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Expression Pattern of Superoxide Dismutase Under Drought Stress in Maize.

Kaliyugam Shiriga[1, 2], Rinku Sharma[1], Krishan Kumar[2], Shiv Kumar Yadav[3],Firoz Hossain[1] and NepoleanThirunavukkarasu[1*]

Division of Genetics, Indian Agricultural Research Institute, New Delhi – 110012, India[1]

School of Life Sciences Singhania University, Rajasthan – 333515, India[2]

Division of Seed Science and Technology, Indian Agricultural Research Institute, New Delhi – 110012, India[3].

[*]Corresponding author

Abstract: Superoxide dismutase (SOD) is one of the major classes of antioxidant enzymes, which protects the cellular and subcellular components against harmful reactive oxygen species. In maize, three types of SODs are present based on their constituent metal ions, namely Cu/Zn-SOD, Mn-SOD and Fe-SOD. We investigated the effect of water stress on SOD isozymes in two contrast maize genotypes, tolerant and susceptible. We found Mn-SOD and Fe-SOD with production of more transcriptomes in the tolerant genotype than susceptible. Cytosolic SOD had heightened expression levels in the tolerant genotype. The expression analyses of cytosolic SOD, Mn-SOD and Fe-SOD would be used as candidate genes for the development of drought tolerant maize.

Keywords: Maize, superoxide dismutase, water stress, qRT-PCR

I. INTRODUCTION

Plants are often exposed to abiotic stresses such as drought, extremes of temperature, and salinity, which reduces yields up to 70% worldwide. Drought is one of the major environmental stresses limiting plant growth, and consequently reduced crop yield [1]. Plants have developed various mechanisms such as modulating the expression of stress tolerance genes and synthesizing compatible solutes [2] to cope with the oxidative stresses caused by unfavourable environments. Antioxidant defense systems are well known for scavenging reactive oxygen species (ROS) produced in different stressful conditions, such as activation of the antioxidant enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and peroxidase (POD) [3,4]. The scavenging capacity of superoxide radicals (O_2^-) is achieved through an upstream enzyme SOD, which catalyses the dismutation of superoxide to hydrogen peroxide (H_2O_2). POD reduces H_2O_2 to water using various substrates as electron donors.

SODs are encoded by a small multigene family and classified into three types based on the metal found in the active site: Mn-SOD, Fe-SOD and Cu/Zn-SOD. In *Arabidopsis*, the SOD family includes cytoplasmic CSD1, chloroplast CSD2, and peroxisomes CSD3, plastidic FSD (*FSD1*, *FSD2 and FSD3*) and mitochondrial MSD1 [5]. SODs constitute the first line of defence against reactive oxygen species (ROS). SODs belong to a large and ubiquitous family of metallo-enzymes in aerobic organisms [6]. In previous studies, different types of SOD showed distinct expression patterns [7] to various stress such as chilling injury [8], oxidative stress and H_2O_2, and temperature [9]. The present investigation is an attempt to analyze the expression of maize metal ionic SODs by qRT-PCR in tolerant and susceptible genotypes to be identified oxidative responsive SODs for the production of tolerant cultivars to reactive oxygen species (ROS).

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II. MATERIALS AND METHODS

A. Plant materials, treatments, and collection of tissues

Two maize inbred lines, HKI532 (drought tolerant) and HKIPC3-3 (drought susceptible) were grown under natural conditions and they were watered daily to field capacity till the stress initiation. Drought stress was imposed on plants at three-leaf stage by withholding water for seven days, while the control plants were irrigated daily to field capacity (Figure 1). Samples for RNA extraction were collected from both the genotypes at two different time periods: on the day the treatment began and seven days later, when the treatment was terminated.

B. RNA isolation, DNase I treatment, and cDNA synthesis

Total RNA was isolated from the harvested samples using Qiagen RNeasy columns (Qiagen, Hilden, Germany) followed by DNase I treatment to remove any contamination with genomic DNA. Concentrations were determined with a Thermo Scientific NanoDrop 1000 spectrophotometer (Thermo Scientific, Delaware, USA). The cDNA was synthesized using ProtoScript first strand cDNA synthesis kit (BioLabs, Massachusetts, USA) from 1 µg of total RNA according to the manufacturer's protocol. The reverse transcription reaction was carried out at 44°C for 60 min followed by 92°C for 10 min.

Fig. 1. Response of HKI532 (drought tolerant) and HKIPC3-3 (drought Susceptible) genotypes to well-watered control and severe drought stages.

C. Quantitative real-time PCR analyses

Gene-specific primers (Table 1) were designed using IDT PrimerQuest (http://www.idtdna.com/scitools/applications/primerquest/default.aspx) and a 18s RNA primer was used as the internal control.

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TABLE I
THE PRIMER SETS USED FOR STUDYING THE SOD GENE EXPRESSION ANALYSIS.

