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Exploring and manipulating the structures and functional properties of wheat seed proteins

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SUMMARY – Transformation has been used to transfer additional HMW subunit genes to genetic stocks, breeding lines and cultivars of bread and durum wheat, resulting in expression levels up to and above those of the endogenous HMW subunits. Analysis of dough properties using a 2 g Mixograph showed that this resulted in some cases in increases in dough strength, indicating that transformation can be used to improve the processing properties for making pasta and bread.

Key words: Gluten, genetic engineering, functionality, HMW subunits.

RESUME – *"Exploration et manipulation des structures et propriétés fonctionnelles des protéines de graines de blé". Des gènes supplémentaires codant pour des sous-unités de Haut Poids Moléculaire ont été introduits par transformation dans des stocks génétiques, des lignées reproductrices et des cultivars de blé dur, résultant en des niveaux d'expression identiques ou supérieurs à ceux des gènes endogènes. L'analyse des propriétés de la pâte à l'aide d'un Mixographe de 2 g a montré que cela résultait dans certains cas en une résistance accrue de la pâte indiquant que la transformation peut être utilisée pour améliorer la qualité de la farine pour la fabrication des pâtes et du pain.*

Mots-clés : *Gluten, amélioration génétique, fonctionnalité, sous-unités de Haut Poids Moléculaire.*

Introduction

Wheat is the major crop in the world, in terms of its geographical distribution, area under cultivation and total yield. It forms a major part of the human diet in many countries, as a source of energy and protein. However, it is rarely consumed by humans without processing into bread or other products. The range of processed wheat products is vast, reflecting cultural preferences as well as differences in the processing properties of wheats adapted to different regions. They include a wide range of leavened and unleavened breads, noodles and pasta made from bread and durum wheats, respectively, cakes and biscuits (cookies). In addition, wheat is used as an ingredient in many other processed foods.

The ability to use wheat for most of these food products depends on the structures and properties of the storage proteins. These account for about half of the grain proteins and form a continuous network in dough which confers the unusual properties of elasticity combined with extensibility or viscous flow. This allows the entrapment of carbon dioxide during the proving of leavened dough but also the cohesiveness required for other food products. However, the precise balance of extensibility and elasticity required varies with different end uses, with highly elastic doughs being preferred for breadmaking. The balance of extensibility and elasticity is also affected by environmental and genetic factors, with poor breadmaking performance often resulting from low elasticity.

Because elasticity is important in determining wheat quality, it has been the subject of a number of studies. These have shown that elasticity is determined by the amount and properties of high M_r glutenin polymers stabilized by inter-chain disulphide bonds. In addition, differences in elasticity are associated with variation in one group of proteins present in these polymers, called the high molecular weight (HMW) subunits of glutenin (Payne, 1987).

Two HMW subunit genes are present on each of the group 1 chromosomes, encoding a low M_r y-type subunit and a high M_r x-type subunit. However, not all of these genes are expressed, with cultivars of hexaploid bread wheat containing 3, 4 or 5 individual subunits (1Dx, 1Dy, 1Bx and 1Ax and/or 1By subunits) but rarely six subunits. Similarly, most cultivars of tetraploid pasta wheat contain only one or two subunits encoded by chromosome 1B (1Bx subunits with 1By subunits also being present in some cultivars). These differences in HMW subunit composition appear to have quantitative and qualitative effects on breadmaking performance in bread wheat, with quantitative effects associated with differences in the number of expressed genes (each subunit accounting for about 2% of the total protein) and qualitative effects associated with allelic variation in expressed subunits (Halford *et al*., 1992; Shewry *et al*., 1992). We have, therefore, attempted to manipulate the processing properties of bread and pasta wheats by transformation with genes to increase the numbers and amounts of the HMW subunits.

Transformation of bread and durum wheat with HMW subunit genes and HMW-*gus* constructs

Immature caryopses of bread and durum wheat and immature inflorescences of durum wheat were transformed by microprojectile bombardment as described previously (Barcelo and Lazzeri, 1995; Barro *et al*., 1997; He *et al*., 1999). Co-transformation was carried out in which the selectable marker (*bar* conferring resistance to BASTA) and screenable marker [*UidA* encoding β-glucuronidase (GUS)] genes under the control of constitutive promoters were present on separate plasmids to the genes of interest.

Three plasmids contained genes of interest: p1Ax1 contained a 7.0 kb *Eco*RI genomic fragment containing the subunit 1Ax1 gene, p1Dx5 contained a 8.7 kb *Eco*RI genomic fragment containing the 1Dx5 gene and pHMW-*gus* contained 1 kb of 5' upstream sequence from the 1Dx5 gene fused to the *UidA* reporter gene. The latter gene was used for transformation without the *UidA* screenable marker gene. Transformation efficiencies for immature embryos of two near isogenic lines of wheat were about 0.9% and for three cultivars and one breeding line of durum wheat about 0.6%.

