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GENETIC AND TECHNOLOGICAL BASIS OF PROTEIN QUALITY FOR DURUM WHEAT IN PASTA

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ABSTRACT

Principal component analysis was carried out from 9 quality tests that were applied to 113 pilot -type durum wheat samples. Rheological-type characteristics were strongly linked together, typically variety-dependent and independent of protein content. State of surface characteristics of cooked spaghetti appeared essentially independent of rheological characteristics, significantly influenced by protein content and growing location but significantly influenced by genotype too. Attention was focussed on the remarkable relationship between gluten rheological characteristics and the particular allele (gamma-gliadin + LMW-glutenin) present at the Gli-B1 chromosomic locus. Allele "45" had marked positive effect while allele "42" was deleterious as far as gluten viscoelasticity was concerned. On the other hand, a weak but significant relationship was found for the first time between the particular allele (HMW-glutenin) present at Glu-B1 locus and state of surface of cooked pasta. Prediction of this pasta characteristic at the breeding stage was discussed with a recommendation to work out simultaneously a small-scale pasta-making test.

INTRODUCTION

Durum wheat (Triticum durum Desf.) is a raw material of choice for the manufacture of pasta products because of the superior rheological properties of durum wheat pasta doughs and the ideally suited color and cooking quality of durum wheat pasta.

Unlike common wheat, an important part of which can be used for animal feed, the only opening of durum wheat is human food, essentially pasta and couscous. Since pasta, at least in countries such as France and Italy, must be manufactured from pure durum wheat semolina, it is especially important that quality of durum wheat meets demands of semolina and pasta-making industries and of consumers. From the breeders point of view, it is necessary to develop new durum wheat lines with high-quality potential.

Among the different parameters of durum quality (semolina yield, pasta color, pasta cooking quality, ...), we shall primarily deal with cooking quality. As a matter of fact, understanding and prediction of the decisive criteria of cooking quality are still a critical problem and, for instance in France, most of the new durum wheat lines that are submitted for registration are currently rejected on the basis of their unsufficient cooking tolerance.

Cooking quality has been shown to depend on two main parameters: rheological characteristics (related to gluten elasticity or strength) and state of surface (absence of surface deterioration: stickiness, mushiness, clumping). According to Feillet (1984), these two parameters do not appear to be directly related, but their independence has never been conclusively demonstrated. Therefore, breeders still need fast and small-scale methods to predict the two main parameters of cooking quality and genetic improvements depend on their capacity to screen efficiently thousands of lines per year.

As far as gluten quality is concerned, biochemical methods (electrophoresis, HPLC) have been recommended for quality assessment or prediction at the breeding stage. Such methods allow to explain quality at the molecular level and to reach a better quality control through an improvement of the knowledge on biochemical basis of quality. Among recent investigations, Damidaux et al. (1978, 1980) found an unbroken association between the presence of a gamma-gliadin (n° 45 in conventional

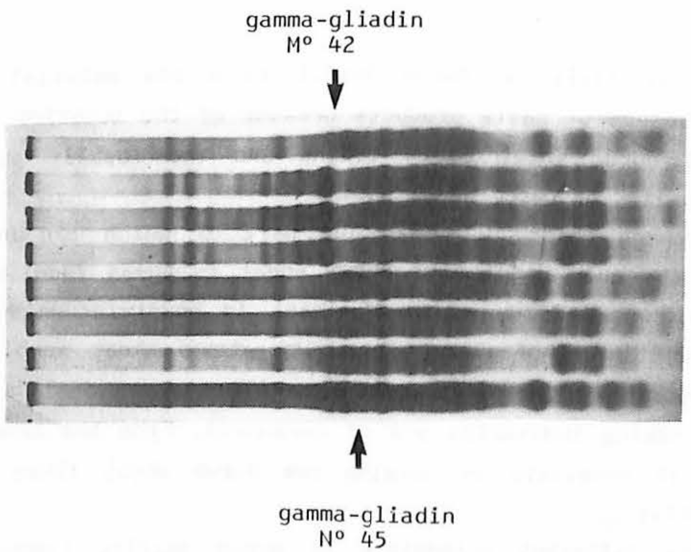

Fig. 1 Polyacrylamide gel electrophoresis (aluminium lactate buffer, pH 3.1) of gliadin fraction from 8 durum wheat varieties.

