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SUMMARY - Stable genetically determined high protein content of durum wheat is of main importance for quality products but difficult to combine with modern varietal traits. Durum wheat world genetic resources, landraces, modern lines and cultivars were analyzed to determine the occurrence of gliadin and glutenin components.The SDS sedimentation volume test and protein content used in the prediction of cooked pasta quality were also controlled. A new negative relation between *Glu A1* HMW null allele and SDS as well as protein content was recently found in *T. turgidum* L. var. *durum*. *Glu A1* HMW alleles 1, 2*, and 2**, which are uncommon in durum wheat, could be related with a better cooked pasta quality. A good amount of variation for carotenoid content of the endosperm related with yellow pasta colour is available. Modern lines with very high levels are used presently for varietal development.

Key words: Protein, gliadin, glutenin, Sodium Dodecyl Sulphate sedimentation test, carotenoid, durum wheat, quality, breeding, genetic resources.

RESUME - *"Pré-amélioration et amélioration du germoplasme de blé dur (Triticum turgidum L. var. durum) pour des produits de qualité". Une teneur en protéine du grain élevée et stable est un caractère génétique d'importance capitale pour l'obtention de produits de qualité, mais elle est difficile à combiner avec d'autres caractères favorables des variétés modernes. Des ressources génétiques mondiales de blé dur, des variétés locales et modernes ont été analysées pour la présence d'unités et sous-unités gliadine et gluténine ainsi que pour le test de sédimentation SDS et la teneur en protéine utilisés pour la prédiction de la qualité des pâtes cuites. Une relation négative nouvelle entre l'allèle nul du locus Glu A1 codant pour les sous-unités de Haut Poids Moléculaire et le test SDS ainsi qu'avec la teneur en protéine a été trouvée. Les allèles du locus Glu A1 codant pour les sous-unités 1, 2*, et 2** pourraient être reliés à une meilleure aptitude à la qualité des pâtes cuites. Une grande diversité pour la teneur en caroténoïdes de l'albumen en relation avec la couleur jaune des produits du blé dur est utilisable. Des lignées modernes avec des niveaux très élevés de caroténoïdes sont en cours d'utilisation pour la création variétale.*

Mots-clés : *Protéines, gliadines, gluténines, Test de Sédimentation SDS, caroténoïdes, blé dur, qualité, sélection, ressources génétiques.*

Superior pasta, couscous and burghul products are presently processed entirely from durum wheat undergoing 3 transformations: milling into semolina, then processing, and finally cooking. The evaluation of durum wheat samples or genotypes for quality related attributes is therefore complex if we want to take into account the various types of industrial processing. This paper will try to cover some of the most important grain quality attributes in durum germplasm. It is generally admitted that gluten quantity and quality are essential for a good quality of durum wheat cooked products. In addition to protein quantity and quality we will discuss the results concerning germplasm breeding for good colour characteristics, i.e. primarily yellow endosperm.

Protein content

Protein content is directly related to pasta quality. Moreover a stable phenotypic and possibly genotypic negative correlation between protein content and yellow berry (soft starchy endosperm) related to low semolina yield was observed (Sombrero and Monneveux, 1989). However, this trait is difficult to breed due to different factors:

(i) Many programs are faced with genotypic and phenotypic negative correlation between grain protein content and yield.

(ii) Modern semi-dwarf bread and durum wheat genotypes generally bear the Rht 1 allele. These cultivars are lodging-resistant under intensive cropping conditions but it was found that their grain protein content was lower (Gale, 1979; Mc Clung *et al.*, 1986).

(iii) Our breeding experience indicates that high durum wheat protein content is not a stable characteristic. For example, we found a non significant (P=0.51) negative correlation between 1990 and 1991 crops for protein content in 109 lines of our breeding nursery at the same location in Mauguio (Table 1). High protein content stability is therefore very interesting to breed in association with other favourable traits. The old medium tall variety, Agathé, is a good standard for high and stable protein content.

Many studies, and particularly the extensive works concerning high protein lines issued from the *T. dicoccoides* species (Avivi, 1978; Joppa and Cantrell, 1990; Cantrell and Joppa, 1991; Joppa *et al.*, 1991) indicate conversely that protein content is a complex genetic trait governed by polygenic quantitative factors. In a study by Chihab (1990), we found good levels of protein content in genetic resources from Northern Africa and Italy. On the contrary resources from Spain and Portugal were often weak in protein content, possibly in relation with *T. turgidum* var. *turgidum* introgressions. Resources from former USSR and Turkey, appeared relatively high in protein whereas the mean protein content of Syrian and Jordan durum landraces was often lower in a world collection.