Expression pattern of pHMW-*gus* in durum wheat

Immature inflorescences of durum wheat cv. Ofanto were transformed with pHMW-*gus* to confirm the expression pattern conferred by the HMW subunit gene promoter. Two plants were recovered which showed similar expression patterns. No expression was observed in vegetative or floral tissues but histochemical staining and RT-PCR showed expression within the starchy endosperm starting at about 10 days after anthesis. An identical expression pattern was observed for the endogenous 1Bx subunit gene present in this cultivar, demonstrating that the HMW subunit promoter can be used to drive endosperm-specific expression of heterologous transgenes in wheat.

Expression of HMW subunits in bread wheat

Two near-isogenic lines were selected for transformation with genes encoding subunits 1Ax1 and 1Dx5. These were line L88-6 containing five HMW subunits (1Ax1, 1Dx5, 1Dy10, 1Bx17, 1By18) and L88-31 expressing only the two chromosome 1B-encoded HMW subunits (1Bx17, 1By18). A number of transformed lines expressing additional HMW subunits were recovered with expression levels ranging up to and above those of the endogenous HMW subunits. The transgene insertion number was estimated to vary from about 1 to 20 (Barro *et al*., 1997; unpublished results of L. Rooke, H.S. Steele, P. Barcelo and P.A. Lazzeri).

Preliminary studies using a 2 g Mixograph showed that transformation of L88-31 with genes for subunits 1Ax1 and/or 1Dx5 resulted in increased dough strength, mirroring the effects observed when subunit number is manipulated by classical genetics. However, transformed lines of L88-6 are of more interest as the addition of HMW subunit transgenes allows the number of expressed genes to be increased above the normal maximum of five. One such line is B73-6-1, in which multiple additional copies (probably about 15) of the subunit 1Dx5 transgene result in an increase in the total amount of HMW subunit protein from about 12.7 to 20% of the total gluten protein (subunit 1Dx5 being increased from about 2.7 to 10.6%) (Rooke *et al*., 1999) (Fig. 1). Mixograph analyses of this line showed highly unusual mixing characteristics, with failure to form a normal dough unless the mixing speed was increased. Blending with flour from a commercial variety (cv. Banks) showed that the incorporation of

B73-6-1 resulted in increased mixing time when blended in proportions up to about 60% but fell when the proportion was increased further (Fig. 1). This may indicate that the line is “overstrong”, but the fact that both the peak resistance and resistance breakdown were reduced at all levels of B73-6-1 suggest that the effect may be more complex. Nevertheless, these results demonstrate that it is possible to produce major changes in the composition and mechanical properties of bread wheat gluten by transformation.

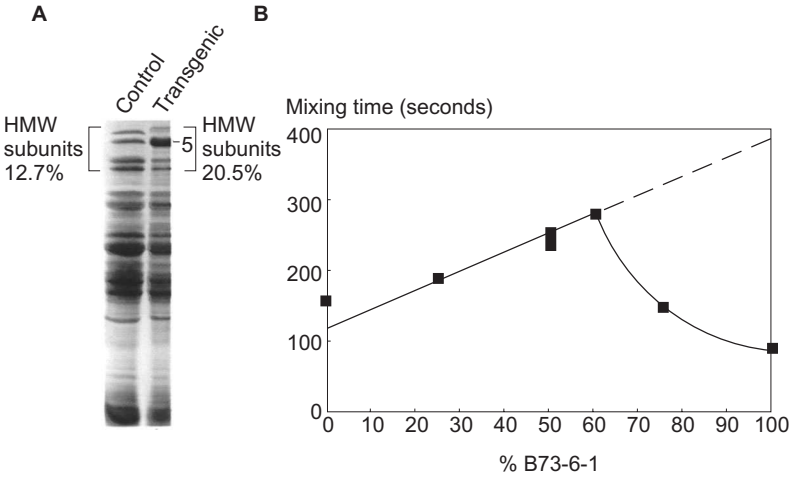

Fig. 1. A: SDS-PAGE of total grain proteins of the transgenic line B73-6-1 (expressing additional copies of the subunit 1Dx5 gene) and the control line L88-6. B: Blending of flours from B73-6-1 and the “normal” cultivar Banks results in increases in the mixing time up to levels of about 60% B73-6-1, above which the mixing time decreases. The results were obtained using the 2 g Mixograph. Based on results in Rooke *et al.* (1999).