Bushuk's P.A.G.E. method) and gluten strength (viscoelasticity) and between the presence of another gamma-gliadin (n° 42) and gluten weakness. This relationship was confirmed by Kosmolak et al. (1980), Du Cros et al. (1982) and more recently by Burnouf and Bietz (1984) using reversed-phase HPLC.

These discoveries gave rise to an extensive use of electrophoresis as a very small-scale breeding tool in view to predict durum wheat cooking quality, more exactly, the rheological component of cooking quality.

However, no equivalent microtest seems to be available for the assessment of surface characteristics of cooked pasta.

In this paper, we shall therefore attempt to discuss some of our recent results on :

1) the respective interest and usefulness of the different technological and biochemical tests that are commonly used for the evaluation of the different aspects of durum wheat cooking quality.

2) biochemical basis of cooking quality of durum wheat in pasta and their implications for durum wheat breeding.

RELATIONSHIPS BETWEEN TECHNOLOGICAL AND BIOCHEMICAL TESTS FOR QUALITY EVALUATION IN DURUM WHEAT.

Correlations studies and principal component analysis were carried out from the results of nine quality tests (including pilot tests) performed on the whole set of french standard varieties (AGATHE, CAPDUR, MONDUR, KIDUR, TOMCLAIR) and of lines submitted to official registration in France in 1983 and 1984 (113 samples consisting in 33 genotypes grown in 2 or 4 locations each).

P.C.A. is a statistical method that allows a creation of synthetic variables called components, which are linear combinations of the original ones and not correlated together. Data are presented on graphical forms showing point locations for tests and for samples. Neighbourhood between two tests points means that these tests are correlated (and as much as the points are distant from the origin).

Figure 2 shows the first principal plan of the P.C.A. for the set of 113 pilot samples of 1983 and 1984 harvests. The most consistent trends are the following :

