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in

Molina-Cano J.L. (ed.), Christou P. (ed.), Graner A. (ed.), Hammer K. (ed.), Jouve N. (ed.), Keller B. (ed.), Lasa J.M. (ed.), Powell W. (ed.), Royo C. (ed.), Shewry P. (ed.), Stanca A.M. (ed.).
Cereal science and technology for feeding ten billion people: genomics era and beyond

Zaragoza : CIHEAM / IRTA
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 81

2008
pages 353-354

Article available on line / Article disponible en ligne à l'adresse :

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http://om.ciheam.org/article.php?IDPDF=800876

To cite this article / Pour citer cet article

Marín S., Pistón F., Barro F. **Expression of two D-hordeins from Hordeum chilense in bread and durum wheat.** In : Molina-Cano J.L. (ed.), Christou P. (ed.), Graner A. (ed.), Hammer K. (ed.), Jouve N. (ed.), Keller B. (ed.), Lasa J.M. (ed.), Powell W. (ed.), Royo C. (ed.), Shewry P. (ed.), Stanca A.M. (ed.). *Cereal science and technology for feeding ten billion people: genomics era and beyond.* Zaragoza : CIHEAM / IRTA, 2008. p. 353-354 (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 81)

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Expression of two D-hordeins from *Hordeum chilense* in bread and durum wheat

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SUMMARY – Two D-hordeins genes from *H. chilense* were introduced into bread and pasta wheat by particle bombardment. The D-hordeins showed levels of expression similar to that of the homeologous high molecular weight glutenin subunits (HMW-GS) of wheat. These transgenic lines increase the genetic variability for storage protein and can expand the end-use of wheat.

Introduction

The HMW-GS of wheat are responsible for the breadmaking quality (Shewry *et al*., 1992). These HMW-GS are coded by single genes located on chromosomes 1A, 1B and 1D of wheat. In barley, the homeologous to the HMW-GS are the D-hordeins. *Hordeum chilense* is a wild barley which has been successfully used in the synthesis of amphiploids by crossing with *Triticum* spp. (Martín and Chapman, 1977; Martín and Sanchez-Monge, 1982). In these amphiploids, called tritordeum, the D-hordeins play an important role in breadmaking quality (Alvarez *et al*., 1999). The aim of this work is to investigate the effect of two D-hordeins in the breadmaking and pasta quality of wheat.

Material and methods

Two D-hordein genes from lines H1 and H7 of *Hordeum chilense* were isolated by "genome walking" and the complete gene, including promoter, amplified by PCR and cloned into a transformation vector. These sequences were introduced in bread and durum wheat by particle bombardment. The HMW-GS composition were analysed in SDS-PAGE gels in a discontinuous Tris-HCl-SDS buffer system (pH: 6.8/8.8) at a 10% polyacrylamide concentration (w/v, C= 1.28%).

Results and discussion

The nucleotide and amino acid sequences showed that both D-hordeins were different in length, due to differences in the repetitive regions. The Blast comparison showed that both D-hordeins are similar to the HMW-GS 1Dx5 and 1Dy10 of bread wheat. Immature embryos of bread wheat 'Perico' and 'Bobwhite', and durum wheat 'Don Pedro' were transformed with the D-hordein genes from *H. chilense*. SDS-PAGE analysis showed similar levels of expression of both D-hordeins in comparison to the endogenous HMW-GS (Figure 1). Both D-hordeins were also transferred to the bread line 'Anza' by crossing with transgenic lines. Transformation with D-hordeins will be useful to expand the genetic variability for storage proteins and to investigate the effect of D-hordeins in the breadmaking and pasta quality of wheat.

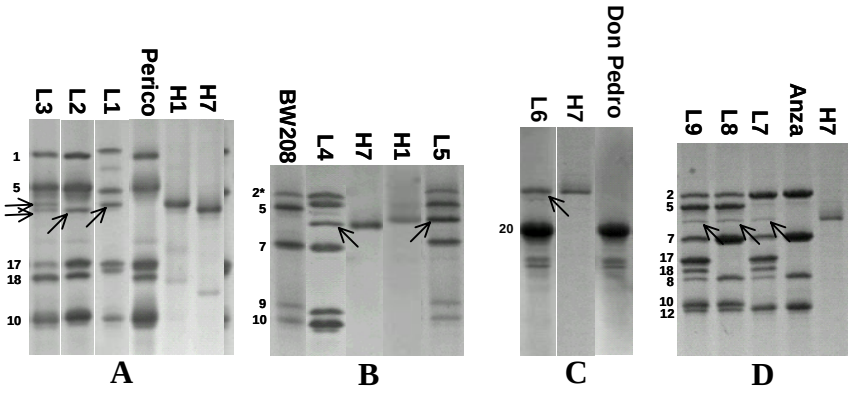

Fig. 1. Expression of D-hordeins in different backgrounds of wheat. **A.** transgenic lines (L1, L2, L3) of bread wheat 'Perico' expressing the D-hordeins from H1 and H7. **B.** Transgenic lines (L4, L5) of bread wheat 'Bobwhite' (BW208) expressing the D-hordeins from H1 and H7. **C.** Transgenic line (L6) of durum wheat 'Don Pedro' expressing the D-hordein from H7. **D.** Expression of the D-hordein from H7 in bread wheat 'Anza'. This D-hordein was transferred by crossing 'Anza' with line L2 (panel **A**). Arrows indicate the transgenic subunits and numbers indicate the corresponding HMW-GS.

Acknowledgments

The authors thank funding by Spanish CICYT (project AGL2004-03361-C02-2).

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