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Use of interspecific hybridisation in quality improvement of cereals

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SUMMARY – Interspecific hybridisation has shown to be a useful tool in the breeding of cultivated species of *Triticeae* tribe. This technique has been mainly used for transferring some interesting agronomic characters as resistance to biotic and abiotic stresses, although this has produced a loss of quality. However, the high variability detected for endosperm storage proteins in this tribe, together with other quality traits as the pigments content, could be used in quality improvement of cereals. We consider that *H. chilense* is a good example of this use, both as genes source for improvement of durum and bread wheat or tritordeum (amphiploid *H. chilense-Triticum* sp.).

Key words: Quality, interspecific hybridisation, *Hordeum chilense,* storage proteins.

RESUME – *"Utilisation de l'hybridation interspécifique pour l'amélioration de la qualité des céréales". L'hybridation interspécifique se révèle comme un instrument très utile pour l'amélioration des espèces cultivées de la tribu* Triticeae. *Cette technique est très utilisée pour le transfert de certains caractères agronomiques comme la résistance au stress biotique et abiotique, même si ce processus, très souvent, produit une diminution de la qualité. Néanmoins, on pourrait profiter de la haute variabilité existante dans les protéines de réserve dans l'endosperme ainsi que d'autres traits de qualité comme le contenu en pigments, pour améliorer la qualité des céréales. Nous considérons que* H. chilense *est un bon choix pour réaliser ce processus, car cette espèce présente une bonne source de gènes pour l'amélioration du blé dur, blé tendre, ou tritordeum (allopolyploïde* H. chilense-Triticum *sp.).*

Mots-clés : *Qualité, hybridation interspécifique,* Hordeum chilense, *protéines de réserve.*

Introduction

The Triticeae tribe has been very important in the human food since ancient times. The domestication of wheat (at diploid and tetraploid level) and barley is linked with the birth of Agriculture in the Fertile Crescent area (*ca.* 8000 BC). This importance is still maintained at present; in fact, wheat is one of the most important of all cultivated plants with respect to human nutrition and represents 1/3 of total production of cereals in the World. Most of the production is consumed directly as flour and constituted 1/6 of world diary diet according to FAO data (FAO, 1999).

Actually two types of wheat are mainly cultivated in the world, one hexaploid (bread wheat – *Triticum aestivum* ssp. *aestivum* L. em. Thell.) and another tetraploid (durum wheat – *T. turgidum* ssp. *durum* Desf. em. Husn.). The durum wheat is best known for its use in pasta industry, where the characteristics of its gluten and yellowish colour are much appreciated. In the Mediterranean zone, this crop is used for other uses as couscous, burghul, frike, home-baked flat-bread... It is important to emphasise that about 90% of the world's durum production has its origin in the Mediterranean region. Likewise the South of Europe (mainly Italy and Spain) was the base of traditional breads. This bread is characterised by a yellowish colour, fine and uniform porosity, characteristic taste and smell, and prolonged storage life (Quaglia, 1988).

What is quality?

The quality in cereals has been mainly associated with the characteristics of a macroproteic structure named gluten, which was probably first described by Beccari (1745). Gluten has been generally defined as *the visco-elastic mass that remains after thoroughly washing out the starch from a dough*. Although it is possible to find gluten in other cereals as barley or rye, gluten is usually produced from wheat flour.

The main components of gluten are proteins that represent between 75 and 85%, together with lipids (5-10%) and traces of other elements as starch, vitamins, etc. (Wrigley and Bietz, 1988).

Traditionally, the endosperm proteins of wheat have been classified into four main groups (albumins, globulins, gliadins, and glutenins) based on solubility criteria in different solvents (Osborne, 1924). The main wheat storage proteins are classified as glutenins (high and low M_r glutenin subunits) and gliadins (α/β, γ and ω gliadins) based on their solubility, molecular weight and amino acid composition (Wrigley and Bietz, 1988). The high M_r glutenin subunits have been studied in detail because of their role in determining bread-making quality of bread wheat (Payne *et al.,* 1984; Kasarda, 1989). Genetic studies have shown that these subunits are encoded by complex loci (*Glu-1*) on the long arms of the group 1 chromosomes of the A, B and D genomes of hexaploid wheat (Shewry *et al.,* 1994). Likewise, several studies have indicated that is possible to establish homoeology between these loci of wheat and some other ones found in different species within the tribe *Triticeae* (Shewry and Tatham, 1990), which is according with the putative common origin of these genes (Kasarda *et al.,* 1984; Rafalski *et al.,* 1984).