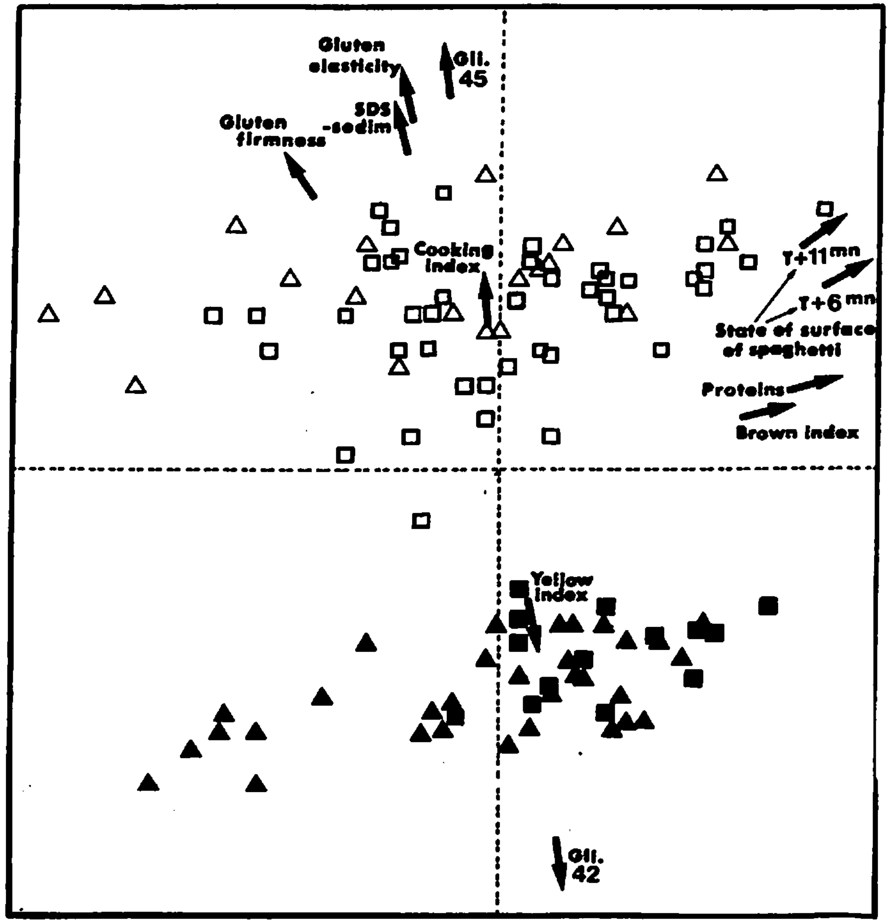

Fig. 2 Principal component analysis of 9 technological tests and gliadin electrophoregrams from 113 durum wheat cultivars and breeding lines (△= spring wheats, □ = winter wheats, white = gliadin type 45, black = gliadin type 42)

1) The three "rheological-type traits (gluten firmness, gluten elasticity and SDS-sedimentation volume) are strongly linked and clearly define a "rheological-quality" component or axis.

2) Protein content corresponds to a second axis, roughly orthogonal to the "rheological" one, corroborating the independence of the two characteristics.

3) Spaghetti state of surface after T+6 and T+11 mn of cooking are strongly correlated between each other and relatively associated to protein content, but no trend between spaghetti state of surface and rheological characteristics is apparent.

RELATIONSHIPS BETWEEN QUALITY TESTS AND PROTEIC MARKERS :

Gamma-gliadins

Figure 2 also shows a most striking result, which is the distribution of the samples in the PCA graphic according to their gliadin electrophoretic composition.

In the first principal plan, all varieties belonging to the "gliadin type 42" (black points) have much lower scores for the different rheological tests than those of "gliadin type 45" group (white points) and the two groups are very clearly separated. From this large and new set of samples, it is therefore a very strong confirmation of the results that Damidaux et al. published as soon as 1978.

Interestingly, when the different growing locations of each genotype are identified on the PCA graphic (results not shown), they are generally not superposed, but they essentially differ from protein content and state of surface score, so that their junction line is also roughly parallel to the "rheological axis". This result certainly means that state of surface of cooked spaghetti is highly influenced by growing location and protein content. It does not go, however, against a possible varietal basis for this parameter as it has been demonstrated by analysis of variance. Accordingly, PCA clearly corroborates the earliest Damidaux's conclusions according which gluten firmness and elasticity are essentially varietal-dependent with very little influence of protein content and growing location.

In addition, PCA evidences the relative independence between the two parameters of durum wheat cooking quality : rheological characteristics and state of surface of cooked pasta. Gliadin electrophoresis can be considered, more than ever, as a powerful tool for rheological quality screening. But breeders must know that they cannot breed efficiently for good surface characteristics of cooked spaghetti through gluten rheological tests or gliadin electrophoresis.

Low-molecular-weight glutenins :

The relationship between gamma-gliadin type and gluten viscoelasticity has been recently extended to the whole allelic block, called Gli-B1 by Payne et al. (1984). This block, located on the short arm of the 1B chromosome, includes gamma-gliadins, omega-gliadins and low-molecular-weight glutenins (figure 3).

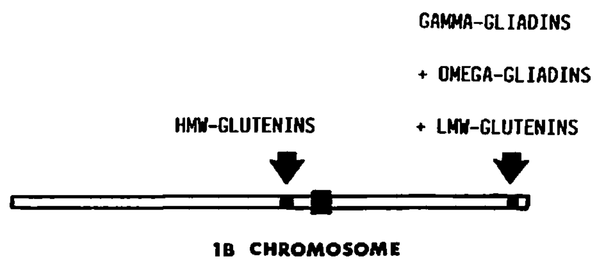

Fig. 3 Chromosomic location of genes coding for proteins that impart durum wheat cooking quality.

