Assessment of grain protein composition in old and modern Italian durum wheat genotypes

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Abstract

The effect of durum wheat breeding on technological quality was mainly investigated in relation to allelic polymorphism, in particular for glutenins, while fewer information are available on the changes in grain protein proportion. In the present investigation, an old and a modern group of durum wheat genotypes, grown in Mediterranean environment, were evaluated for grain protein composition, according to Osborne extraction procedure. In modern genotypes, a higher relative content of soluble glutenin was observed which might contribute to their better technological performance. Moreover, a slight decrease both in the amount of gliadin fraction, mainly responsible for gluten related disorders, and in the monomeric to polymeric protein ratio was observed in the modern durum wheat varieties. Among the genotypes investigated, Svevo and Saragolla, showed the lowest gliadin and the highest glutenin content, while the old genotypes Cappelli showed an opposite behaviour.

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Introduction

The Mediterranean basin represents the main geographical region of durum wheat (*Triticum turgidum* L. spp. *durum*) cultivation, with a contribution of about 60% of the world production (Casolani *et al*., 2016). Semolina obtained from kernel milling process is mostly used for pasta production and secondary for bread and other minor products. Breeding programs started in Italy in 1915 with the release of cultivar Cappelli by Nazareno Strampelli; the initial aim was to select lines and cultivars characterised by higher grain yield, earliness and reduced lodging. A secondary target of breeding was to improve grain quality, especially in terms of protein content and technological end-use.

The aptitude of wheat to be processed for the production of different foods is mostly determined by grain proteins accumulated in the endosperm. The protein fractions are generally classified on the basis of the sequential extractability proposed by Osborne (1924) in two main groups: water-soluble albumins and globulins, that generally include metabolic polypeptides, and water-insoluble (or alcohol-soluble) gliadin and glutenin also known as storage proteins (Shewry and Tatham, 1990). The unique properties to form the dough depend on the structures and interactions of the storage proteins (Shewry, 2009), called also gluten-forming proteins. Gluten proteins include a large number of sub-fractions, generally classified on the basis of their mobility in electrophoresis. Polymeric glutenins are classified in high (HMW-GS) and low (LMW-GS type B, C and D) molecular weight glutenin subunits. In durum wheat (AABB) HMW-GSs are mainly encoded by *Glu-B1* and *Glu-A1* genes, while LMW-GS by *Glu-B3* and *Glu-A3* genes. A genetic polymorphism for glutenin proteins is known (Payne *et al*., 1979; Pogna *et al*., 1990), including alleles associated to good and poor dough properties (Peña *et al*., 1994). Genetic improvement programs on durum wheat during 20th century were predominantly focused on the selection of genotypes associated with a strong gluten, generally determined by gluten index or alveographic characteristics (Sissons, 2008). Studies performed on lines bred from Mediterranean area demonstrated that the most of them are characterized by *Glu-B1* 7+8 or 6+8 and *Glu-B3* type II alleles configuration (Raciti *et al*., 2003; De Vita *et al*., 2007; Subira *et al*., 2014; Nazco *et al*., 2014). In addition, the higher relative expression of the B-type LMW-GS in the modern durum wheat varieties has been recently reported by De Santis *et al*. (2017). While the effect of breeding in terms of subunit composition was deeply investigated (De Vita *et al*., 2007; Ribeiro *et al*., 2016), lacking information is available on the changes in the proportion of the different protein fractions due to breeding activity (Juhasz *et al*., 2003), in particular on durum wheat (Fois *et al*., 2011). In order to deep insight into the changes in relative composition of grain protein fractions due to breeding activity during 20th century, in this paper an old and a modern group of Italian

durum wheat genotypes were compared in a two-years field trial under Mediterranean conditions.