The low M_r glutenin subunits have been classified in three groups B, C and D, group B being the best studied. These proteins are coded at the *Glu-3* loci on the short arms of chromosomes of homoeologous group 1. In durum wheat, the low M_r glutenin subunits have been better studied than in bread wheat because of their relationships with the gluten strength and pasta quality (Pogna *et al.,* 1988, 1990). With respect to group D, some work has suggested that probably the proteins present in this zone may be considered as contaminants and classified as ω-gliadins (Branlard *et al.,* 1992; Khelifi and Branlard, 1991).

Gliadins are synthesised by genes of complex loci on the short arms of chromosomes 1 and 6. Because their role in quality has been strongly disputed (Pogna *et al.,* 1988, 1990; Metakosvky *et al.,* 1997a,b), these proteins have been mainly used for genealogical and identification studies, showing as excellent molecular markers.

The search of new quality genes

First studies of the molecular aspects of quality in the world collections of wheat revealed low variability for some protein components with sharp influence on bread-making performance (Shewry *et al.,* 1994). This loss of variability has been related with the change of the landraces or old varieties by new varieties of high yield and wide adaptability. Actually, the revival of the traditional food, together with the new uses of cereals, has promoted the search for new alleles present in landraces that could be useful in contributing genes for quality improvement, mainly in the glutenin or gliadin fractions (Impiglia *et al.,* 1998a,b). This search has been extended to the wild relatives of cereals (Jauhar, 1993).

Triticeae tribe is formed for species with three levels of polyploidy (diploids, tetraploids and hexaploids) showing high degree of crossability between them, which has allowed the natural transference of genes. This process has been a useful tool in the breeding of cultivated species of this tribe. In durum wheat, the cross with *T. turgidum* ssp. *dicoccoides* Körn. em. Thell., has been widely used as a way of transference of genes. Another source of genes less used is *T. turgidum* ssp. *dicoccum* Schrank em. Thell., together with other wild tetraploid wheats as *T. turgidum* ssp. *polonicum* L. em. Thell., *T. timopheevi* ssp. *armeniacum* Jakubz. em. MK, etc. These wild wheats have been mainly used for transferring other interesting agronomic characters such as resistance to biotic and abiotic stresses (Srivastava and Damania, 1989). Unfortunately, in most of these cases, the transference of these traits has produced a loss of bread making quality of wheat (Dhaliwal *et al.,* 1987; Koebner and Shepherd, 1988). A good example of the detrimental effects, is the use of 1BL/1RS rye wheat translocation. The significant improvements in agronomic characters that this translocation conferred to wheat (resistance to rusts or high grain yield) have contributed to its broad use in breeding programmes. However, dough made from 1BL/1RS wheat genotypes are intolerant to overmixing and exhibit increased stickiness, which are both undesirable traits in bread-making (Dhaliwal *et al.,* 1987; Peña *et al.,* 1990; Graybosch *et al.,* 1993, 1999).

We think this could be reduced by the use of allelic variants of endosperm storage proteins with positive effects on quality. Furthermore, because these proteins are likewise molecular markers, it would permit beginning the selection in early generations. To this respect, the works performed with *T. turgidum* ssp. *dicoccoides* are good example. Joppa *et al.* (1991) indicated that this species could be a source of proteins with good quality. These results have been confirmed in wheat-dicoccoides 6B subtitucion lines

(Kovacs *et al.,* 1998). Furthermore, Ciaffi *et al.* (1995) used this species for transferring to durum wheat an allelic variant of *Glu-A1* locus, that presented two active components while one only active component has been found in all the cultivated wheats (Shewry *et al.,* 1994). This material showed higher quality than the donor and host wheats.

Development of new species

A direct approach for exploiting genetic variability of wild species is the synthesis of amphiploids or artificial polyploids by means of chromosomal duplication of hybrids with colchicine; in fact, polyploidy is a natural mechanism of evolution of cereals, such as durum and bread wheats. Although numerous attempts have been performed (Jauhar, 1993), only the genome of rye (*Secale cereale* L.) has been successfully integrated with the wheat genomes to produce one man-made crop, triticale (×*Triticosecale* Wittmack). This crop has already proved useful in several regions (Varughese, 1996), which China, Poland, Germany and Australia that represent 77% of world area harvesting of this crop (FAO, 1999).