Autran and Berrier (1984) have shown that the presence of gamma gliadin 45 in durum wheats is always linked to the presence of a strong component (identified to a low molecular weight subunit : LMW n° 2 by Payne and coworkers) in SDS-PAGE patterns while the presence of gliadin 42 is linked to the presence of faint LMW n° 1 bands (figure 4).

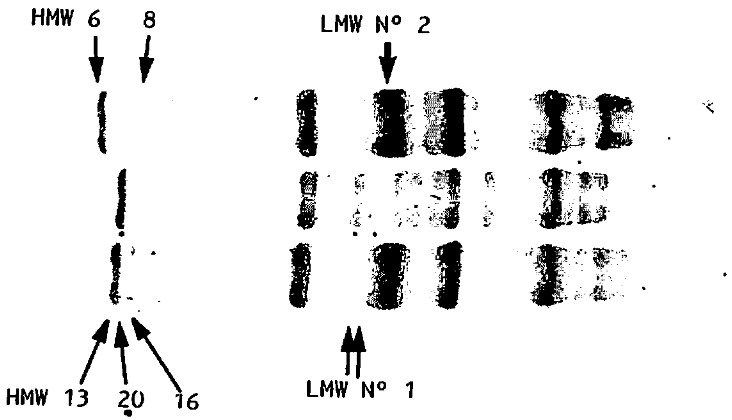

Fig. 4 SDS-polyacrylamide gel electrophoresis (tris-glycine buffer, pH 8,4) of whole proteic extracts (glutenin + gliadin) from 3 durum wheat varieties.

Accordingly, screening for gluten rheological characteristics can be carried out as well through gliadin PAGE as through LMW glutenin analysis in SDS-PAGE.

As far as gluten rheological characteristics are concerned, the different quality levels of the two durum wheat groups seem to be essentially imparted by the allele present at the Gli-B1 locus : either the "45" allele (which includes gamma gliadin 45 + omega gliadins + LMW

glutenin n° 2) and which has a positive effect, or the "42" allele (gamma gliadin 42 + omega gliadins + LMW glutenins n°1). Since it has been shown that the world durum wheat collection consisted of these only two alleles at this locus (excepted a few very rare "41" or "44" types), a such situation explains the remarkable bimodal distribution of gluten characteristics in the durum wheat species (Figure 5).

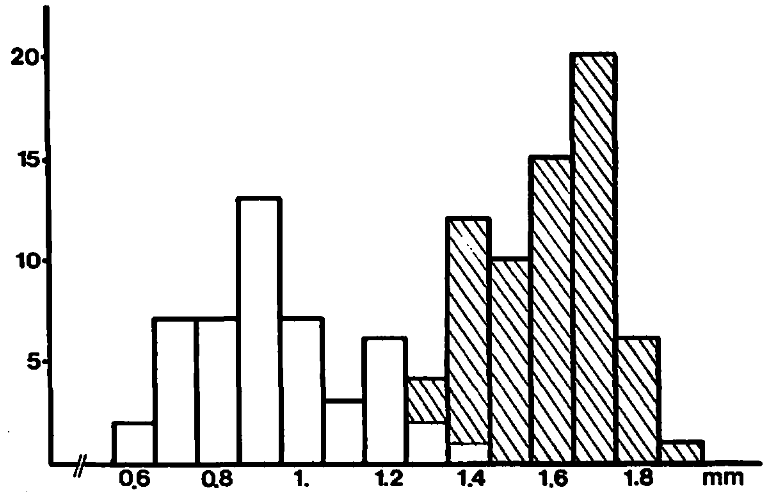

Fig. 5 Distribution of 113 durum wheat cultivars and lines according to gluten elasticity (white : gliadin type 42: hachured : gliadin type 45)

<u>High molecular weight glutenin subunits</u> :

In bread wheats, some particular HMW subunits have been reported by Payne and Lawrence (1983) and by Branlard and Leblanc (1985) to impart baking quality. Until now, no similar relationship has been found in durum wheats.