Materials and methods

Plant material

Two groups of Italian durum wheat (*Triticum turgidum* L. spp. *durum*) genotypes were chosen on the basis of their release dates: old genotypes (1900-1949) and modern ones (1985-2005). The year of release, pedigree and genetic information on *Glu-B1* and *Glu-B3* genes have been previously reported in De Santis *et al*. (2017). Briefly the old group consists of four old Italian landraces (Dauno III, old Saragolla, Russello, Timilia R.B. *reste bianche*), genotype Cappelli and two cultivars (Garigliano and Grifoni 235) obtained by selection from Cappelli. Relative to the modern genotypes, they were all bred after the introduction of the dwarfing (Rht) genes, from 1985 to 2005. All modern genotypes are relatives with Cappelli.

Field trials

Plants were grown in the field on a clay-loam soil at Foggia (Italy, 41° 28' N, 15° 32' E and 75 m a.s.l.), in two years (2013 and 2014) as reported in De Santis *et al*. (2017). Briefly, a randomised block design with three replications was used; 80 kg ha^{-1} of nitrogen and 70 kg ha^{-1} of phosphorous were applied in each crop season. The two crop seasons, 2012/13 (2013) and 2013/14 (2014), showed a comparable thermal and rainfall distribution. Differences were observed only in the amount of rainfall during grain filling, with a lower values in 2013 (53.8 mm *vs* 152.8 mm).

Grain protein analysis

Semolina was obtained by laboratory mill (4 cylinders, sieve 180 µm, Bona). Semolina protein content (SPC) was determined by NIR (Infratec 1241 Analyzer, Foss, Hillerod, Denmark, ICC159). Endosperm proteins were extracted according to De Santis *et al*. (2017). Briefly, 100 mg of semolina were suspended in 0.4 mL of KCl buffer (pH 7.8) and centrifuged at 14,500 g (4°C, 10 min) and the supernatant, containing albumin and globulin (A/G), was collected. The KCl-insoluble fraction was suspended in 1-propanol solution (50% v/v) and centrifuged for 10 min at 4500 g (repeated twice), then supernatant (gliadin) was recovered and collected. Soluble glutenins were extracted from the pellet by extraction solution (1-propanol 50% v/v, 1% DTT), after centrifugation at 10,000 g for 10 min (room temperature). All fractions were concentrated by SpeedVac™ and then suspended in 8 M urea solution. Protein quantification was performed by Biuret method. Residue glutenin content (from the pellet of glutenin) was determined as difference between the total protein content and sum of A/G, gliadin and soluble glutenin, as reported in Zilic *et al*. (2011). Total glutenin was then determined as sum of the amount of soluble and residue glutenin fractions. Monomeric to polymeric storage protein ratio (mon:pol) was determined as the proportion of the amount of gliadin to total glutenin.

Statistical analysis

Data from the two years were analysed using analysis of variance (ANOVA) after testing the variance homogeneity of the investigated parameters by Bartlett's test. The significant differences among the mean values were assessed by Tukey's test. Comparison of the mean values of the two groups was performed by Student's *t*-test. Principal component analysis (PCA) was carried out on all samples with regard to the six considered variables (SPC, A/G, gliadin, soluble and total glutenin, mon:pol). The varimax method was adopted to obtain the best factor rotation. Statistical analysis was performed by JMP (SAS Institute) software.

Results

Grain protein composition

The analysis of variance generally showed a significant effect of genotype, crop season and of their interaction (GxY) on all investigated parameters, except for total glutenin (Table 1). Within the old group, SPC ranged from 11.0% (Grifoni 235) to 15.4% (old Saragolla, Cappelli) and within the modern one from 10.7% (Claudio) to 14.9% (Svevo); mean values resulted significantly higher in the old group. A/G content represented from 17.2% (old Saragolla) to 23.9% (Dauno III) of the total semolina protein content in the old group and from 16.0% (Preco) to 27.7% (PR22D89) in the modern group. A significant higher content was observed in 2014 only for the old genotypes. Moreover, the modern group showed a wider range of variation with respect to the old one, with no significant difference between the two groups (Figure 1).

Gliadin content showed a slight higher mean value in the old group, which also showed a higher variability from 26.4% of Grifoni 235 to 47.2% of Cappelli, with respect to the modern group ranging from 21.6% (Saragolla) to 39.4% (Simeto). A general significant increase occurred in 2014 in the old group with the exception of Timilia RB that showed a decrease in that crop season. Within the modern cultivars, a significant increase in 2014 was observed only for Iride and PR22D89.

