the last century [32]. Even now, in some areas of Sicily, the landraces or historical varieties of wheat make it possible to guarantee the production of artisanal pasta, bread and other typical bakery products that are also Italian specialties in the world. For example, the bread named 'Pane Nero di Castelvetrano' (Castelvetrano Black Bread), made with at least 20% of cultivar Timilia, has been declared a Slow Food Presidium, a recognition awarded to safeguard high quality artisan products (Slow Food Foundation for Biodiversity, International Presidia) [33]. Other durum wheat breads have been acknowledged by the denomination *Traditional Agricultural Food Product*. Among them, 'Pane di Monreale' is made with Timilia and Russello flour [34].

Mono-varietal flours and pasta related to some of these local Sicilian varieties are already available on the market but the lack of a traceability system starting from seed propagation does not give the guarantee that the transformed product is 100% the variety signed on the label.

3.2. Chemical Composition of Commercial Sicilian Wheat Flours

Table 2 shows the chemical characteristics of Sicilian durum wheat landraces labelled as Timilia, Russello, and Perciasacchi, of one historical durum wheat variety Margherito and of the bread wheat Sicilian landrace Maiorca deriving from commercial batches from the Sicilian organic farm. In particular, there is a high fiber content in all samples, since they are wholemeal flours, while the total protein content is at least 13 g 100 g^{-1} flour, which is considered a good value for technological quality for all the semolina samples. Specifically, Perciasacchi showed a very high protein content (around 16 g 100 g^{-1} flour),

indicating a good quality in regards to gluten constituent and protein content for the pasta making process [19].

Table 1. Taxonomy, origin and main local uses of Sicilian wheat landraces/historical and commercial varieties analyzed in this study.

Taxonomic Classification	Genotypes	Geographic or Genetic Origin and Historical Information	Year of Release	Local Use
T. turgidum ssp. *durum*	Cappelli	Historical variety selected in Apulia from Tunisian landrace Jenah Rhetifah by Strampelli	1915	Dry pasta, artisanal breads
T. turgidum ssp. *durum*	Margherito (syn. Bidì)	Historical variety selected in Sicily from Tunisian landraces 'Mahmoudi and Bidì' by Santagati (Eastern Sicily) with name Margherito and Tucci (Western Sicily) with name Bidì	<1915	Dry pasta, fresh pasta, artisanal breads
T. turgidum ssp. *turanicum*	Perciasacchi (syn. Strazzavisazzi)	Indigenous landrace from Sicily	<1900	Dry pasta, fresh pasta, artisanal breads, sweet and savory baked goods
T. turgidum ssp. *durum*	Russello (syn. Priziusa)	Indigenous landrace from Sicily	1927	Dry pasta, fresh pasta, artisanal breads, sweet and savory baked goods
T. turgidum ssp. *durum*	Ruscìa (syn. Russello ibleo)	Indigenous landrace from Sicily	<1900	Hard dough natural leaved bread, fresh pasta
T. turgidum ssp. *durum*	Timilia (syn. Tumminia)	Indigenous landrace from Sicily	<1900	'Castelvetrano Black bread', hard dough natural leaved bread, fresh pasta, craft beer
T. aestivum. ssp. *aestivum*	Maiorca	Indigenous landrace from Sicily	<1900	Homemade cakes, typical Sicilian sweets
T. turgidum ssp. *durum*	Aureo	Commercial variety (Italy) genealogy: Kofa/Svevo	2009	Mono-varietal dry pasta (Voiello®)
T. turgidum ssp. *durum*	Biensur	Commercial variety (France) genealogy: not available	2001	Dry and fresh pasta

As far as the total gluten proteins are concerned, similar amounts were found in Margherito, Perciasacchi and Russello, while significantly lower amounts were found in Timilia ($p \leq 0.05$) (Table 2). Besides the total protein amounts, by sequential gluten protein extraction, the relative abundance of the different gluten protein classes (gliadins, HMW-GS and LMW-GS) were also calculated. Margherito and Perciasacchi showed higher relative abundance of HMW-GS with respect to the other durum wheat landraces and, in addition, Margherito has the highest LMW-GS relative abundance. The bread wheat Maiorca showed the highest amount of HMW-GS as expected for hexaploid wheat. Both HMW and LMW-GS are important parameters affecting gluten strength, which is the ability of the proteins to form a tenacious network able to promote better extrusion properties and thus pasta with superior cooking properties [19]. Timilia instead showed the higher relative abundance of gliadin fraction with respect to the other landraces (Table 2), which render this variety more suitable for the production of leavened bread, since gliadins are mainly responsible for giving bread the ability to rise properly during baking [19].