Accordingly, in this work we have analyzed SDS-PAGE patterns of total reduced proteins extracted from durum wheat semolina in order to make a first inventory of the different HMW glutenin types blocks present in our set of cultivars and breeding lines (see figure 4).

Among the 113 genotypes, 5 HMW subunits types has been identified (Figure 6), including 3 that are widely distributed and 2 rare types (present in only 1 or 2 genotypes).

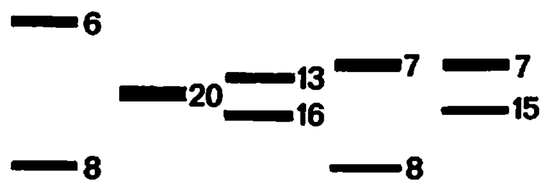

Fig. 6 HMW-glutenin types identified in the 113 french cultivars and lines.

Upon introducing these HMW data in the previous PCA analysis (Figure 7), it can be observed :

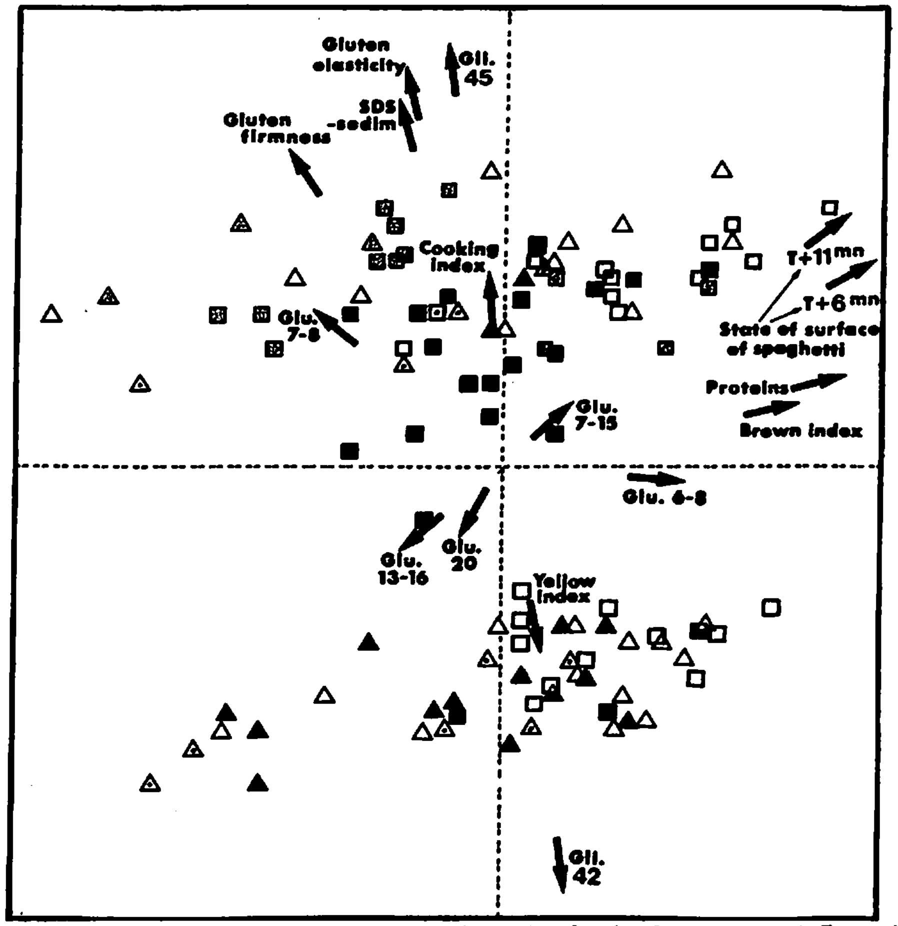

Fig. 7 Principal component analysis of 9 technological tests and 7 traits of gliadins and glutenins electrophoregrams from 113 durum wheat cultivars and breeding lines (O = spring wheat; □ = winter wheats, white : HMW type 6-8; black : HMW type 20; point : HMW type 13-16, grey : other types)

1) a relative independence between 42/45 gliadin types and HMW blocks what could be expected since genes coding for these two proteic fractions are respectively located on the short arm and on the long arm of the group 1 chromosomes.

