Purple grain colour genes in wheat

Khlestkina E., Shoeva O., Börner A., Gordeeva E.

in

Porceddu E. (ed.), Damania A.B. (ed.), Qualset C.O. (ed.).
Proceedings of the International Symposium on Genetics and breeding of durum wheat

Bari : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 110

2014
pages 533-539

Biochemical and molecular approaches for the technological quality assessment of durum wheat varieties

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Abstract. Durum wheat (*Triticum turgidum ssp. durum*), one of the most important cultivated species in North-Africa, is mainly used for pasta and, to a lesser extent, as flat-bread and couscous for human consumption. A lot of breeding programs concentrate mainly on the dough quality. Quality value is determined by protein content, glutenin, and gliadin allelic composition, the different ratios between those subunits and with less importance the expression of other loci. The aims of our study are to determine some genetic tools of selection, from a choice of high quality wheat genotypes, and propose a breeding improvement program. Among thirty-seven varieties, we assessed the dough quality based on some technological parameters (such as, grain protein content, SDS-sedimentation volume, mixogram) and analysed the variability of gluten subunits (HMW and LMW glutenins and gliadins). Based on the results, we have characterized and selected varieties with better gluten strength and some landraces that displayed the best grain protein contents. The relationships between technological parameters and protein electrophoretic patterns showed that the presence of some *Glu-B1* subunits correlated, with a positive influence, on SDSS parameters value. In contrast other subunits of the locus *Glu-A1* have a negative influence on gluten strength.

Keywords. *Triticum turgidum* var. *durum* – Prolamins – Gluten quality.

Approches biochimiques et moléculaires pour l'évaluation de la qualité technologique des variétés de blé dur

Résumé. *Le blé dur (Triticum turgidum ssp. durum), l'une des espèces cultivées les plus importantes de l'Afrique du Nord, est principalement utilisé pour la fabrication des pâtes et, dans une moindre mesure, du pain plat et du couscous destinés à la consommation humaine. Bon nombre de programmes d'amélioration se concentrent essentiellement sur la qualité de la pâte. La valeur de qualité est déterminée par la teneur en protéines, en gluténine et la composition allélique de la gliadine, les différents rapports entre ces sous-unités et à un degré moindre, l'expression d'autres loci. Cette étude a pour objectif de déterminer des outils génétiques de sélection, à partir d'un choix de génotypes de blé de haute qualité, et de proposer un programme d'amélioration génétique. Parmi trente-sept variétés, nous avons évalué la qualité de la pâte sur la base de certains paramètres technologiques (tels que la teneur en protéines du grain, le volume de sédimentation en milieu SDS, le mixogramme) et analysé la variabilité des sous-unités du gluten (gluténines et gliadines de faible et de haut poids moléculaire). Les résultats obtenus nous ont permis de caractériser et sélectionner des variétés ayant une meilleure force du gluten et des variétés locales qui présentaient les meilleures teneurs en protéines du grain. Les relations entre les paramètres technologiques et les profils électrophorétiques des protéines ont montré que la présence de certaines sous-unités Glu-B1 est corrélée positivement à la valeur des paramètres SDSS. A l'inverse, d'autres sous-unités du locus Glu-A1 ont une influence négative sur la force du gluten.*

Mots-clés. *Triticum turgidum var. durum – Prolamines – Qualité du gluten.*

I – Introduction

Wheat endosperm proteins, namely prolamins are subdivided into gliadins and glutenins, according to their polymerisation properties (Miguel *et al.,* 2011). Durum wheat (*Triticum turgidum* var. *durum*), the preferred raw material for the production of pasta worldwide, is usually cultivated under rainfed conditions in the Mediterranean Basin, which often imposes a number of environmental stresses on the crop. Terminal drought stress during the grain-filling period, usually results in yield reductions, but in most cases results in high grain quality (Nazco *et al*., 2013). Several studies have shown the existence of variability for quality traits in durum wheat landraces (Moragues *et al*., 2006; Aguiriano *et al*., 2008; Nazco *et al*., 2013). Our capacity to gain profit from this diversity depends on the identification of accessions containing genes and alleles demonstrated to be useful in breeding programmes. Differences in dough properties and baking quality are largely determined by the superimposed effects of protein content, the size distribution of the polymeric glutenin, the allelic compositions of the HMW-GS and the LMW-GS, and on the relative amounts of the different glutenin subunits (Ruiz and Carrillo, 1995; Vazquez *et al.*, 1996; Patil *et al.*, 2006). Previous studies applied the γ-gliadins 45 and 42 as markers for good and poor quality of gluten quality, respectively (Damidaux *et al.*, 1978; du Cros *et al.*, 1982). This is due to the genetic linkage with LMW-GS (Payne *et al.*, 1980, 1983, 1984). In fact, pasta cooking quality and gluten strength were initially related to the negative and positive effects of the low Mr glutenin subunit patterns LMW-1 and LMW-2, respectively (du Cros, 1987; Pogna *et al.*, 1990).The HMW glutenin appears to have less critical effects than the LMW glutenin on the gluten strength of durum wheat (Vazquez *et al.*, 1996; Brites and Carillo, 2001). Nevertheless, this has not been clearly established due to limited genetic variability at the *Glu-1* loci present in modern durum wheat cultivars used in published studies (Sisson *et al.*, 2005). It has been suggested that the identification of genes influencing dough quality, other than those controlling the gluten fraction, might be useful way of recognizing others factors (Law *et al.*, 2005).The aims of this study are to analyse the variability of HMW-GS and B-LMW-GS and determinate the effect of *Glu-B1* subunits of HMW on gluten strength.