As for soluble glutenins, mean values ranged from 6.1% (Garigliano) to 19.4% (Russello) within the old group and from 13.9% (Preco) to 24.4% (Iride) within the modern group; so the modern group was characterized by higher values (Figure 1) distributed in a narrower range of variation. Furthermore, higher values in the modern genotypes were observed in 2013.

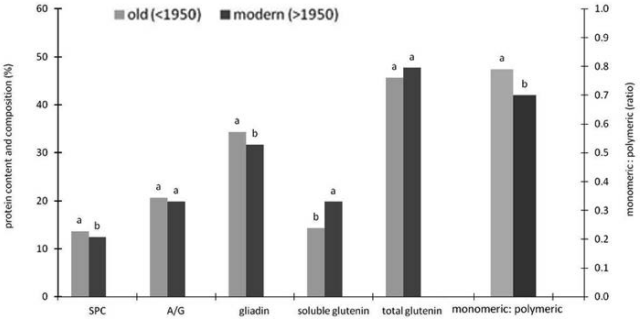

Figure 1. Protein content and composition of old and modern groups of durum wheat genotypes. Values are mean of the two crop seasons. SPC, semolina protein content; A/G, albumin and globulin. Different letters indicate values significantly different at P≤0.05 according to Student's T-test.

Overall glutenin content (polymeric storage proteins) ranged from 34.0% (Cappelli) to 55.0% (Grifoni 235) in the old group and from 36.7% (Iride) to 59.1% (Saragolla) in the modern group. The effect of the crop season was not significant, both in old and in modern genotypes. Cultivars Svevo and Saragolla were characterised by the highest total glutenin content. The mon:pol ranged from 0.50 (Grifoni 235) to 1.39 (Cappelli) in the old group and from 0.37 (Saragolla) to 1.04 (Iride) in the modern group. Furthermore, a slight lower mean values was observed in the modern group (Figure 1).

Principal component analysis (PCA) was performed on the correlations matrix of the different investigated parameters, as reported in Figure 2. The first two components explained 82% of the total observed variability. The first factor (50% of the total variability) was positively associated with gliadin and mon:pol and negatively with total glutenin content. Along the second factor (32% of the total variability) a positive association was observed with SPC, while a negative association was found with A/G and the soluble glutenin content. While no marked discrimination between the two genotype groups was observed along factor 1, a better separation was found along the factor 2, with the modern genotypes grouped in the lower part of the score box (Figure 2).

Discussion

In this paper the differences between an old and a modern group of Italian durum wheat genotypes released in the 20th century were explored in relation to the proportion of grain protein fractions. The observed values of the metabolic proteins (albumin and globulin), complementary with the storage proteins, were not different between old and modern groups and were in a range in accordance with the literature (Shewry, 2009). The observed vari-

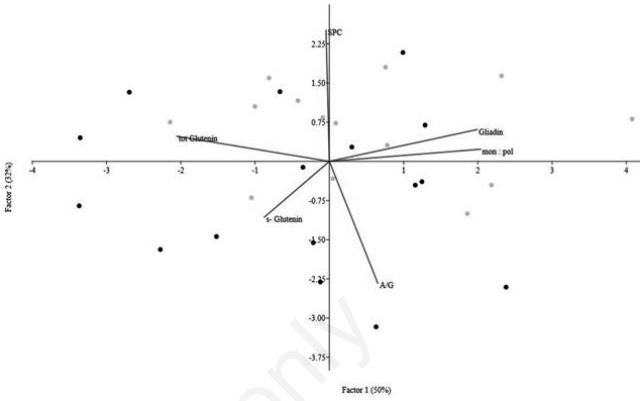

Figure 2. Principal component analysis of grain protein composition of old and modern durum wheat genotypes grown in two crop seasons. Old genotypes (grey) and modern genotypes (black). SPC, semolina protein content; A/G, albumin and globulin; s-glutenin, soluble glutenin; tot glutenin, total glutenin; mon:pol, monomeric to polymeric storage proteins ratio.