2) interestingly, a weak but significant relationship between HMW types and quality, for both gluten rheological properties and state of surface of cooked pasta : HMW block 6-8 appears positively associated to quality, block 20 appears neutral or slightly negative, while block 13-16 is negatively associated to quality (no conclusion, can be drawn for the 2 other because of too small sizes of class). This ranking of the 3 main blocks is essentially conserved whether the considered set of samples is the "42" group, the "45" group, or the whole set (figure 8).

GLUTEN ELASTICITY

TYPE 45 VARIETIES : BLOCK 13-16 < BLOCK 20 (AMIDUR) < BLOCK 6-8 (PRIMADUR, MONDUR)
(CAPDUR)

TYPE 42 VARIETIES : BLOCK 13-16 ≤ BLOCK 20 (CARGIVOX) < BLOCK 6-8 (CANDO)

ALL VARIETIES: BLOCK 13-16 < BLOCK 20 < BLOCK 6-8

STATE OF SURFACE OF COOKED PASTA

TYPE 45 VARIETIES : BLOCK 13-16 < BLOCK 20 (DURELLE) < BLOCK 6-8 (AGATHE, MONDUR)

TYPE 42 VARIETIES : BLOCK 13-16 ≤ BLOCK 20 (TOMCLAIR) < BLOCK 6-8 (KIDUR, CANDO)

ALL VARIETIES: BLOCK 13-16 < BLOCK 20 ≤ BLOCK 6-8

Fig. 8 Relationships of the major HMW-glutenin blocks to durum wheat quality.

Therefore, for the breeders point of view, in spite of the little influence of HMW, it could be advisable to select those lines which have the "45" type allele and "6-8" type HMW block. This choice should improve the probability to develop varieties with both good gluten rheological properties and good state of surface of cooked pasta. For example, it is important to notice that most of the best french varieties (for both aspects of cooking quality) : AGATHE, MONDUR, PRIMADUR, MONTFERRIER... belong to type "gliadin 45" + HMW 6-8 and that even those with "gliadin 42" + HMW 6-8 : KIDUR, LAKOTA, CANDO,... use to give results higher than the average of "42 type" varieties. Conversely, varieties with HMW 20 and having "45-type" (AMIDUR, CASOAR, DURELLE,...) or "42-type" (TOMCLAIR, CARGIVOX, Z11, 28,...) give frequently lower results compared to the respective averages of the "45 group" or of the "42 group".

Possible nature of the relationship between proteic markers and quality:

The exact nature of the relationship between proteic markers and quality is still under question. It can be recalled that :

1) From gliadins 45 and 42 isolation (Cottenet et al.(1983, 1984) only small differences in amino acid composition and surface hydrophobicity characteristics could be noticed, from which it seemed difficult to explain entirely the opposite behaviours of "45-type" and "42-type" glutens.

2) In view to approach gluten functional properties, Autran and Berrier (1984) recently shown that gliadin and glutenin fractions had very different behaviours upon gluten heating, pasta cooking, or high temperature pasta drying.

They hypothesized that gamma gliadins 45/42 were essentially genetic markers, while LMW and HMW glutenins could have strong functional properties (capacity to aggregate into a continuous network upon pasta processing). In addition, LMW glutenins (locus Gli-B1) could explain differences in quality, while HMW glutenins (locus Glu-B1) could impart quality expression through technological treatments but with a lower contribution to the varietal differences.