Table 2. Proximate composition and gluten proteins quantification (Gliadins, high molecular weight glutenins—HMW-GS and low molecular weight glutenins -LMW-GS) of Sicilian commercial wheat flours.

Landrace/Historical Variety	Total Protein Content [1]	Total Gluten Proteins [2]	Gli [3]	HMW-GS [3]	LMW-GS [3]	HMW/LMW-GS	Total GS/Gli	Ash [1]	Lipids [1]	Fibre [1]	Starch [1]
Margherito	12.9 b	31.3 a	81.4 d	3.1 b	15.4 a	0.2 b	0.2 b	2.0 a	2.3 a	13.1 a	66.3 b
Perciasacchi	15.9 a	31.2 a	83.7 b	3.7 b	12.6 c	0.3 b	0.2 b	1.8 b	2.0 a	9.8 c	70.3 a
Russello	13.0 b	31.2 a	82.6 c	1.6 c	15.8 a	0.1 c	0.2 b	1.6 b	2.2 a	12.1 b	64.2 b
Timilia	13.6 b	29.4 b	85.7 a	1.3 c	13.1 b	0.1 c	0.2 b	1.8 b	2.2 a	12.8 b	65.1 b
Maiorca	13.2 b	23.1 c	74.8 d	9.3 a	15.2 a	0.5 a	0.3 a	1.9 b	2.1 a	10.6 c	68.6 b

Within each parameter, different letters indicate significant differences (Tukey test, $p < 0.05$; $n = 5$). [1] Expressed as g 100 g^{-1} of dry matter. [2] Total gluten proteins correspond to the sum of the three different protein fractions (gliadins, HMW-GS and LMW-GS) expressed in mg g^{-1}. [3] Percentage of the different gluten classes with respect to the total amount extracted[2].

3.3. Identification of HMW-GS Profiles in the Pure Seeds of the Durum Wheat Sicilian Landraces/Historical Varieties

HMW-GS were extracted both from pure seeds of each local variety from the CREA collection and from seeds of the three durum wheat commercial samples (Cappelli, Aureo and Biensur) with different known HMW-GS assets and separated in SDS-PAGE at 8%. These latter commercial varieties with different HMW-GS protein assets were utilized in order to compare protein profiles and to assign specific protein profiles to our unknown samples [35]. In particular, HMW-GS of the following durum wheat varieties were used as a comparison: Biensur (Bx7 + By8), Aureo (Bx6 + By8), Cappelli (Bx20 + By20). Based on the electrophoretic distribution of the HMW-GS of type Bx and By, it was possible to identify and attribute the asset similar to HMW-GS Bx20 of Cappelli to Margherito and Perciasacchi, the variant Bx13 + By16 to Russello (syn. Priziusa) as previously indicated by other authors [36], and an asset similar to Bx6 + By8 to Ruscìa (syn. Russello ibleo) and Timilia (Figure 1 and Table 3). For Timilia, different authors report an asset of Bx20 at locus B1 [37,38], while Muccilli et al. [39], analyzing 13 Timilia accessions from the gene bank of Experimental Wheat Crop Consortium Station of Sicily, showed that all of them contained HMW-GS assets of 75.2 kDa and 86.4 kDa, similar to a possible Bx6 and By8 asset, found also in our reference seed sample deriving from the same gene bank. Moreover, the presence of many accessions of Timilia (Timilia reste bianche, Timilia reste nere, Timilia SG1, Timilia SG2, Tumminia SG3) in the South of Italy with different HMW-GS assets could not be excluded. The data on the different landraces analyzed in this work by SDS-PAGE were also validated by the exact *m/z* calculated by MALDI/TOF-MS (Figure 2 and Table 3).

