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ISOLATION AND CHARACTERIZATION OF APR (ADENOSINE 5' - PHOSPHOSULFATE REDUCTASE) AND APR-LIKE GENES IN WHEAT

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The thioredoxin (TRX) superfamily includes redox proteins sharing a common structural motif named thioredoxin domain or "thioredoxin fold" with a consensus catalytic site sequence CxxC/S. The secondary structure of TRX domain consists of 5 β-strands surrounded by α-helices forming a central pleated β-sheet. The plant TRX superfamily is extremely more complex than in other eukaryotes. Phylogenetic analysis of 104 Arabidopsis protein sequences containing TRX domains formed five major clades, consisting of subfamilies with putatively distinct functions: thioredoxins, glutaredoxins (GSXs), protein disulfide isomerases (PDIs), peroxiredoxins and ferredoxins. The major clade containing PDI and PDI-like proteins included also two groups having different enzymatic activities. One of these consisted of two proteins of the quiescin-sulfhydryl oxidase (QSOX) family, which associate an oxiding TRX domain with an FAD containing ERV domain. The other group included seven proteins, three of them containing a TRX domain and a domain responsible for adenosine 5'- phosphosulfate (APS) reduction. These three proteins belong to the adenosine 5'- phosphosulfate reductase (APR) family, including key enzymes for plant sulfate assimilation through reduction of APS to sulphite. The four remaining APR-like proteins showed significant homologies and structural similarities with the TRX domains of APR but lacked the APS reductase domain.

The aim of this study was the cloning and characterization of APR and APR-like genes in wheat. A BLAST search of the DFCI Wheat Gene Index (TaGI, version 12) database using the seven available sequences of *APR* and *APR*-like genes of Arabidopsis and the six sequences of rice fetched four distinct contigs (TC, tentative consensus sequences), which were used for cloning the full-length cDNAs of four non-homoeologous APR and APR-like wheat genes: *TaAPRL1*, *TaAPRL3*, *TaAPRL5* and *TaAPRL6*. Southern and PCR analyses of DNA from Chinese Spring and its nulli-tetrasomic lines showed that the three homoeologous sequences of the four genes were located in the following chromosome homoeologous groups: 1) *TaAPRL1,* group 2; 2) *TaAPRL3*, group 3; 3) *TaAPRL5,* group 5*; 4) TaAPRL6,* group 7. The search in different protein databases for conserved motives in the deduced amino acid sequences of the four non-homoeologous genes revealed significant structural differences between the protein encoded by *TaAPRL1* and the three remaining APR-like proteins. TaAPRL1 (460 aa) was the largest among the APR-like proteins identified in wheat (TaAPRL3: 317 aa; TaAPRL5: 300 aa; TaAPRL6: 300 aa) and, in addition to a C-terminal TRX domain, it possessed the domain responsible for APS reduction and a chloroplast/plastid transit peptide sequence at the N-terminus. The remaining three APR-like proteins showed a high level of conservation of their structural features, in terms of both size and domain composition. They had a central TRX domain, a C-terminal trans-membrane segment and an N-terminal signal peptide. The structural analysis of the deduced amino acid sequences of the

four isolated genes suggests that only TaAPRL1 (the true wheat APR protein) is involved in the sulfate assimilation pathway, whereas the other three proteins (APR-like) may be implicated in redox reactions within the secretory pathway. Genomic and cDNA sequences of the three homoeologous *TaAPRL1* genes were cloned, sequenced and the most relevant differences are discussed.