Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 9.05

CLONING AND CHARACTERIZATION OF THREE HOMOEOLOGOUS WHEAT PDI-LIKE GENES LOCATED ON GROUP 5 CHROMOSOMES

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Gene structure, gene promoter, Triticum, protein folding, wheat quality

PDI and PDI-like proteins are responsible for multiple metabolic functions, including secretory protein folding, chaperone activity and redox signalling. In plants the proteins of the PDI family cluster into eight phylogenetic classes, five of them include proteins with two thioredoxin (TRX)-like active domains, whereas the other three classes own a single TRX-like domain. The first class includes the typical PDI, which in cereals may be involved in the folding of secretory proteins during the formation of endosperm protein bodies. The three homoeologous genes coding for the typical PDI and their promoter sequences had previously been isolated and characterized. Their exon/intron structure is highly conserved and includes 10 exons. Recently we reported the characterization of the whole set of nine non-homoeologous PDI-like gene sequences of wheat; since phylogenetic analysis assigned them to the eight plant subfamilies, at least one wheat gene has been cloned for each group. In this study we report the characterisation of the genomic and cDNA sequences of three homoeologous PDI-like genes (TaPDIL5-1A, TaPDIL5-1B and TaPDIL5-1D) of the V phylogenetic group and located in chromosome arms 5AL, 5BL and 5DL of hexaploid wheat. The coding region and the exon/intron structure, consisting of nine exons, of their genomic sequences were highly conserved. The most relevant differences were detected in the length of the second (1393 bp in TaPDIL5-1B, 1076bp in TaPDIL5-1A and 1026 bp in TaPDIL5-1D) and fifth (967 bp in TaPDIL5-1D, 723 bp in both TaPDIL5-1A and TaPDIL5-1B) introns. Their ORFs consisted of 1323 bp, corresponding to polypeptides of 440 aa, with an estimated Mw of 47.2 KDa and pI of 5.3. The three encoded proteins possessed two tandem TRX active domains (***a°-a),*** each containing the typical tetra-peptide site -CGHC-, an inactive TRX ***b*** domain at its C-terminus, the signal peptide and a modified NDEL signal for retention in the ER. The comparison of the wheat sequences with the PDI-like genes of the V phylogenetic group from *Ararabidopsis*, rice and the moss *Physcomitrella patens* revealed a high level of conservation of their structural features, in terms of intron pattern and exon number, size and position of the active sites. The promoter sequence of the PDI-like gene located in 5A chromosome of bread wheat cv Chinese Spring was cloned using the inverse PCR (IPCR) tecnique. The sequence analysis showed that the fragment cloned by IPCR included about 1400 bp located upstream of the coding sequence. The promoter sequences of the PDI-like genes located in 5B and 5D chromosomes of C. Spring were cloned through PCR amplification using two primer pairs. One of the primers, the same for both pairs, was designed on the basis of a sequence in the distal region of the previously cloned promoter of the 5A chromosome, whereas the second specific primer of each pair was chosen within regions of the

second intron. The search of cis-acting regulatory elements within the promoters of the three genes was performed using the databases of plant promoters PlantCARE and PLACE and the differences between the three sequences will be discussed. Finally, transgenic durum wheat lines (cv Svevo) over-expressing the gene located in 5A chromosome, which was put under the control of the ubiquitin promoter of maize, were produced for the functional characterization of this gene. PCR analyses of 40 plants regenerated from 800 bombarded embryos showed that 5 of them were transgenic for the PDI over-expression construct.