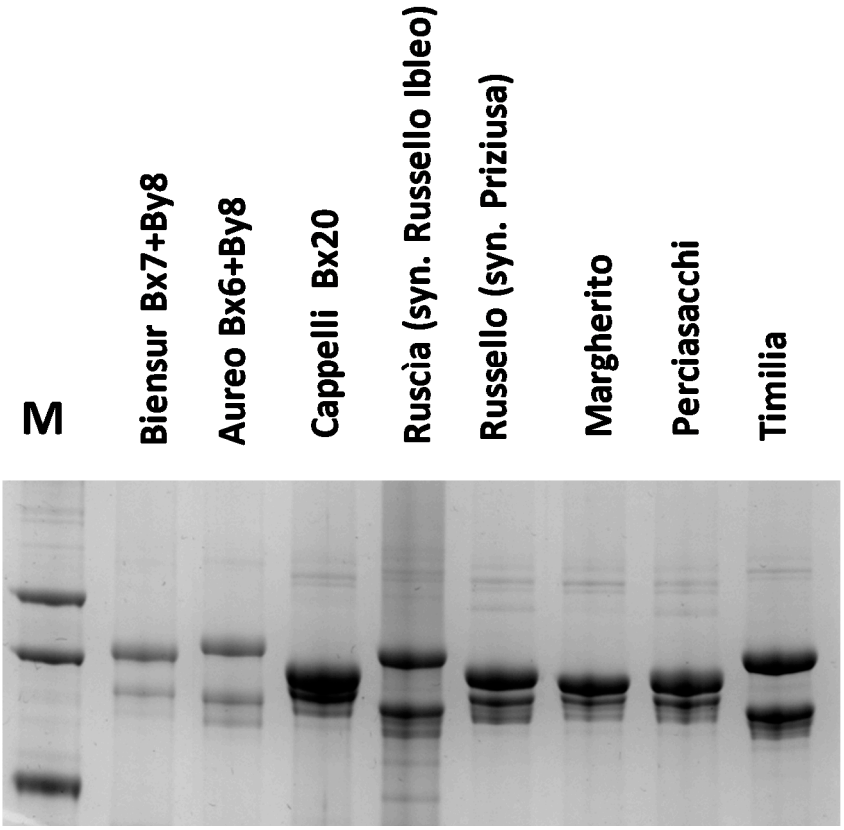

Figure 1. HMW-GS extracted from three commercial varieties of durum wheat (Biensur, Aureo, Cappelli) and from the durum wheat reference seeds (Ruscìa, Russello, Margherito, Perciasacchi and Timilia) obtained from CREA. M: markers weight.

Table 3. HMW-GS profiles assigned to the reference seeds of landraces and historical cultivars both with SDS-PAGE profiles in comparison with commercial durum wheat varieties and by MALDI-TOF/MS analyses.

Modern and Historical Cultivars and Landraces	HMW-GS [1]	*m/z* [2]
Biensur	Bx7 + By8	82.927; 74.862
Aureo	Bx6 + By8	86.510; 74.899
Cappelli	Bx20 + By20	82.467; 75.304
Margherito	Bx20 + By20	82.475; 75.488
Perciasacchi	Bx20 + By20	82.423; 75.060
Ruscìa	Bx6 + By8	86.026; 75.060
Russello	Bx13 + By16	82.612; 76.944
Timilia	Bx6 + By8	86.618; 74.374

[1] High molecular weight glutenin subunit assets (HMW-GS) assigned by SDS-PAGE. [2] *m/z* obtained by spectra from MALDI/TOF-MS analysis in linear mode.

