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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 447-449

Comparative transcriptional and proteomic profiling of bread wheat cv. 'Bobwhite' and its derived transgenic line over-expressing a *lmw-gs* gene

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SUMMARY – A parallel transcriptional and proteomic comparison of seeds from a transformed bread wheat line over-expressing a transgenic low molecular weight glutenin subunit gene relative to the corresponding non-transformed genotype was performed. Proteomic analyses showed that, during seed development, several classes of endosperm proteins were differentially accumulated in the transgenic genotype. As a result of the strong increase in the amount of the transgenic protein, the endogenous glutenin subunit, all sub-classes of gliadins, and metabolic as well as Chloroform/Methanol soluble proteins were reduced in the transgenic genotype. This result was paralleled by the corresponding changes in transcript levels detected by microarray experiments. These findings suggest that the most evident effect of the strong over-expression of the transgenic glutenin gene consists in a global compensatory response involving a significant decrease in the amounts of polypeptides belonging to the prolamin superfamily.

Introduction

The quality of the wheat end-use products is largely determined by the gluten proteins, a complex mixture of polypeptides accounting for up to 80% of the total seed proteins. Gluten proteins are the determinants of the visco-elastic properties of the wheat doughs, and their quantity in the seeds is directly correlated with the quality of end-use products.

Recent efforts to increase the quantity of gluten proteins in the seeds focused on the introduction of additional gene copies by means of genetic engineering technology. With the aim of improving the quality traits of wheat-derived end products, we have produced and characterized a transgenic bread wheat line overexpressing a LMW-GS (Low Molecular Weight-Glutenin Subunit), whose relative quantity in the seed is expected to positively affect the technological properties of the flour.

In our previous studies (Masci *et al.*, 2003) it was shown that the transgenic polypeptide was incorporated into the glutenin fraction, but the SDS sedimentation test values were lower than expected in the transgenic line compared to the untransformed one, likely because glutenin polymer size and composition were largely altered.

In order to define the consequences of transgene(s) insertion/expression and the effects of genetic transformation on the global endosperm gene and protein expression, and thus with the aim to verify the principle of substantial equivalence, we carried out a comparative proteomic and transcriptional profiling between the seeds of the transgenic line with their non-transformed counterpart.

Materials and methods

Both the transgenic line (BWT) and the untransformed bread wheat cultivar Bobwhite (BW) were grown in a glasshouse under the same conditions. Developing seeds were collected at 10, 20, 30, and 35 days post anthesis (DPA).

In the microarray experiment, comparisons were made between BW and BWT at the same time point. Total RNA was isolated using Trizol reagent (Invitrogen) and indirect labelling and hybridizations were performed on three biological replicates, each one derived from a pooled, independent group of individuals. Dye-swap hybridizations were repeated for each biological

replicate. A cDNA wheat microarray was used representing 7457 unigenes, based on 68,000 ESTs coming from 47 different wheat cDNA libraries derived from different tissues (Zhang *et al.*, 2004). The differentially expressed genes were identified using the software package LIMMA (Linear Models for Microarray Data) (Smith, 2004).

The proteomic comparison was performed on developing and mature seeds of BW and BWT, but also including the negative control seeds derived from the transgenic line, in order to rule out the effect of genetic transformation.

Gliadins, total gluten proteins, B- and C-type LMW-GS, metabolic and CM-like proteins were selectively extracted from developing and mature seeds following published procedures (Masci *et al.*, 2002, 2003; Hurkman and Tanaka, 2004) and analyzed by means of two-dimensional gel electrophoresis (IEFxSDS-PAGE or Acid-PAGExSDS-PAGE). Image analyses and matching was performed using the computer software ImageMaster 2D Elite (Amersham Biosciences). N-terminal sequencing and/or MS analyses were used to identify the differentially expressed spots (Vensel *et al.*, 2005).

Results and discussion

Microarray analysis showed that, during the seed development, 542 unigenes were significantly differentially expressed (DE) between the wild-type and the transgenic genotype. These have been filtered according to their mean expression fold-change (≥2) thus retaining only 250 candidate, differentially expressed genes. All DE genes, both those up- and down-regulated in the transgenic with respect to the wild-type genotype, were clustered in several functional categories, and are summarized in Table 1. The majority of the DE genes encodes the various classes of seed-storage proteins, or are genes encoding proteins involved in trafficking/secretion, defense, glutamine biosynthesis, and transcription and translation-related processes. Interestingly, and most probably as a direct consequence of LMW-GS overexpression, all classes of seed-storage related genes (HMW-GS, α/β-, γ- and ω-gliadins) were heavily down-regulated in the seeds of the transgenic genotype.

Table 1. Summary of the microarray experiment and functional classification of DE genes. Fold change refers to the mean intensity ratio of Transgenic/Wild-type. The column "Timepoint" reports when differential expression was detected. DPA = days post anthesis

Up-regulated genes			
Functional category	Representative clone names	Maximum fold change	Timepoint
Seed storage proteins	LMW glutenin subunit (transgene)	>10	10, 20 and 30 DPA
Transport	Sarcoplasmic reticulum protein, SEC1 gene	>7	10 DPA
Transcription & Translation	Zinc finger protein, putative transcription factor, putative chromosome decondensation factor, bZIP transcription factor, ribosomal proteins	>13	10, 20 and 30 DPA
Down-regulated genes			
Functional category	Representative clone names	Maximum fold change	Timepoint
Seed storage proteins	HMW glutenin subunit α/β-gliadins γ-gliadins ω-gliadins	~0.15 ~0.10 ~0.15 ~0.12	20, 30 and 35 DPA
Defense	α/β-amylase/trypsin inhibitors, Chloroform/methanol soluble proteins (CM-like proteins), other protease inhibitors, peroxidases, lipid transfer proteins	~0.20	20, 30 and 35 DPA
Glutamine biosynthesis	Glutamine synthetase, Glutaminyl-tRNA synthetase	~0.30	20, 30 and 35 DPA

Transcript abundances assessed by the comparative microarray analyses were completely confirmed by proteomic data on selected classes of endoperm proteins. Metabolic and CM-like proteins (two classes including several allergenic components), for example, whose specific transcripts were down-regulated in the transgenic genotype during seed development, showed to be, in mature seeds, less abundant in the transgenic genotype with respect to the wild-type.

Acknowledgements

This work was supported by MIUR (Italian Minister of University and Research), project FIRB RBNE01TYZF Gene expression and accumulation of agronomically important proteins in the plant cell: transcriptional and post-transcriptional mechanisms.

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