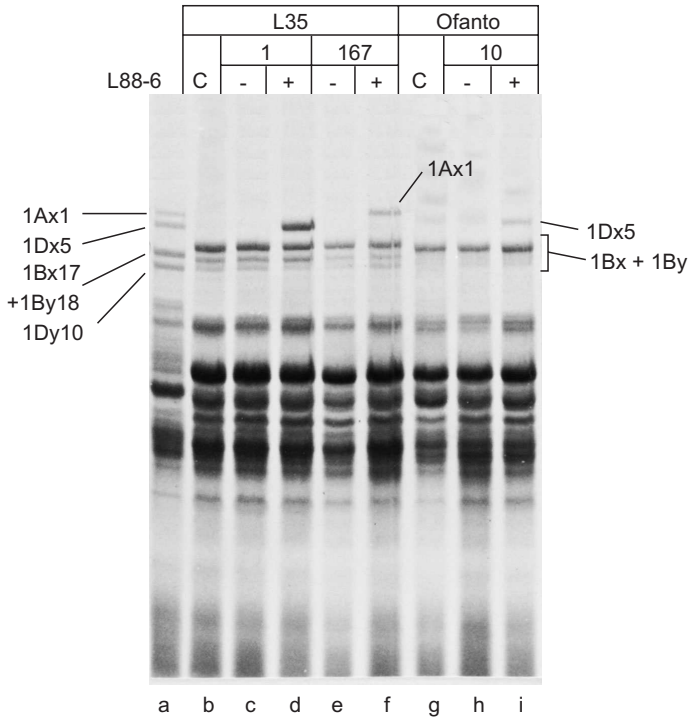

Fig. 2. SDS-PAGE of total flour proteins from seed of sibling T_2 plants which are negative (-) or positive (+) for the expression of the 1Dx5 transgene in L35 (line 1), the 1Ax1 transgene in L35 (line 167) and the 1Dx5 transgene in Ofanto (line 10). Tracks C are from control plants of the parental lines. The bracket indicates the endogenous 1Bx + 1By subunits present in the durum wheat lines (taken from He *et al.,* 1999).

Transformation of durum wheat with HMW subunit genes

The level of gluten visco-elasticity is also a major quality character in durum wheat used for either pasta making or breadmaking. Genes for subunit 1Ax1 and 1Dx5 were therefore used to transform immature embryos of three cultivars (L35, Ofanto, Svevo) and one breeding line (Latino x Lira) of durum wheat, with an efficiency of about 0.6%. Ten independent transgenic lines were isolated, including lines of L35, Svevo and Latino x Lira expressing subunit 1Ax1 and lines of L35 and Ofanto expressing subunit 1Dx5 (He *et al.*, 1999). Three lines showing segregation for HMW subunit expression were used to isolate sibling T_2 plants which were positive or negative for transgene expression (Fig. 2), allowing precise analyses to be made of the effects of the additional HMW subunits on the Mixograph parameters (Fig. 3).