S. No.	Gene name	Primer Sequence	Primer T_M
1	Zm Cytosolic SOD F	CGTGTTGCTTGTGGGATCATTGGA	59.6
	Zm Cytosolic SOD R	TCGGTGGCTACAGGTGCATAATGA	59.9
2	Zm Cu-Zn SOD F	TGTTGCAAATGCTGAGGGCATAGC	60.2
	Zm Cu-Zn SOD R	CCAACAACACCACATGCCAGTCTT	59.8
3	Zm FeSOD F	AGCACAGGTCTGGAACCATCACTT	60.1
	Zm FeSOD R	ACAGCTGTAAGGCTGAGCGGATAA	59.9
4	Zm MnSOD F	TTGTGTACCTGCTGGACCAAGTGT	60.2
	Zm MnSOD R	ACTACGAGCAGCAGAAAGTGGAGT	59.7

Dissociation curve testing was carried out for each primer pair that showed only one melting temperature for all samples. The qRT-PCR reactions were carried out at 95°C for 5 min followed by 40 cycles of 95°C for 15s, 60°C for 30s and 72°C for 1 min. Expression data for the SOD genes were normalized by subtracting the mean reference gene CT value from individual CT values of the corresponding target genes (ΔCT). The fold-change value was calculated using the expression $2^{-\Delta\Delta CT}$ where ΔΔCT is the difference between the ΔCT condition of interest and ΔCT control.

III. RESULTS AND DISCUSSION

A. *Expression of SOD genes*

Reactive oxygen species (ROSs) are produced under stress conditions of plant cells. SOD catalyzes the first step in the ROS scavenging system. The response of metal ionic SOD gene expression analysis was done under water deficit condition using qRT-PCR compared with normal condition in contrasting maize genotypes. Plants were subjected to water stress by withholding water supply and the expression of SOD was characterized during stress induction. ROS generated in stress conditions leads to Membrane damage, chlorophyll damage and leaf bleaching due to superoxide, this oxidative damage controlled by Mn-SOD [10]. The expressions of Mn-SOD gene was strongly induced intolerant than susceptible at the drought stress treatment. This result suggests that expression of the Mn-SOD could protect tolerant genotype upon drought stress. While Fe-SOD gene was up regulated to some extent by drought treatment, the chloroplast Cu/Zn-SOD gene was not induced by drought treatment (Figure 2).

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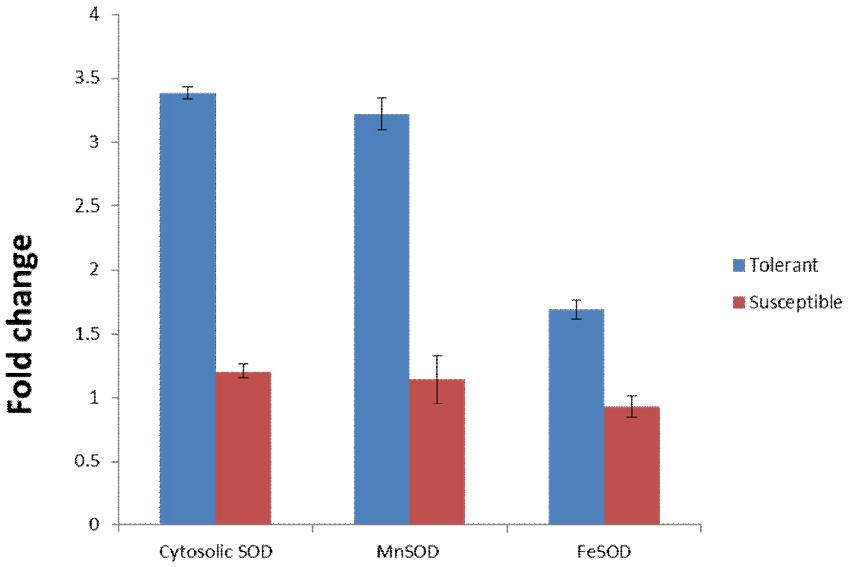

Figure 2. Differential regulation of superoxide dismutase isoforms in maize leaves under water stress condition.

In the tolerant genotype, increased number of Mn-SOD, Cytosolic SOD transcripts were associated with protection of the plant against oxidative stress. Wang *et al*., has reported that in presence of Mn-SOD drought tolerance was in increased in transgenic Rice. SOD can protect PS II from superoxide generated by oxidative and water stress [11]. Our data also suggest that higher expression Mn-SOD, Fe-SOD can protect the photosynthetic apparatus via protecting the relevant proteins and lipids, leads to tolerance under drought stress. These protective mechanisms appeared to reach a limited magnitude at severe water stress intensities, may be due to the possible alteration of the expression of other genes associated with stress tolerance when SOD activity increases or to the involvement of excessive oxidative agents. On the contrary, the susceptible genotype has shown limited expression of Mn-SOD and/or Fe-SOD isoforms, which showed their sensitivity to water stress.

IV. CONCLUSION

Mn-SOD and Fe-SOD were expressed more in the tolerant genotype than the susceptible. Cytosolic SOD had highest triggered under water stress condition in the tolerant genotype confer protection from oxidative stress, while chloroplast Cu/Zn-SOD expression was not affected. The differential regulation of SOD isoforms under water deficit conditions makes these enzymes as biochemical indicators of oxidative stress resulting from unfavorable environmental conditions. The gene expression study in contrasting genotypes leads to greater understanding of SODs roles under water stress.

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