One principal handicap of triticale has been the above-mentioned negative effect on bread-making quality of the R genome. This has been modified by selectively incorporating the high molecular weight glutenin alleles into the R genome from the D genome. The introduction of chromosome 1D, mainly the substitutions 1D(1A), 1D(1B) or 1D(1R), improves sensitively the bread-making quality of triticale (Kazman and Lelley, 1996).

This process has also been performed for the synthesis of new bread wheats, involving the genome of *T. turgidum* ssp. *durum* and *Aegilops tauschii* Coss. (the D genome donor in bread wheat), as a means of introducing desiderable traits into bread wheat (Multani *et al.,* 1988; Schachtman *et al.,* 1992) and studying the relationship between *Ae. tauschii* and bread wheat (Lagudah *et al.,* 1987; Lagudah and Halloran, 1988; Peña *et al.,* 1995).

Plant breeders have been interested in crossing wheat and barley since the beginning of the century (Farrer, 1904), but amphiploids, ×*Tritordeum* Ascherson et Graebner, were obtained only when wild relatives of *H. vulgare* L. were used. *H. chilense* Roem. et Schult. is a wild South American diploid barley included in the Section Anisolepis (Bothmer *et al.,* 1995). It occurs exclusively in Chile and Argentina and it is highly polymorphic both morphologically and biochemically (Giménez, 1995). It is a weak perennial and is a component of natural pastures where it is greatly appreciated by cattle (Valderrama *et al.,* 1991).

The first amphiploid between *H. chilense* and *T. aestivum* (cv. Chinese Spring) showed extremely low fertility. Afterwards, when additional *H. chilense* accessions and different bread wheat cultivars were used, some of the resulting amphiploids were highly fertile. Nevertheless, these materials showed poor initial growth and high frequencies of aneuploids, traits that could limit their possible direct use as a crop. In contrast, hexaploid tritordeum (*H. chilense* × *T. turgidum* ssp. *durum*) shows low frequency of aneuploids, a wide variation in rate of growth, and good fertility (Martín and Sanchez-Monge Laguna, 1982). These and other favourable agronomic traits such as biomass, number of spikelets/spike, seed size, or protein content, led us to consider the potential of this amphiploid as a possible new crop, which have been confirmed with later studies (see Martín *et al.,* 1999 for revision).

Quality improvement of a new specie

Breadmaking quality

Preliminary analyses seemed to indicate that the quality characteristics of tritordeum were the results of the amphiploidy itself. However, further investigations indicated that interactions between the storage proteins from both genomes, barley and wheat, are responsible for the quality characteristics of tritordeum (Alvarez and Martín, 1994; Alvarez *et al.,* 1994). The end use properties of tritordeum were tested using the same approaches as used to evaluate barley and wheat quality. Malting quality was evaluated by a micromalting test according to Molina-Cano (1975), indicating that tritordeum is similar in its malting properties to other naked-grained cereals (Alvarez *et al.,* 1992).

Analysis of the hexaploid tritordeum for processing quality, showed that the dough viscoelasticity was similar to that of some medium quality bread wheat cultivars currently grown in Spain, e.g. cv. 'Anza'

(Alvarez *et al.,* 1992). This, together with the fact that tritordeum has low levels of vitreousness and hardness and high flour yield, suggests that tritordeum is more suitable for breadmaking than for pastamaking. These results are more interesting if we consider that hexaploid tritordeum lacks the **D** genome, which has been associated with the viscoelastic properties of bread wheat (Orth and Shellenberger, 1988).

The comparison of hexaploid tritordeum with wheats and triticale confirmed that tritordeum has quality traits similar to those of bread wheat, but different from those of durum wheat and triticale (Alvarez *et al.,* 1994; Sillero *et al.,* 1997). The differences between the wheat parents and their derived tritordeums were greater in hexaploid than in octoploid tritordeums. In octoploid tritordeums, the presence of the **D** genome, appeared to moderate the effects of the $\mathbf{H^{ch}}$ genome.