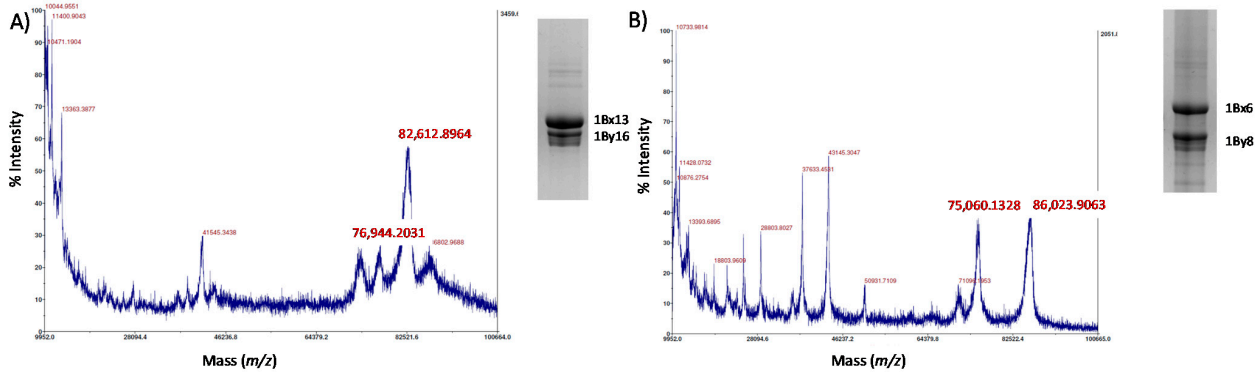

Figure 2. Examples of HMW-GS spectra of: (**A**) Russello (syn. Priziusa) and (**B**) Ruscìa (syn. Russello ibleo) durum wheat landraces. Mass spectra obtained by MALDI-TOF/MS analysis in linear mode and the corresponding bands separated by SDS-PAGE.

3.4. Analysis of HMW-GS Profiles in Commercial Flours and in the Corresponding Seed Batch of Each Wheat Sicilian Landrace/Historical Variety

Following the identification of the HMW-GS of the pure seeds of the gene bank of CREA in durum wheat local varieties, a characterization of the HMW-GS was carried out in the samples of commercial flours labeled as mono-varietal and in the corresponding batches of wheat grains, both supplied by the Sicilian farm. This latter approach was necessary to verify that all the seeds of the same grain batch, from which flour labeled as mono-varietal is produced, are characterized by the same HMW-GS profile. For this purpose, 15 to 20 seeds were randomly selected from the seed batches from which the flours labelled as mono-varietal derived. Figure 3 shows, for each Sicilian local variety, an example of the HMW-GS profile of the commercial flour labelled as mono-varietal, the HMW-GS profile of the reference pure seed deriving from the CREA gene bank and a number of HMW-GS profiles of single seeds from the different commercial batches labelled as Margherito, Perciasacchi, Russello, Timilia. The bread wheat Maiorca flour and seed batch was also analyzed, as bread wheat was cultivated in the same farm.

