

## **PROTEIN DISULFIDE ISOMERASE GENE FAMILY IN WHEAT: GENOMIC STRUCTURE, PHYLOGENETIC AND EXPRESSION ANALYSES**

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PDI and PDI-like proteins are responsible for multiple metabolic functions, including secretory protein folding, chaperone activity and redox signalling. Most studies on their diversified metabolic roles have been carried out in mammals, whereas in plants the knowledge on the structural and functional features of these proteins and of their encoding genes is much less extensive. The purpose of the present research was the cloning and characterization of the genes encoding PDI and PDI like proteins in bread wheat and the comparison of their structure and expression with those of homologous genes isolated in other plant species. Former studies in wheat and other cereal species had been restricted to the genes encoding the typical PDI, which may accomplish an important role in the folding and deposition of seed storage proteins. Since wheat flour quality is strongly affected by composition and structure of seed storage proteins, the potential involvement of the PDI and of some PDI-like proteins of wheat in the seed storage protein folding and in the formation of intra- and inter-molecular disulfide bonds makes their study particularly interesting.

Fourteen wheat cDNA sequences of PDI-like genes were amplified and cloned; eight of them were relative to distinct PDI-like genes, whereas six corresponded to homoeologous sequences. Also the genomic sequences of the eight non-homoeologous genes were amplified and cloned. Phylogenetic analyses, which included the eight PDI-like genes cloned in this research and the typical PDI gene, assign at least one of them to each of the eight major clades identified in the phylogenetic tree of the PDI gene family of plants. Although not probable, the presence of additional wheat genes of the PDI family can not be ruled out. The genes of the wheat PDI family were located in chromosome regions syntenic with the chromosome locations of their rice homologs, confirming their close syntenic relationships. Within the same phylogenetic group a high level of conservation, in terms of sequence homology, genomic structure and domain organization, was detected between the wheat sequences and those of the compared plant species. The wheat proteins of five groups (I-V) have two thioredoxin-like active domains and show structural similarities to the corresponding proteins of higher eukaryotes, whereas those of the remaining three groups (VI-VIII) contain a single thioredoxin-like active domain. Phylogenetic analysis showed that the complete set of PDI and PDI-like genes was already present in *P. patens* and that extended phenomena of duplication events have characterized the evolution of this gene family in different plant taxa. The comparison of the exon/intron structure showed a very similar genomic organization across the analysed species, including *P. patens*, whereas the alga *C. reinhardtii* showed a different intron/exon structure. The high conservation level of sequence and genomic organization within the

PDI gene family, even between distant plant species, might be ascribed to the key metabolic roles of their protein products. The expression analysis of the nine non-homoeologous wheat genes, which was carried out by quantitative real time RT-PCR (qRT-PCR) in a set of 29 samples including tissues, developmental stages and temperature stresses, showed their constitutive, even though highly variable transcription rate. The comprehensive structural and expression characterization of the complete set of *PDI* and *PDI*-like genes of wheat performed in this study represents a basis for future functional characterization of this gene family in the hexaploid context of bread wheat.