CONCLUSION

1) Unlike what is observed in bread wheats, where HMW subunits (coded by genes located on the long arm of group 1 chromosome) are considered to have a major effect on baking quality, in durum wheat, the major effect is obviously played by Gli-B1 locus : gamma + omega gliadins + LMW glutenins (short arm of 1B chromosome). In addition, contrary to bread wheat where several loci, with several alleles each, seem to influence more or less positively or negatively baking or gluten quality, durum wheat species has only two alleles with quite opposite effects at the locus involved in gluten rheological characteristics (Figure 9). From which it appears that : a) in a practical point of view, this

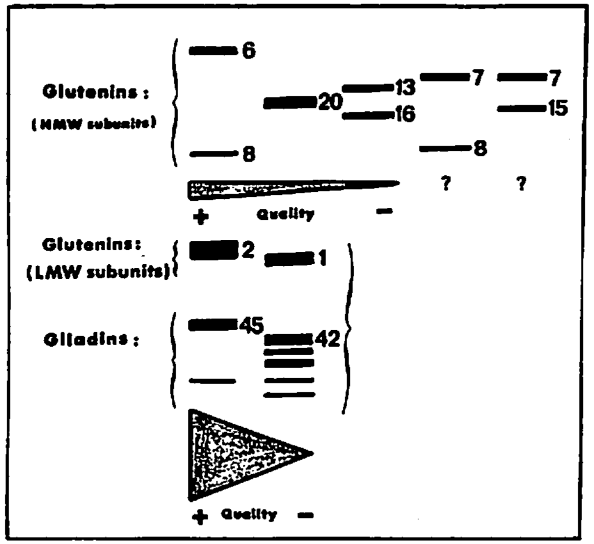

Fig. 9 Relationship of the different proteic alleles to rheological properties of durum wheat gluten.

parameter of cooking quality is easy to predict at the breeding stage and b) that we deal with a simple and unique model which could be helpful for basic studies on functional properties of proteins.

2) Biochemical basis of cooked pasta state of surface - second critical parameter of cooking quality - turn out to be more complex than those of gluten rheological characteristics, in spite of the significant relationship found, for the first time in this work, with some types of HMW subunits (Figure 10). Further investigations are needed on proteins structure and physico-chemical properties and certainly also on components other than proteins, such as lipids or carbohydrates. Therefore, looking forward to any improvement in this field of knowledge, we found especially important to work out simultaneously a new microtest based on a small-scale milling, pasta making and pasta cooking, in order to be able to screen a larger number of growing locations of each genotype and to be able, for the first time, to specify the heritability of state of surface characteristic and take it into account in durum wheat breeding programs.

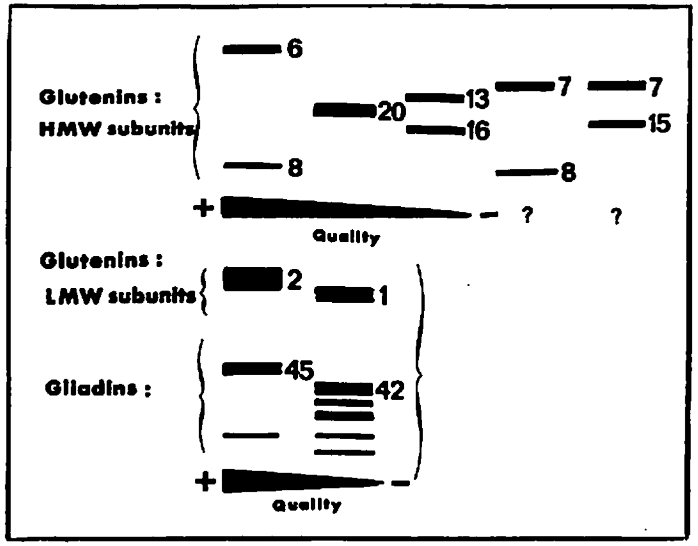

Fig. 10 Relationship of the different proteic alleles to state of surface of spaghetti

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