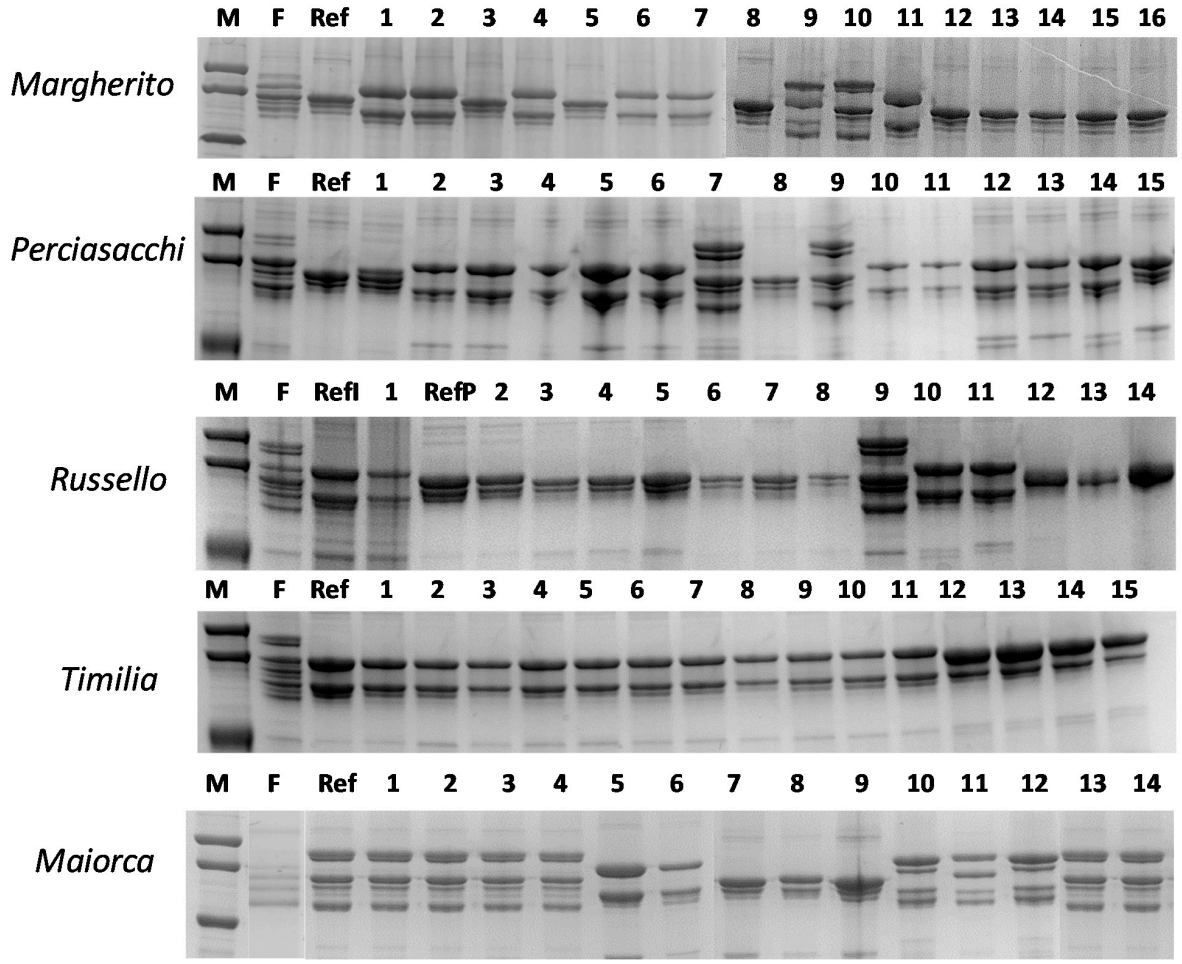

Figure 3. SDS-PAGE of single seed HMW-GS extraction from batches of three durum wheat Sicilian landraces (Perciasacchi, Russello, Timilia), one historical durum wheat variety (Margherito) and one bread wheat Sicilian landrace (Maiorca). F = flour labelled as mono-varietal; Ref: pure seed HMW-GS reference; RefI = pure seed HMW-GS reference Ruscìa (syn. Russello ibleo); RefP = pure seed HMW-GS pure seed Russello (syn. Priziusa).

As far the commercial flour samples labelled as mono-varietal are concerned, many protein bands were present in the samples and it was not possible to assign a specific HMW-GS profile corresponding to any of the pure seeds of the wheat landraces (Figure 3). In addition, a contamination of bread wheat was evident in many of the commercial flours of the durum wheat landraces, which is visible by the presence of high molecular weight HMW-GS subunits typical of D genome in the gels.

In particular, for Margherito, many different profiles of both durum and bread wheat are present and only a few seeds from the seed batch corresponded to the reference profile Bx20 present in Figure 1. As an example, in Figure 3, five seeds have the Bx6 + By8 profile, and in two seeds, five HMW-GS bands were found, some of those corresponding to proteins encoded by the D genome typical of bread wheat. These data support the hypothesis that the grain of Margherito certified as mono-varietal not only has contamination by other durum wheat varieties, but also contamination from Maiorca, bread wheat landrace cultivated in the same farm (Figure 3).

For Perciasacchi, most of the seeds are characterized by a profile different from the reference Bx20 (Figure 3). Two seeds have a Bx20 profile, while the remaining have the Bx6 + By8 profile (Figure 3). Bread wheat contamination was also present in the batch.

Single seeds of the Ruscìa batch are characterized by two protein profiles shown in Figure 3. Most of the seeds have the Bx13 + By16 profile, associated with the reference Russello (Figure 1), while some samples have a profile Bx6 + By8 corresponding to Ruscìa

(Figure 1). However, although most of the seeds appear to belong to one of the two landraces, there are some seeds having a different profile than the reference ones. In fact, the presence of seeds showing a profile similar to Bx20 and others with 5 bands typical of bread wheat Maiorca was also highlighted.

As far as Timilia is concerned, the image of the gel in Figure 3 showed that most of the seeds are characterized by the HMW-GS Bx6 + By8 profile, consisting of 2 bands, which correspond to the reference profile in Figure 1. However, some samples have a different profile, corresponding to Bx7 + By8, as shown in the reference gel (Figure 1), which do not belong to any of the landraces analyzed in this study. The same analysis was performed on a seed batch from bread wheat Maiorca cultivated in the same farm. Most of the HMW-GS protein profiles of Maiorca seeds are made up of 5 bands. However, few seeds have the Bx6 + By8 profile; while other seeds have a Bx20 profile. Both of these profiles were ascribed to durum wheat landraces.

