

Keywords: APX; MDHAR; DHAR; SOD; GR;  ROS

Introduction

The present study was aimed to examine *in silico* sequences concerned in plant stress defence mechanism were analysis of promoters of (APX), superoxide dismutase (SOD), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione peroxidase (GR) sequences present up to 1000 bp upstream region and evolutionary relationship of major ascorbate glutathione pathway genes of *Oryza sativa* involve plant defence responses against plant stress tolerance.

Materials and Methods

Database search

The sequences of rice genes that are involved in the ascorbate glutathione pathway such as Ascorbate peroxidase (APX), Monodehydroascorbate reductase (MDHAR), Dehydroascorbate reductase (DHAR), Super oxide dismutase (SOD) and Glutathione reductase (GR) and their upstream regions were retrieved from NCBI database (<http://www.ncbi.nlm.nih.gov>).

Identification of 5' regulatory region of antioxidant genes involved in ascorbate glutathione pathway in rice genome

All the upstream nucleotide sequences and coding domains

of *Oryza sativa* genome using Basic local alignment tool (<http://blast.ncbi.nlm.gov/blast/cgi>) Locus link was used to identify genomic sequences of 1 kbp extending 5' from the translation start site of each antioxidant gene family involved in plant stress defence mechanism. These sequences were used for the computational analysis.

Cis acting regulatory element analysis (CAREs)

1.0 kbp of 5' upstream region of each antioxidant gene family involved in plant stress defence mechanism were scanned for the presence of putative cis-regulatory element with registered in Plant CARE (<http://bioinformatics.psb.ugent-be/webtools/plantcare/html/>) and PLACE (<http://www.dna.affrc.go.jp/PLACE/signalscan.html>) tools. The details of sources of the upstream sequences of antioxidant protein/ enzymes encoding genes of *Oryza Sativa* are given in Table 1.

Evolutionary relationship analysis

For evolutionary relationship, maximum parsimony trees for all the protein sequences were created using Molecular Evolutionary Genetics Analysis Version 6.0. The relationships between adjacent nodes were based on bootstrap support from 5000 replicates. The number indicated percentages against each node.

In vitro analysis

Plant leaves were collected and treated with sterile solutions of 5.2 mM Methyl Viologen (MV), 5.2 mM H₂O₂, 5.2 mM ethephon (ET) and 5.2 mM salicylic acid (SA), for 12 h. Each treatment was performed in triplicate. Total cellular extracts were prepared and used in activity assays. The activities of APX, GR and SOD were determined through spectrophotometer.

Results

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