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Poster Abstract – E.07

VERIFICATION OF THE PRINCIPLE OF SUBSTANTIAL EQUIVALENCE IN A TRANSGENIC BREAD WHEAT LINE OVER-EXPRESSING A LOW-MOLECULAR-WEIGHT GLUTENIN SUBUNIT BY MEANS OF A PROTEOMIC ANALYSIS OF ENDOSPERM PROTEINS

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Recent efforts to increase the quantity of specific wheat gluten proteins, which are directly correlated with the quality of end-use products, have focused on the introduction of additional gene copies by means of genetic engineering. With the aim of improving the quality traits of wheat-derived end products, we have produced and characterized a transgenic bread wheat line over-expressing a LMW (low-molecular-weight) glutenin subunit.

In order to define the consequences of transgene(s) insertion/expression and the effects of genetic transformation on the global abundances of the various classes of endosperm proteins, we have carried out an extensive, comparative proteomic profiling between the seeds of the transgenic line with their corresponding non-transformed counterpart. The investigation of the consequences of this transgenic event on the global endosperm protein expression is also of particular interest in the context of defining the "substantial equivalence" of this particular transgenic wheat line, since major concerns of genetic modification regard the potential of unintended side effects, which could originate depending on transgene integration site(s), spatial and temporal expression and/or from its putative interactions within several pathways of plant metabolism.

Proteomic analyses showed that, during the seed development, several classes of endosperm proteins were significantly differentially regulated. As a result of the high over-expression, and subsequent accumulation, of the transgenic LMW glutenin subunit, HMW (high-molecular-weight) glutenins and all sub-classes of gliadins were heavily down-regulated during seed filling in the transgenic genotype. Similarly, we also had evidences that CM-like proteins, a class including several components involved in food allergy to wheat, are less abundant in the transgenic genotype with respect to the wild-type. We also investigated the relative abundances, in developing endosperms, of the metabolic protein fraction, which showed only minor differences between the transgenic and the wild-type genotype. Protein identification of differentially expressed spots is currently underway, using a combination of N-terminal sequencing and MS.

In all protein classes, the differential expression detected by proteomic analyses, both in mature and developing seeds, had also been suggested by the corresponding transcript abundances assessed by comparative microarray experiments (Scossa et al., SIGA 2005).

In our opinion, this particular transgenic bread wheat line does not seem to contradict the principle of substantial equivalence, at least for that regarding the development of novel allergenic polypeptides in the endosperm, although showing significant transcriptional and proteomic

differences. Such differences, in fact, do not involve polypeptides implicated in differential allergenic properties with respect to its comparator. In conclusion, the global magnitude of the differential expression observed in this transgenic bread wheat line seems not greater than the natural variability usually observed in the cultivars obtained by traditional breeding methods.