The MALDI-TOF/MS analyses performed on a further 40 seed samples from the different seed batches allowed us to define the percentages of contamination found in the different grain batches (Figure 4). The Margherito batch has 47% of Margherito grain, 40% of Timilia grain and 13% of bread wheat. Perciasacchi batch has only 13% of Perciasacchi seed, 74% of Timilia profile and 13% of bread wheat. Russello constitutes 70% of the grain called Russello, although only the "word" Priziusa appears on the label, 70% is made up of 50% of the Priziusa and 20% of the Ruscìa accession; a 5% contamination is from bread wheat. Timilia constitutes 70% of the grain called Timilia, while 20% was from durum wheat and 10% was bread wheat. The bread wheat Maiorca is also represented by 67%, but otherwise has contamination from durum wheat Timilia and Margherito.

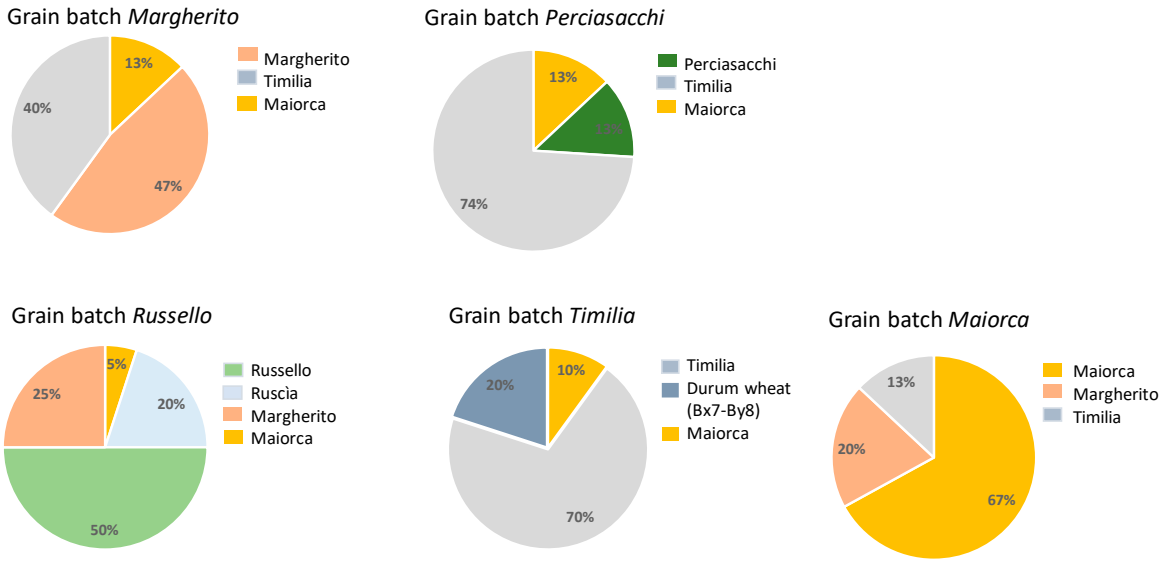

Figure 4. Percentages of contamination found in the different grain batches of three durum wheat Sicilian landraces (Perciasacchi, Russello, Timilia), one historical durum wheat variety (Margherito) and one bread wheat Sicilian landrace (Maiorca).

4. Conclusions

The environmental and historical characteristics of Italy guarantee a high degree of agricultural biodiversity. The benefits of biodiversity are reflected in services relating to the food supply chain and cultural interest. Indigenous varieties and historic wheat varieties are agronomical and nutritionally interesting [40] and there is currently a strong interest from consumers, farmers and producers. However, the production and marketing of certified seeds, as well as the correspondence with derived products, is not guaranteed due to a lack of traceability along the supply chain. Therefore, the flours labeled as mono-

varietal for the production of pasta/bread (reporting the specific genotype on the label), may not fully correspond to the declared variety.

The results of this study showed that each flour examined (labeled as mono-varietal) is cross-contaminated by the other local varieties of durum wheat and by the native Maiorca bread wheat grown on the same farm. Therefore, contaminations may be due either to the exchange of genetically non-pure seeds between custodian breeders or to the mixing of seeds during harvesting by mechanical means, or, to a lesser extent, to cross-pollination between different genotypes. All that has been highlighted therefore opens new opportunities to improve the supply chain of local wheat varieties through a traceability system to verify the varietal identity from the seed to the final product. Finally, a solid certification system for these products will be able to protect both the farmer and the consumer: the farmer will have an economic advantage as the consumer will be willing to pay a higher price if he has the guarantee on the product that he expects to have nutritional and health benefits.

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