Protein quality and SDS sedimentation test

SDS sedimentation test is a simple and rapid technique (Dexter *et al.*, 1980; Quick and Donnelly, 1980). It is somewhat dependent on protein content and possibly on the vitreousness of the kernels and on the milling apparatus. Milling kernels into smaller particles could induce higher SDS sedimentation ratings. However, we observed a good reproducibility of SDS ratings compared with protein content (Table 2). Recent unpublished results (J. C. Autran, pers. comm.) confirm the interest of this test in relation to cooked pasta quality rheological test. We observed that landraces and other genetic resources from Northern Africa, Italy, Turkey are often high in SDS volume (Chihab, 1990).

Protein quality and glutenin subunits

The importance of storage protein units and glutenin subunits is a well established fact in bread wheat breeding. However, a relationship between cereal end product quality (cooked pasta) and storage protein unit type analyzed through gliadin electrophoresis pattern was first found in durum wheat (Damidaux *et al.*, 1978). Investigations developed rapidly into wheat genetic diversity concerning the main storage protein components High Molecular Weight (HMW) Glutenin subunits (Payne and Lawrence, 1983; Branlard and Le Blanc, 1985) and also gliadins and Low Molecular Weight (LMW) Glutenin subunits. A set of reliable relations between HMW glutenin subunits coded by multigenes on 3 homologous loci and breadmaking quality was found in bread wheat. These effects of various intensity were considered to be broadly additive, thus enabling the build up of easy to compute breeder quality indexes.

Conversely, the relation between storage protein components and durum pasta cooking quality was not evidenced at the same rate. However, Pogna *et al.* (1990) showed the effect of LMW glutenin subunits 1 and 2; they are coded by 2 alleles of the *Glu B3* locus strongly linked to the *Gli B1* chromosome 1B locus specifying for gliadins 42 and 45, already studied by Damidaux *et al.* (1978). A genetic recombination study between the 2 loci indicated that the LMW 2 allele could be the causal effect of superior gluten and cooking quality, the gliadin 45 allele is merely a quality marker in durum breeding programs.

Studies of the relations between HMW glutenin subunits and quality for durum products are not numerous. The associations reported are weak or contradictory. Vallega (1986) evidenced no clear relation between HMW glutenin subunits and cooked spaghetti quality for a sample of 19 varieties. Autran and Feillet (1987) evaluated 113 French advanced lines with 5 different HMW glutenin electrophoregrams. A weak but significant relation was found between HMW glutenin subunits and the rheological or surface properties of cooked pasta. HMW 6_8 lines are somewhat superior to HMW 20 lines. HMW 20 lines appear better than HMW 13_16 lines. The same year Du Cros (1987) studied 102 Australian breeding lines. She found 12 different HMW glutenin electrophoregrams and compared their gluten strength through mixograph data. A HMW glutenin subunit comparable to 2* (Payne and Lawrence, 1983) could be related with high quality scores. Autran and Galterio (1989) analyzed 95 French varieties and breeding lines. They found an association between HMW glutenin subunit 2** and a poor cooking quality, between subunit 7_8 and better gluten rheological qualities, between subunit 13_16 and inferior gluten firmness or cooked pasta surface properties.

Boggini and Pogna (1989) evaluated 37 Italian durum varieties for bread-making properties. LMW-2 glutenin, HMW 7_8 or 20 are associated with the highest bread volumes. Pogna *et al.* (1990) grew the offspring of 3 biparental crosses segregating for *Glu B1* HMW alleles coding for HMW 20, HMW 7_8, HMW 6_8. HMW 7_8 subunit was associated with superior SDS sedimentation volume and to better rheological gluten properties. Carrillo *et al.* (1990) evaluated 39 Spanish grown cultivars. The varieties with HMW 20 appear statistically inferior for SDS volume sedimentation. In the offspring of a cross between an accession of *T. dicoccoides* with the cultivar Creso, Ciaffi *et al.* (1991) discovered a *Glu A1* HMW allele coding for low mobility HMW subunit associated with superior SDS sedimentation volume which contrasted to the null *Glu A1* HMW allele from Creso.

Taking these results into account, the relation between specific HMW glutenin subunits and the quality of durum wheat appear relatively weak or inconsistent. Our approach aimed at obtaining a broad genetic based view of the genetic diversity of storage protein factors of durum wheat species thus trying to minimize sampling, linkage, recent breeding trends and genetic drift effects.