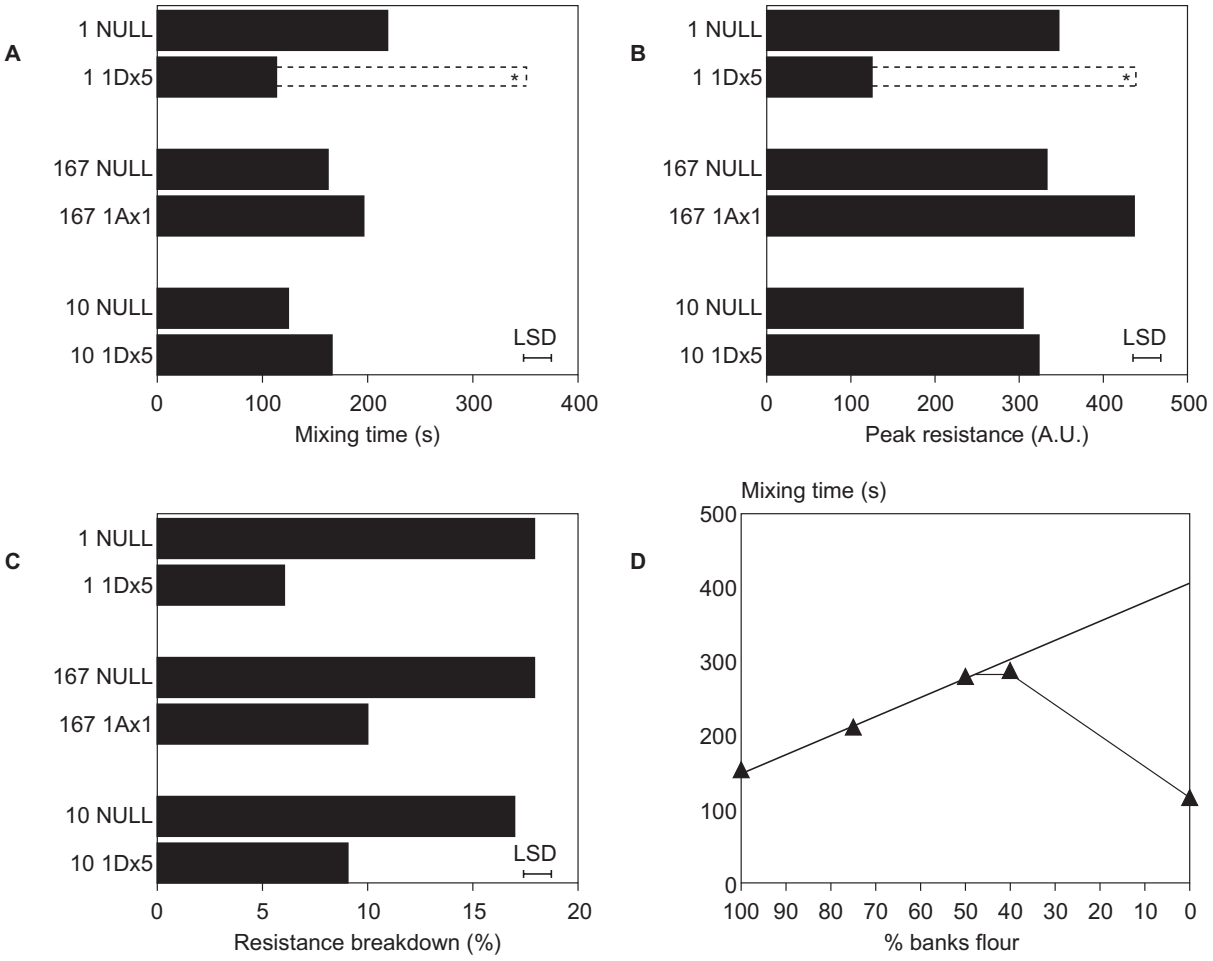

Fig. 3. Analysis of the mixing properties of dough from transgenic durum lines 1 (1Dx5), 167 (1Ax1) and 10 (1Dx5). A. Mixing time (seconds) for doughs made from negative and positive sibling T_2 seeds of lines 1, 167 and 10. For line 1, the presence of the 1Dx5 subunit resulted in flour that was "overstrong" resulting in a low mixing time. In order to obtain data for this line analyses were carried out on flour samples blended with flour from cv. Banks (dotted area on graph, see also graph D). B. Peak dough resistances (arbitrary units) for doughs made from negative and positive sibling T_2 seeds of lines 1, 167 and 10. For line 1, the presence of the 1Dx5 subunit resulted in flour that was "overstrong" resulting in a low peak resistance. In order to obtain data for this line analyses were carried out on flour samples blended with flour from cv. Banks (dotted area). C. Resistance breakdown (%) for doughs made from negative and positive sibling T_2 seeds of lines 1, 167 and 10. With all three lines the presence of either the 1Ax1 or 1Dx5 HMW subunit led to a decrease in the rate of breakdown indicating greater gluten stability. D. Mixing time (seconds) for a series of flours blended from positive T_2 seeds of line 1 and cv. Banks (% of Banks given on x axis). Extrapolation allows the true mixing time for line 1 to be calculated (taken from He *et al.,* 1999).

The expression levels of the transgenes in lines 10 (1Dx5 in Ofanto) and 167 (1Ax1 in L35) were similar to those of the endogenous HMW subunits and the positive lines had increased dough strength as measured by increased mixing time and peak resistance and decreased resistance breakdown. A higher level of transgene expression was observed in line 1 (1Dx5 in L35) and the Mixograph parameters were more similar to those of the "overstrong" bread wheat line B73-6-1 (see above). In particular, blending of flour of this line with flour of the normal cultivar Banks resulted in increases in mixing time at levels up to about 50% above which the mixing time decreased.

Conclusions

Our studies of bread and pasta wheats have demonstrated that it is possible to manipulate the biomechanical and functional properties of wheat gluten by transformation with additional genes for HMW subunits. This includes increasing the dough strength and conferring new properties beyond those currently available in bread or durum wheat. The existing bread wheat lines are now being evaluated in more detail using material grown in replicated field trials and the work extended to transform varieties grown commercially in the UK. We are also using transformation to explore the molecular basis for the role of the HMW subunits in determining gluten visco-elasticity by transforming with genes encoding a range of mutant forms as part of an EU FAIR programme.

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