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ARTICLE

Transcriptional changes in ascorbate-glutathione cycle under drought conditions

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ABSTRACT Ascorbate-glutathione cycle has an important role in defensive processes against oxidative damage generated by drought stress. Changes in expression patterns, subjected to reduced amount of irrigation solution, of ascorbate peroxidase (APX), monodehydroascorbate reductase (MDAR) and dehydroascorbate reductase (DHAR) as enzyme families of this cycle were studied comparing a drought tolerant (*Triticum aestivum* cv. Plainsman V) and a drought sensitive (*Triticum aestivum* cv. Cappelle Desprez) wheat genotype. Relative transcript level of isoenzymes localized in distinct subcellular organelles showed significant differences between the two genotypes at the beginning of treatment. Among APX isoenzymes, a thylakoid-bound (tAPX), two stromal (sAPX1, sAPX2), one of the two cytosolic (cAPX1) and a peroxisomal (mAPX) APXs displayed higher relative transcript level in the drought tolerant genotype. The same was observed in case of cytosolic (cDHAR) and stromal (sDHAR) DHARs. However, relative transcript levels of MDHAR isoenzymes were similar in both genotypes. Under drought conditions, the initial relative transcript levels of distinct isoenzymes changed differently comparing the two genotypes leading to the final conclusion that the drought tolerant genotype up-regulates mostly the cytosolic APXs and MDARs to maintain the cellular ascorbate redox state, however in the drought sensitive genotype, sAPX and sDHAR are induced to fill the same function.
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A continuous oxidative attack on plants during drought stress leads to the presence of an arsenal of enzymatic and non-enzymatic plant antioxidant defenses to resist the oxidative stress in higher plants (Shao et al. 2008). Ascorbate is one of the main antioxidants in plants; its presence is important in many physiological processes including protection of the cell against H_2O_2 and other toxic derivatives of oxygen. Therefore, maintenance of available amount of reduced ascorbate is fundamental in the plant defense system. In ascorbate-glutathione or Halliwell-Asada cycle (Fig. 1) ascorbate peroxidase (APX) is the reduced ascorbate consuming enzyme in reduction of H_2O_2 to water during oxidative stress. The oxidized ascorbate forms (monodehydroascorbate and dehydroascorbate) are recycled to reduced ascorbate via two pathways catalyzed by monodehydroascobate reductase (MDAR) and dehydroascorbate reductase (DHAR), respectively, consuming NAD(P)H and reduced glutathione as electron donors. Ascorbate is present in most cellular compartments together with ascorbate-glutathione cycle enzymes which can be classified according to their subcellular location. Several classifications of APX family are well-known depending on the examined species (rice Teixeira et al. 2006; *Arabidopsis* Panchuk et al. 2005) but generally we can distinguish cytosolic (cAPX), chloroplastic (chlAPX) and peroxisomal (mAPX) APXs, furthermore chlAPXs can be divided into two subclasses, namely thylakoid-bound (tAPX) and stromal (sAPX) APXs. In addition, MDAR activity has been described in several plant cell compartments such as chloroplast (sMDAR), mitochondria and peroxisomes (mMDAR) and cytosol (cMDAR) (Eltayeb et al. 2007). DHAR isoenzymes can be found in cytosol (cDHAR), chloroplast (sDHAR) and mitochondria (Mittler et al. 2004). The aim of this study was to determine the participation of isoforms of APX, MDAR and DHAR enzyme families in water stress tolerance of wheat comparing a drought tolerant and a drought sensitive genotype.

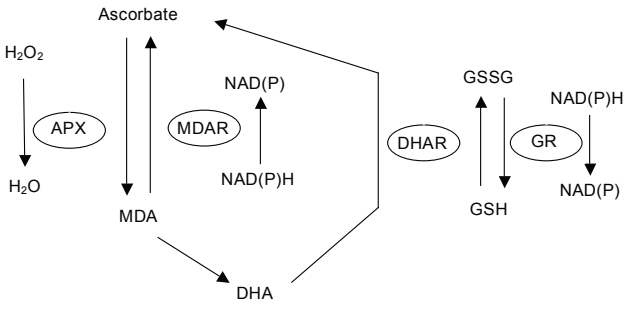

Figure 1. Scheme of ascorbate-glutathione cycle. Abbreviations: MDA: monodehydroascorbate; DHA: dehydroascorbate; GSH: reduced glutathione; GSSG: oxidized glutathione; GR: glutathione reductase.