Our first study was based on a world collection of 502 durum genetic resources mainly collected by and maintained at the INRA Genetic and Plant Breeding Station in Montpellier, evaluated for the occurrence and frequency of HMW glutenin subunits (Branlard *et al.*, 1989). *T. durum* species appear more polymorphic than *T. aestivum* at *Glu A1* HMW and *Glu B1* HMW concerned loci (18 alleles against 14). Frequencies are equally very different. At the *Glu A1* HMW locus, the null allele associated with inferior bread-making quality of *T. aestivum* is present for 84% of this collection (97% of French durum wheats) against 44% of a world collection of 1019 bread wheats (Branlard *et al.*, 1990). At the *Glu B1* HMW locus, 3 alleles coding for 6_8, 7_8, and 20 subunits are predominant with a cumulated frequency of 86% against 44% in the preceding world bread wheat collection.

In a recent study (Kaan *et al.*, 1993) we investigated a world collection of 280 durum genetic resources including modern varieties and landraces for the relationship between storage protein components and 2 simple criteria (kernel protein content and SDS sedimentation) associated with cooked pasta quality.

The frequency of gliadin and HMW glutenin subunit alleles was comparable with the preceding results of Branlard *et al.* (1989). Gliadin 45 and correlated LMW 2 glutenin are strongly predominant in this collection (Table 1).

The *Glu A1* HMW null allele is less frequent in this collection compared to the results of Branlard *et al.* (1989) where recent French breeding lines and varieties are more represented and nearly monomorphic for null allele.

Table 1. Relations between quality traits in a nursery of 109 breeding lines of durum wheat in 1990 and 1991 at the Mauguio station

Variable	Protein %	Protein %	SDS volume (ml)	SDS volume (ml)	Carotenoid (ppm)	Carotenoid (ppm)
Season	1990	1991	1990	1991	1990	1991
Mean	13.09	10.25	39.18	36.34	7.11	6.61
Minimum value	10.4	7.5	14	22	5.1	4.3
Maximum value	18.1	13.6	60	52	12.8	10.9
Standard error	0.2	0.2	0.7	0.7	1.8	2.0
Correlation with protein crop 1990	+1.0	-0.063 ns	+0.066 ns	-0.210 **	+0.044 ns	+0.128 ns
Correlation with protein crop 1991	-0.063 ns	+1.0	+0.189 *	+0.273 **	+0.023 ns	+0.197 *
Correlation with SDS crop 1990	+0.066 ns	+0.189 *	+1.0	+0.708 ***	-0.095 ns	-0.060 ns
Correlation with SDS crop 1991	-0.210 *	+0.273 **	+0.708 ***	+1.0	-0.038 ns	-0.071 ns
Correlation with carotenoid crop 1990	+0.044 ns	+0.023 ns	-0.095 ns	-0.038 ns	+1.0	+0.728 ***
Correlation with carotenoid crop 1991	+0.128 ns	+0.197 *	-0.060 ns	-0.071 ns	+0.728 ***	+1.0

Significance of correlations
ns: Probability > 0.05
*: Probability < 0.05
**: Probability < 0.01
***: Probability < 0.001

Table 2. Relation between quality related characteristics and specific storage protein units and subunits in a durum wheat collection of 280 accessions

Locus	Unit or subunit	Frequency[†] (%)	Frequency[††] (%)	SDS[††] mean (ml)	SDS mean Signification	Protein[††] mean (%)	Protein mean Signification
Gli B1	*γ-42*	*31.8*	*36.4*	*30.09*	***	*16.51*	*ns*
Gli B1	*γ-45*	*68.2*	*63.2*	*34.06*	***	*16.72*	*ns*
Glu A1	*null*	*83.5*	*71.1*	*31.90*	*	*16.54*	*
Glu A1	*1*	*7.0*	*13.2*	*35.32*	*	*16.87*	*ns*
Glu A1	*2**	*4.6*	*11.4*	*33.25*	*ns*	*16.87*	*ns*
Glu A1	*2***	*2.9*	*4.2*	*34.33*	*ns*	*17.10*	*ns*
Glu B1	*7*	*0.8*	*1.1*				
Glu B1	*7_8*	*25.9*	*30.4*	*33.06*	*ns*	*16.74*	*ns*
Glu B1	*6_8*	*26.3*	*19.3*	*32.19*	*ns*	*16.44*	*ns*
Glu B1	*20*	*33.5*	*33.9*	*31.61*	*ns*	*16.69*	*ns*
Glu B1	*13_16*	*5.5*	*3.6*	*34.80*	*ns*	*15.94*	*ns*
Glu B1	*14_15*	*0.6*	*0.7*				
Glu B1	*22*	*0.0*	*0.7*				
Glu B1	*23_18*	*3.4*	*5.0*	*33.93*	*ns*	*17.52*	**
Glu B1	*19*	*0.4*	*0.7*				
Glu B1	*7_15*	*2.2*	*2.9*				
Glu B1	*6_16*	*1.2*	*0.7*				
Glu B1	*23_22*	*0.2*	*1.1*				
Mean				*32.61*		*16.65*	

