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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 445-446

To cite this article / Pour citer cet article

Pistón F., Martín A., Barro F. **Three genes from the anther-flavonoid pathway are down-regulated in male sterile alloplasmic 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. 445-446 (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 81)

Three genes from the anther-flavonoid pathway are down-regulated in male sterile alloplasmic wheat

F. Pistón, A. Martín and F. Barro
Dpto. de Agronomía y Mejora Genética Vegetal, IAS-CSIC, Apdo 4084, 14080 Córdoba, Spain

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SUMMARY – In this work the subtractive hybridization was used to clone and characterize genes that are differently expressed in two lines of alloplasmic wheat. One line was sterile and the other containing an addition of the chromosome 6HS of *Hordeum chilense*, fertile. Three genes that coded for key enzymes of the flavonoid pathway were monitored by real time PCR, confirming that the three genes are down-regulated in the sterile line.

Key words: Subtractive hybridization, alloplasmic wheat, restorer genes.

Introduction

Cytoplasmic male sterility (CMS) is a maternal heredity character that causes the abortion of pollen development. The CMS has been used satisfactorily in the genetic breeding of several species to facilitate the production of hybrids at great scale (Touzet *et al.*, 2004). The nature of the CMS has been related in many cases with mitochondrial defects. However, in many species, the lethal effects of the mitochondrial defects can be avoided by the action of nuclear genes, calls nuclear restorer (Liu *et al.*, 2001). Nevertheless, neither the specific mechanisms nor the genes implied in the restoration are well known.

Material and methods

In our laboratory two alloplasmic lines of bread wheat (Chinese Spring) in cytoplasm of *Hordeum chilense* have been obtained. One line was sterile while the other, that contains an addition of an arm of the chromosome 6H of *H. chilense*, was fertile. The populations of messenger RNA of immature anthers from both lines were compared themselves by subtractive hybridization. The expression patterns of the different genes were monitored by quantitative real time PCR (QRT-PCR).

Results and discussion

Twenty-two clones that were expressed in the fertile line but not in the sterile were identified and sequenced. Three of the sequences coded for key enzymes of the flavonoid biosynthetic pathway: Chalcone syntase (EC 2.3.1.74), Dihidroflavonol 4-reductase (EC 1.1.1.219) and Flavonone 3-hidroxylase (EC 1.14.11.9). The flavonoid pathway in anthers is essential for the pollen development (Napoli *et al.*, 1999). The expression patterns of the three genes were monitored and quantified by QRT-PCR in both the fertile and sterile lines and in one control line of Chinese Spring (Fig. 1). The results showed that the three genes were down-regulated in the sterile line. These genes could be responsible for the CMS in these alloplasmic lines of wheat.

Acknowledgments

This work was supported by Spanish CICYT (AGL2004-03361-C02-2).

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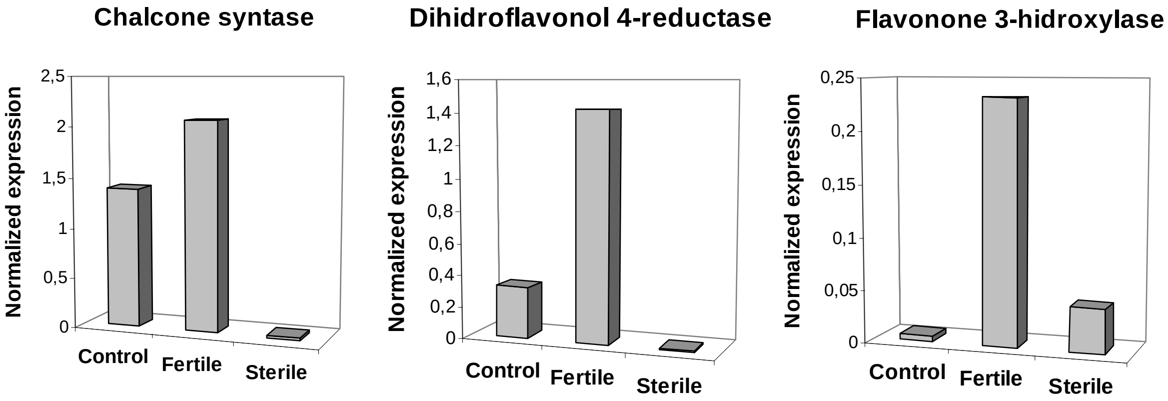

Fig. 1. Normalized expression of three genes in the flavonoid pathway of wheat. The expression was monitored by QRT-PCR from total RNA of immature anthers. Control, Chinese Spring; Fertile, alloplasmic line with addition of one arm of chromosome 6H; Sterile, alloplasmic line.