II – Material and methods

Thirty-seven durum wheat (*Triticum durum Desf*.) varieties (30 landraces and 7 modern cultivars) were included in this study.The varieties were sown in a randomised complete-block, with two repetitions, design under rainfed condition in two different localities. Proteins were extracted from crushed endosperm following a sequential procedure (Singh *et al.*, 1991). Electrophoresis of reduced and alkylated proteins (high and low molecular weight glutenin subunits) was performed on sodium dodecyl sulphate polyacrylamide gels (SDS-PAGE) according to Payne *et al.* (1980). Gliadins were fractionated by A-PAGE (Lafiandra and Kasarda, 1985). B-LMW glutenin alleles were designated according to Nieto-Taladriz *et al.* (1997) nomenclature. Protein content at 14% moisture basis, was estimated by a near-infrared reflectance analysis (NIR) using a Technicon Infralyzer 300. Gluten strength was estimated by SDS-sedimentation (SDSS) test according to Dick and Quick (1983). Rheological properties were determined by Mixograph-10g whole wheat meal (Finney and Shogren, 1972). The mixograph parameters measured were: mixing development time (MT), maximum peak height (MH), height at 3 min after the peak of the curve (H3), and resistance to breakdown (BDR) (difference in percentage between MH andH3). Values represent means of four repetitions (two repetitions in each locality).

III – Results and discussion

Durum wheat samples analysed in this study displayed a wide range in dough strength and gluten properties. In order to analyse the variability of *Glu-A1* and *Glu-B1* (HMW-GS), *Glu-A3, Glu-B3*

and *Glu-B2* (B-LMW-GS), and *Gli-B1* (γ-gliadin) loci, we were able to calculate a genetic diversity with the D index, according to the following formula:

$$Dj = 1 - \Sigma p^2_{ij}$$

Where p is the frequency of the i^{th} allele at j^{th} locus.

Considerable diversity was found in landraces from Spain and with a lesser degree within Tunisian landraces. The modern varieties had a poor genetic diversity index (Table 1). Diversity in gluten loci varied from D= 0,29 to D= 0,66. A high diversity was also recorded in *Glu-B1 and Glu-A3* loci, and a minor diversity in *Gli-B1.* Among the 37 genotypes, four were γ-gliadin 42 type, 31were γ-gliadin 45 type and two are rare alleles, Null and γ-44. The well-established tendency for γ-gliadin 42 types to be consistently weak is clearly evident. The γ-gliadin 45 types exhibited a wide range of strength from weak to very strong. Previous studies applied the γ-gliadins 45 and 42 as marker for good and poor quality of gluten quality, respectively (Damidaux *et al.*, 1978; du Cros *et al.*, 1982). ANOVA analysis for quality parameters has indicated that significant effect was recorded for B-LMW glutenin loci variation, while γ-45 pattern is present, accounting for 17.8%, 39.8% and 19.0% of the variation for SDSS, MT and BDR, respectively. A second ANOVA analysis of quality parameters showed a significant effect of all allelic prolamins variation. This last model explains 60.3%, 61.3% and 50.4% of the variance of SDSS, MT and BDR parameters respectively. When comparing these values with the previous model, which only took account of the variation of the B-LMW glutenin loci, we can deduce the importance of HMW glutenin loci *via* the increasing and improvement of the qualitative parameters. The gliadin influence is lower in this study, because most varieties possess γ-45. While the allelic variance (*F* values) of *Glu-A1, Glu-B1*, *Glu-A3*, *Glu-B3* and *Glu-B2* loci and the variation of quality parameters measured, demonstrate a high significant effect of *Glu-B1* (HMW-GS) on gluten strength (SDSS) and Mixograph test. Moreover, interaction between this locus (*Glu-B1)* and *Glu-A3* is recorded in SDSS volume and Mixing time. The second interaction between *Glu-B1* and *Glu-B3 allelic variation* marked a significant influence only on SDS-sedimentation volume (Table 2).

Table 1. Genetic diversity indices.

	Total Varieties	Landraces (Spain)	Landraces (Tunisia)	Modern Varieties
D (*Glu-A1*)	0,48	0,66	0,22	-
D (*Glu-B1*)	0,66	0,55	0,53	0,57
D (*Glu-A3*)	0,60	0,70	-	0,45
D (*Glu-B3*)	0,45	0,45	0,47	0,25
D (*Glu-B2*)	0,25	0,50	0,47	0,25
D (*Gli-B1*)	0,29	0,30	0,22	0,25
D-index	0,45	0,42	0,31	0,29

Table 2. The Analysis of variance (F values) of HMW and B-LMW loci and Quality parameters.

Source	Protein	SDSS (mm)	Mixing time (s)	Breakdown (%)
Glu-A1	1.40	0.75	0.36	6.10**
Glu-B1	0.93	6.41**	3.77*	3.55*
Glu-A3	1.05	9.02**	18.63**	1.81
Glu-B3	0.70	0.57	1.09	0.38
Glu-B2	0.34	2.43	6.04*	0.37
Glu-B1 x *Glu-A3*	0.66	6.35*	6.18*	0.03
Glu-B1 x *Glu-B3*	0.03	4.29*	1.17	2.32

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