[†]Refers to the study by Branlard *et al.* (1989)
[††]Refers to the publication of Kaan *et al.* (1993)
Statistical significance is not given for the rarest alleles
ns: not significant; *: significant at 5% level; **: significant at 1% level; ***: significant at 0.1% level

The *Glu B1* HMW locus allele coding for the 22 subunit yet unknown in durum wheat is observed on Diyarbakir (Turkey) and Kurzahriger Sommer (Germany). The statistical independence between alleles at different loci was investigated. Linkage disequilibrium was found for some alleles; however, the statistical significance of the results was not modified.

The levels and differences in SDS tests are low for durum in comparison to bread wheat where the range is commonly from 40 to 100, but corresponds to other durum observations (Carrillo *et al.*, 1990; Ciaffi *et al.*, 1991).

The well established positive relation between *Gli B1* specified gliadin and superior SDS sedimentation volume (Damidaux *et al.*, 1978) is confirmed. A new negative relation between *Glu A1* HMW null allele and SDS as well as protein content was evidenced in *T. durum*. *Glu A1* HMW alleles 1, 2*, and 2** which are uncommon in durum wheat could be related with a better cooked pasta quality. 27 genetic resources predominantly from Southeastern Europe and Turkey with the promising *Glu A1* HMW 1 allele were found in addition to our preceding results (Branlard *et al.*, 1989). In contrast, the negative relation between *Glu A1* HMW null predominant allele and quality evaluated by SDS sedimentation volume as well as protein content was not yet observed in durum wheat but is accepted in bread wheat (Branlard and Dardevet, 1985) and is in agreement with some results from interspecific crosses with *T. dicoccoides* (Ciaffi *et al.*, 1991) and with preliminary observations of Du Cros (1987).

The relation between the recently found *Glu B1* HMW 23_18 allele and high protein content is new but observed on only 14 resources of particular *Glu A1* HMW allele composition and of various origins (5 countries). However, it could be interesting to confirm this association on crosses and offspring studies, eventually on interspecific or isogenic line studies.

We obtained recent unpublished results on 310 durum accessions collected in Morocco. They indicated very different glutenin subunit and gliadin allele frequencies. The negative relation between the predominant null *Glu A1* HMW allele and SDS sedimentation was not statistically confirmed, possibly due to the scarcity of other alleles. Conversely the rare *Glu B1* HMW 14_15 allele present in 7 accessions was found to be associated in a statistically significant way with the best SDS sedimentation score (40.86 compared to a general mean of 33.55).

In conclusion to our present work on protein quality germplasm, the genetic basis of the observations concerning the possible interest of specific storage protein units and subunits is broad but needs to be confirmed by specific crosses studies, particularly in the case of rare alleles. F 4 are presently under study for crosses involving parents bearing *Glu A1* HMW 1 allele. Other HMW Glutenin alleles considered to be favourable for the breadmaking quality of bread wheat such as *Glu B1* HMW 7_9, 17_18 are yet unknown on durum wheat. Their possible effect on durum quality could be evaluated after interspecific crosses.

Yellow colour of products

This component of quality durum products is economically important. The carotenoid pigments of endosperm are essential for a good yellow colour after processing. We are using the classical AACC method (1983). There is some environmental variation, however genotypic correlations are high as illustrated by results presented in Table 2. The recent rate of genetic progress in Europe is very impressive. Many landraces and other genetic resources varieties from Northern Africa, Italy and Iberian Peninsula are typically low in carotenoid (from 3 to 5 ppm level). The varieties and landraces from former USSR, USA and Canada are much better (about 8 ppm). Recent and promising breeding lines are nearing 15 ppm level. This character appear therefore relatively easy to breed and it is doubtful at present that new prospections and evaluations of specifically yellow endosperm coloured genetic resources are needed. Unfavourable correlations between high carotenoid content and yellow berry (soft starchy endosperm) was found as well as with small kernels, but could be related with the Eastern European (former USSR) origin of the high yellow-coloured endosperm material.

Conclusion

A good amount of prospection and evaluation was performed by various laboratories on durum wheat genotypes for storage protein quantity and diversity. We will have a better idea of their possible use in the short term. New storage or small proteins related to quality could conversely be evidenced. A rapid rate of genetic progress for yellow coloured endosperm was obtained through the use of classical genetic resources. Much remains to be inquired about the use of germplasm for the other components of colour, brightness of pasta and absence of blackpoint.

Abbreviations

HMW: High molecular weight; LMW: Low molecular weight; SDS: Sodium Dodecyl Sulphate.

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