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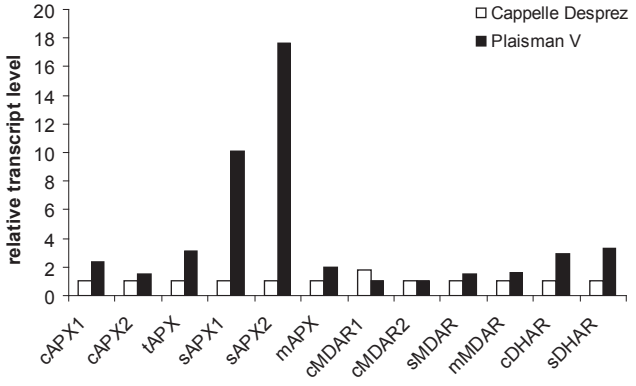

Figure 2. Pairwise comparison of initial relative transcript levels of ascorbate peroxidase (APX), monodehydroascorbate reductase (MDAR) and dehydroascorbate reductase (DHAR) gene groups in the two wheat genotypes.

Materials and Methods

Plant material was obtained from the experiment described by Secenji et al. (2005). RNA isolation, cDNA synthesis and real-time qPCR were done the same way as written in the above mentioned paper except the reference gene during real-time qPCR was 18S rRNA. Nucleotide sequences of wheat *APX*s, *MDHAR*s and *DHAR*s were selected according to BLAST searches against GenBank and TIGR (Plant Transcript Assemblies) databases. Phylogenetic trees were composed from 273 (APX), 315 (MDAR) and 200 (DHAR) amino acid long sequences. Group specific primer pairs were designed to detect relative expression level of certain groups.

Results and Discussion

Ascorbate-glutathione cycle plays a key role in defense system against oxidative stress. Our aim was to examine this cycle in details in shoot tissue looking for subcellular isoforms of APX, MDAR, DHAR enzyme families which are involved in stress defense by detection of their gene expression level subjected to water stress.

Examining the *APX* gene family, 23 nucleotide sequences were found using BLAST search against TIGR wheat database followed by their classification into six groups according to multiple alignment of their ORFs. At the beginning of the treatment, Plainsman V showed significantly higher relative expression levels in case of *cAPX1*, *tAPX*, *sAPX1*, *sAPX2* and *mAPX* than the drought sensitive genotype. During the treatment, up-regulations were detected in relative expression levels of *cAPX1* and *cAPX2* in Plainsman V, while in Cappelle Desprez *cAPX1* and *sAPX2* showed increased relative transcript level. Furthermore, *sAPX* isoform was down-regulated in Plainsman V however, its total relative transcript level was still higher than in Cappelle Desprez.

Analyzing the *MDAR* gene family, 10 nucleotide sequences were found in TIGR wheat database which were grouped into four classes according to multiple alignment of their ORFs. In contrast to APX isoenzymes, none of the MDAR isoforms displayed distinct relative transcript level comparing the two genotypes at the beginning of the treatment. In addition, only *cMDAR2* showed significant up-regulation in both genotypes emphasizing that up-regulated expression level of *sMDAR* did not follow the increased transcript level of *sAPX2* in Cappelle Desprez.

Examining *DHAR* gene family, 9 nucleotide sequences were found in TIGR wheat database collecting them into two groups according to multiple alignment of their ORFs. Similar to APX isoforms, the two isoenzymes of DHAR family showed higher relative transcript level in drought tolerant genotype at the beginning of the treatment however, chloroplastic *sDHAR* was up-regulated during the treatment only in Cappelle Desprez showing similar relative transcript level than the initial one in Plainsman V.

Comparing the two genotypes, Plainsman V as a drought tolerant genotype has higher initial transcript level of isoforms from APX and DHAR families (Fig. 2) nevertheless, it up-regulates only cytosolic *APXs* and *MDARs* under drought conditions. However, Cappelle Desprez as a drought sensitive genotype has less initial transcipts from isoforms of APX and DHAR enzyme families completed with up-regulated gene expression of chloroplastic *APXs* and *DHARs* which cell compartment is the nest of oxidative stress.

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