Table 1. Semolina protein content composition of old and modern durum wheat genotypes grown in 2013 and 2014 crop seasons (A), and F significance level from analysis of the variance (B).

A) Genotypes	YR	SPC (%)		A/G (%)		Gliadin (%)		Glutenin Soluble (%)		Glutenin Total (%)		mon:pol (ratio)	
		2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Old<1950													
Dauno III	Landrace	14.1^{DE}	13.2^{GH}	20.3^{GH}	23.9^{D}	33.2$^{H–L}$	37.5$^{A–D}$	13.9^{L}	13.0^{L}	46.5$^{a–e}$	38.6$^{c–e}$	0.72$^{D–H}$	0.99^{B}
Old Saragolla	Landrace	13.7^{EF}	15.4^{A}	17.2^{N}	18.2$^{K–M}$	31.8$^{J–M}$	31.8$^{J–M}$	17.2^{IJ}	17.2$^{H–J}$	51.0$^{a–d}$	50.1$^{a–d}$	0.62$^{G–K}$	0.64$^{F–J}$
Russello	Landrace	14.6^{BC}	12.4^{JK}	18.3$^{K–M}$	20.2^{GH}	33.4$^{G–L}$	34.0$^{F–J}$	17.7$^{A–E}$	19.4$^{D–I}$	48.3$^{a–e}$	45.9$^{a–e}$	0.69$^{D–I}$	0.74$^{C–H}$
Timilia RB	Landrace	14.9^{B}	12.9^{HI}	19.1^{IJ}	20.7^{FG}	40.5^{B}	34.0$^{F–K}$	12.3^{L}	8.5^{M}	40.4$^{b–e}$	45.3$^{a–e}$	1.03^{B}	0.76C$^{–H}$
Cappelli	1915	15.4^{A}	13.6^{FG}	17.9^{LM}	18.8^{JK}	36.7$^{C–F}$	47.2^{A}	14.6^{KL}	18.4$^{D–J}$	45.3$^{a–e}$	34.0^{e}	0.84$^{B–F}$	1.39^{A}
Garigliano	1927	13.9^{EF}	14.4$^{O–Q}$	20.7^{FG}	23.8^{D}	30.7$^{L–N}$	35.3$^{E–I}$	7.0^{M}	6.1^{M}	48.6$^{a–e}$	40.9$^{b–e}$	0.64$^{F–J}$	0.86$^{B–E}$
Grifoni 235	1949	12.0^{CD}	11.0^{KL}	18.6$^{J–L}$	21.1^{F}	26.4^{OP}	29.1$^{M–O}$	18.0$^{E–J}$	17.2$^{I–J}$	55.0^{ab}	49.8$^{a–d}$	0.50$^{I–L}$	0.59$^{G–K}$
Modern >1950													
Adamello	1985	12.6^{IJ}	14.9^{B}	19.6^{HI}	18.1$^{K–M}$	32.5$^{I–M}$	32.4$^{I–M}$	16.5^{JK}	17.3$^{H–J}$	47.9$^{a–e}$	49.5$^{a–d}$	0.69$^{D–I}$	0.66$^{E–J}$
Simeto	1988	14.0^{DE}	12.0^{KL}	18.8^{JK}	21.9^{E}	39.4$^{B–D}$	36.7$^{C–G}$	19.6$^{D–H}$	20.2$^{C–F}$	41.8$^{b–e}$	41.4$^{b–e}$	0.94^{BC}	0.89$^{B–D}$
Preco	1995	11.7^{LM}	14.7^{BC}	19.6^{HI}	16.0^{N}	39.3$^{B–E}$	39.3^{BC}	17.9$^{F–J}$	13.9^{L}	42.5$^{b–e}$	44.7$^{a–e}$	0.89$^{B–D}$	0.88$^{B–D}$
Svevo	1996	14.9^{B}	13.9^{EF}	17.2^{N}	18.1$^{K–M}$	24.6^{PQ}	24.0^{Q}	19.9$^{D–J}$	22.4$^{A–C}$	58.2^{a}	58.8^{a}	0.47$^{I–L}$	0.41^{KL}
Iride	1996	11.4^{MN}	10.9^{PQ}	25.2^{C}	26.5^{B}	30.8$^{K–N}$	36.8$^{C–G}$	24.4^{A}	17.4$^{H–J}$	43.9$^{a–e}$	36.7^{de}	0.70$^{D–I}$	1.04^{B}
Claudio	1998	10.7^{Q}	10.9$^{O–Q}$	20.9^{FG}	20.8^{FG}	33.3$^{H–L}$	28.2^{NO}	23.4^{A}	20.6$^{B–D}$	45.8$^{a–e}$	51.1$^{a–d}$	0.73$^{D–H}$	0.55$^{H–L}$
Saragolla	2004	11.3$^{M–P}$	11.3$^{M–P}$	22.0^{E}	19.3^{IJ}	24.6^{PQ}	21.6^{Q}	22.9^{AB}	20.4$^{C–E}$	53.5$^{a–c}$	59.1^{a}	0.46$^{J–L}$	0.37^{L}
PR22D89	2005	10.8^{Q}	12.3^{JK}	27.7^{A}	17.6^{MN}	31.0$^{J–N}$	36.0$^{D–H}$	22.4$^{A–C}$	17.1$^{I–J}$	41.3$^{b–e}$	46.5$^{a–e}$	0.77$^{C–G}$	0.77$^{C–G}$
B) Genotype		**		**		**		**		**		**	**
Year		**		*		**		**		**		ns	*
G x Y		**		**		**		**		**		*	**