One consistent characteristic of the tritordeum (both hexa- and octoploid) flours is the intensive yellow colour due to the high content of carotenoid pigments (Alvarez *et al.,* 1994, 1995a). In fact, the pigment contents of tritordeums are between two and three times those of their wheat parents. However, although this results in a yellow colour, no effect on baking quality has been found to date. This trait has been located to the α-arm of chromosome $7H^{ch}$ (Alvarez *et al.,* 1998) and there it may be possible to transfer this high carotene content to durum wheat by chromosome manipulation (Alvarez *et al.,* 1999a).

Several recombinant lines of hexaploid tritordeum have been analysed by rheological and baking tests according to standard methods (AACC, 1983; ICC, 1986; Campaña *et al.,* 1993). The gluten strength, as estimated by the alveograph parameter W, indicated that tritordeums generally have lower values than the bread wheat control (cv. 'Yecora'). However, some of the tested lines showed reasonably high W values, close to 200. Similarly, the extensibility of the tritordeum doughs was substantially greater than that of the wheat control. A similar tendency was observed for dough springiness and shortness. In fact, the alveograph G values were slightly higher for the tritordeums than for bread wheat. In contrast, the dough tenacity of the tritordeum doughs was clearly lower than that of cv. 'Yecora'. However, wide variability in these traits has been found in other tritordeum lines. In fact, the F_3 progeny of the cross HT31/HT48 showed a very atypical alveograph for tritordeum with tenacity of 103.5 mm and extensibility of 20 mm (Alvarez *et al.,* 1995b).

Other rheological properties were measured using the Brabender micro-Farinograph. In general, the Farinograph characteristics of tritordeum flours were poorer than those of wheat, although some lines of tritordeum had relatively high water absorption values, which were comparable with that of the bread wheat. However, the Farinograph stability of these lines was lower than that of the wheat In the baking test, the tritordeum lines showed loaf volumes between 92 and 60% of the loaf volume of cv. 'Yecora' (579 ml), although it is emphasised that all these lines are genetically related and represent only a small proportion of the available variation in tritordeum. Likewise an important print is that none of the tritordeum lines were deliberately selected for breadmaking quality.

Storage proteins

In 1987 Payne *et al.* (1987), using wheat-*H. chilense* addition lines, found 25 major components by two-dimensional electrophoresis in *H. chilense* grain. The chromosomal location of these components were determined indicating that most prolamins were synthesised by the $1H^{ch}$ chromosome, although some α-prolamins genes were coded at the other chromosomes (chromosomes $5H^{ch}$ and $7H^{ch}$). The results of Fernández (1989) and Tercero *et al.* (1991) confirmed these findings and identified some loci. One locus, named $\textit{Glu-H}^{ch}\textit{1}$, was found on the long arm of chromosome $1H^{ch}$. This locus is homoeologous of the *Glu-1* locus of wheat and *Hor3* locus of barley (Tercero *et al.,* 1991), encoding the high M_r prolamins. A second locus on the short arm of this chromosome designated $\textit{Hor-H}^{ch}\textit{1}$, encoded up to 11 subunits belonging to the ω-, β- and α-hordeins (Tercero *et al.,* 1991). Further loci on chromosomes $5H^{ch}$ and $7H^{ch}$, designated $\textit{Hor-H}^{ch}\textit{2}$ and $\textit{Hor-H}^{ch}\textit{3}$, encode α-hordeins.

The storage protein composition of several lines of hexaploid tritordeum, together with their *H. chilense* and durum wheat parents, were analysed by SDS-PAGE (Alvarez *et al.,* 1993). The banding pattern of each tritordeum results from the addition of the patterns of the two parental lines. Although the tritordeum tested lines represented only a proportion of the genetic variability available, some variation in the prolamin subunits derived from the $\mathbf{H^{ch}}$ genome was detected (Alvarez *et al.,* 1993).

All the prolamins (monomerics and polymerics) of *H. vulgare* L. are usually named hordeins. For this reason some authors have named hordeins to the prolamins of *H. chilense* (Payne *et al.,* 1987; Tercero *et al.,* 1991); however, no evidence has been found that identified these proteins to the prolamins of *H. vulgare.* In fact, biochemical comparison between *H. chilense* and *H. vulgare* shows great differences between species (Fernández *et al.,* 1987). Some results, such as the similarity on chromosome banding pattern after *in situ* hybridisation with probe pAs1 between *H. chilense* and *Ae. tauschii* (Cabrera *et al.,* 1995) or cytoplasm compatibility (Millán and Martín, 1992), suggested that the *H. chilense* genome could be more similar to wheat than to barley. On basis of these results, the H^{ch} prolamins have been considered as glutenin-like or gliadin-like proteins according their solubility in alcohol solutions.