YR, year of release; SPC, semolina protein content; A/G, albumin and globulin; mon:pol, monomeric to polymeric storage protein ratio. For each parameter, values followed by different letters are significantly different at P≤0.05 (small letters) and at P≤0.001 (capital letters), according to the Tukey's test for A) and B). *,**F significance level at 0.05 and 0.001 probability level; ns, not significant.

ability was mainly related to differences in protein content (Triboi *et al*., 2003). As for gliadin fraction, the higher proportion found in the old group might be explained by both a higher protein content (Triboi *et al*., 2003) and a higher subunit expression (De Santis *et al*., 2017); two modern genotypes, Svevo and Saragolla showed the lowest amount of gliadins, while Cappelli the highest one. The slight differences observed between old and modern genotypes may be probably due to the fact that this protein fraction was not a direct target for breeders, since gliadins do not have a key role in gluten strength (Ribeiro *et al*., 2016).

Instead a marked higher content of soluble glutenin was found in the modern group of varieties. This increase might also be responsible for the technological quality improvement due to 20th century breeding, in addition to the favorable polymorphism (De Vita *et al.,* 2007). Indeed, Sapirstein *et al*. (2007) found a positive correlation between the extracted glutenin (50% propanol + DTT) and the alveographic parameters in durum wheat varieties. Also Fois *et al*. (2011), evaluating by RP-HPLC the gluten extractable fractions, observed a higher gluten index consistent with a lower gliadin/glutenin ratio in cultivar Svevo with respect to old durum wheat genotypes. In addition, an increase in the glutenin sub-fractions expression (in particular B-type LMW-GS) in modern Italian durum wheat cultivars was recently observed (De Santis *et al*., 2017). As for the ratio between monomeric and total polymeric (sum of soluble and residue glutenins) storage proteins, the variability observed within groups was in accordance with the literature (Zilic *et al*., 2011). The lower values observed in the modern group might be explained by the slight reduction in gliadin content.

Conclusions

In this study, a contribution to the comprehension of the changes in grain protein composition due to durum wheat breeding during 20th century was given. In particular, in modern genotypes a higher relative content of soluble glutenin was observed which might also be responsible for their better technological performance. Furthermore, a slight decrease in the amount of gliadin fraction, mainly responsible for gluten related disorders, was observed in the modern durum wheat varieties.

Among the old genotypes, Cappelli was characterised by the highest protein and gliadin content, while the modern cultivars Svevo and Saragolla showed the lowest gliadin content consistent with the highest glutenin content in both crop seasons.

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