Recently, we have found up to eight loci encoding prolamin genes in *H. chilense* (Alvarez *et al.,* 1999b). The analysis was performed on the advanced progenies from two crosses of hexaploid tritordeum by acid-PAGE and SDS-PAGE. Two of these loci encoded glutenin proteins, one encoding high M$_r$ subunits (the *Glu-H*^{ch}*1* locus) and the second the low M$_r$ C-subunits (the *Glu-H*^{ch}*4* locus). The work carried out by Payne *et al.* (1987) and Tercero *et al.* (1991) indicated that the *Glu-H*^{ch}*1* locus is located on the long arm of the chromosome 1H^{ch}. Three allelic variants at this locus, named H^{ch}a, H^{ch}b and H^{ch}c, have been detected. The allele H^{ch}a corresponded with the component 1 identified by Payne *et al.* (1987); while the other two alleles have not been described previously. Data obtained with a world collection of *H. chilense* suggest that this variability could be sensitive larger (Alvarez *et al.,* unpublished results). Three allelic variants at the *Glu-H*^{ch}*4* locus were also found in the tritordeums studied.

Tercero *et al.* (1991) reported that the ω-type hordeins together with some α-hordeins are encoded by one locus located on the short arm of chromosome 1H^{ch}, which was named *HorH*^{ch}*-1*. We have found that these proteins are encoded by at least six loci (Alvarez *et al.,* 1999b), three for the ω-hordeins (*Gli-H*^{ch}*1*, *Gli-H*^{ch}*5* and *Gli-H*^{ch}*6*) and three for α-hordeins (*Gli-H*^{ch}*2*, *Gli-H*^{ch}*3* and *Gli-H*^{ch}*4*).

Analysis of SDS-sedimentation values showed that all the loci identified had some effect on gluten strength (Alvarez *et al.,* 1999b). This effect was greater for the *Glu-H*^{ch}*1*, *Gli-H*^{ch}*1* and *Gli-H*^{ch}*5* loci than for the other loci (*Glu-H*^{ch}*4, Gli-H*^{ch}*2, Gli-H*^{ch}*3* and *Gli-H*^{ch}*4*). All these data suggest that the **H^{ch}** genome have similar effect on gluten strength to the **D** genome from *Ae. tauschii*. Consequently, the search of the new allelic variants at the loci studied in the *H. chilense* collection, (mainly *Glu-H*^{ch}*1*, *Gli-H*^{ch}*1* and *Gli-H*^{ch}*5*) could have great importance for quality of tritordeum, and indirectly for increasing the genetic variability for quality traits in wheat.

On the other hand, Payne *et al.* (1987) indicated that the endosperm proteins of *H. chilense* detected between the high M$_r$ and B-low M$_r$ prolamin subunits (D-zone) are prolamins encoded at the chromosome 1H^{ch}. Our data suggest that the D-prolamins that showed high degree of polymorphism, must be considerate as glutenin-like proteins (Alvarez *et al.,* 1999c). At present, these proteins from *H. chilense* are been analysed with the same method as used in this report for establishing the relationships between them (allelism or linkage) and their importance in bread making quality of hexaploid tritordeum.

Conclusions

We think that the high variability detected in *Triticeae* tribe for the storage proteins, together with their role on quality of cereal products, could be doubly used in the breeding process by interspecific hybridisation. One way is the synthesis of new species as triticale or tritordeum. Another way is the search for species that could be used for transferring interesting traits to wheat without detrimental effects on bread making quality.

H. chilense could contribute to widening the genetic basis of quality of bread and durum wheats, mainly by the increase of variability for endosperm storage proteins or the pigments content. In this respect, tritordeum could be used as bridge species for the transfer of these useful traits to wheat. Another possibility is the development of tritordeum as a new man-made crop. In this way, we consider that the role of tritordeum in the food industry could be similar to that of bread wheat, although the ultimate end-uses will depend on the quality characteristics that could be obtained in the breeding programmes.

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