

Changes in Activity of Antioxidative Enzymes in Leaves of Zea mays under Heat Stress Condition

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Abstract: Heat stress is one of the major abiotic stresses affecting agriculture production worldwide which causes an array of physiological, biochemical and morphological changes in plants. Maize crop is unique among cereals on account of wider flexibility and adaptability in growing conditions, ranging from Kharif, Rabi and Spring seasons. Further, the response of plants to high temperature stress is reflected in apparent morphological traits like leaf firing, tassel blasting etc. The composite varieties in general, are known to be better equipped to sustain wider temperature differences, due to their inherent genetic constitution, and plants with typical contrasting symptoms can easily be identified. This study was carried out to analyse the effects of heat stress on soluble protein, free amino acids, total antioxidants, catalase (CAT), and peroxidase activities in six maize genotypes from the Pusa Composites-3 and 4, during spring season-2014 planted in IARI field at Delhi. Heat stress resulted in significantly increased levels of soluble protein content, catalase and peroxidase. However these increases were relatively high in tolerant lines compared to susceptible lines. Results showed that antioxidants act as major defense against radical mediated toxicity by protecting the damage caused by free radicals. Plants with high levels of antioxidants have been reported to have greater resistance to oxidative damage, and thus, the results are found to be informative and interesting.

Keywords: Heat, catalase, peroxidase, peroxidise, Maize.

INTRODUCTION

Heat stress causes an array of physiological, biochemical and morphological changes in plants, which affect plant growth and development. Heat stress is one of the major abiotic stresses affecting many biological processes, including agriculture productivity worldwide. Abiotic stress is the major factor that affects productivity of plants. Heat stress often causes adverse alterations in plant growth, development, physiological processes, and lead to reduction of yield. Plant responses to heat stress vary with the degree of temperature, duration and plant type. At extreme heat stress, cellular damage or cell death may occur within minutes, which may lead to a catastrophic collapse of cellular organization [1].

Heat stress affects all aspects of plant processes like germination, growth, development, reproduction and yield [2, 3]. One of the major consequences of heat stress is the excess generation of reactive oxygen species (ROS), which leads to oxidative stress [4, 5]. A plant is able to tolerate heat stress up to some level by physical changes within the plant body and frequently by creating signals for changing metabolism. Plants alter their metabolism in various ways in response to heat stress, particularly by producing compatible solutes that are able to organize proteins and cellular structures, maintain cell turgor by osmotic adjustment, and modify the antioxidant system to re-establish the cellular redox balance and homeostasis [6, 7]. The excess electrons

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are transferred to oxygen molecules, thus causing the accumulation of toxic reactive oxygen species (ROS) like superoxide radical, hydrogen peroxide, hydroxyl radical, alkoxyl radical and singlet oxygen [8, 9]. Purpose of the present study was to contribute to a better understanding of the physiological responses of specific genotypes from two Pusa Composite maize cultivars to heat stress. We investigated the influence of heat stress on the contents of proteins, catalase, peroxidase and free amino acids in the maize plants under heat stress.

MATERIAL AND METHODS

Two Pusa composite maize cultivars and component maize genotypes constituting them were grown in spring season 2014. Six Maize genotypes were selected from the Pusa Composites-3 and 4, planted in IARI field at Delhi, on the basis of distinct responses to heat stress during flowering stage. All maize plants were grown in the same farm and in the same natural environment. Leaf samples were harvested and immediately frozen in liquid nitrogen. Enzymes were extracted from maize leaves following the standard protocols as outlined below.

SOLUBLE PROTEINS

Soluble proteins were estimated according to Bradford *et al.*, 1976[10]. 0.2g of plant sample was homogenized in 2ml of phosphate buffer pH 7.0 and centrifuged at 12,000g for 15min. 50 μ l of supernatant 950 μ l water was incubated with 5ml Bradford reagent for 5min and absorbance was measured at 595 nm. Protein was calculated by BSA standard.

ESTIMATION OF TOTAL FREE AMINO ACIDS

Free amino acids were extracted and estimated according to Moore and Stein, 1954[11]. 100 mg of plant material was homogenized in 5ml of 80% ethanol and centrifuged at 8000 rpm for 10min. 100 μ l of supernatant was diluted up to 1ml with ethanol and 2ml Ninhydrin reagent was added. Mixture was kept for 30 min in hot water bath at 95°C. Reaction was terminated in ice and absorbance was taken at 570 nm. Total free amino acid was calculated by plotting standard glycine.

CATALASE

Catalase activity was measured by the method of Aebi, 1974[12]. 0.1 ml supernatant was added to cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). Reaction was started by the addition of

1.0 ml of freshly prepared 30 mM H₂O₂. The rate of decomposition of H₂O₂ was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of catalase was calculated by extinction coefficient and expressed as units/mg protein.

Ascorbate peroxidase

Ascorbate peroxidase activity was estimated by observing the decrease in absorbance due to ascorbic acid at 290 nm [13] 0.1 ml of enzyme extract was added to the 3 ml reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 1 mM EDTA and 0.1 mM H₂O₂. The reaction was started with the addition of 0.1 mM hydrogen peroxide. Decrease in absorbance for a period of 1min was measured at 290 nm in a UV visible spectrophotometer. Activity is expressed by calculating the decrease in ascorbic acid content by extinction coefficient.

RESULT AND DISCUSSION

The composite varieties in general, are known to be better equipped to sustain wider temperature differences, due to their inherent genetic constitution. When subjected or exposed to high temperature, maize plants exhibit usually distinguishable responses. For example, tassel Blasting and flag leaf drying are the prominent features which are easily identified. On the other hand tolerant plants either do not exhibit such responses or may show milder symptoms. Hence maize genotypes comprising of plants with typical contrasting symptoms can easily be identified and subjected to further analysis. Catalase activity in tolerant lines were relatively high, ranging from 40.19 to 45 mM/min/mg protein in comparison to susceptible lines which had lower values (from 16.5 to 18.79 mM/min/mg protein). Maximum high free amino acid content was detected in susceptible lines (ranged from 10.38 to 10.66 mg/gm fresh wt) as compared to tolerant lines (ranged from 8.0 to 9.33 mg/gm fresh wt). Total soluble protein in tolerant lines ranged from 0.70 to 0.98 mg/gm fresh wt as compared to susceptible lines (ranged from 0.75 to 0.80 mg/gm fresh wt). Similarly peroxidase activity in tolerant lines ranged from 0.023 to 0.03 mM/min/mg protein as compared to susceptible lines (from 0.025 to 0.028 mM/min/mg protein). Increase in catalase and peroxidase activity is supposed to be an adaptive trait possibly helping to overcome the damage to the metabolism by reactive oxygen species and play important role in heat tolerance.

Table 1
Comparison of different parameters in six maize composite lines

S. No	Genotypes	Total protein (mg/gm fresh wt)	Catalase (mM/min/mg protein)	Peroxidase (mM/min/mg protein)	Free amino acid (mg/gm fresh wt)
1	MS1	0.750	16.500	0.028	10.383
2	MS2	0.800	16.866	0.027	10.667
3	MS3	0.807	18.799	0.025	10.583
4	MTT1	0.700	45.000	0.030	9.150
5	MTT2	0.986	44.447	0.023	9.333
6	MTT3	0.818	40.179	0.027	8.000

It is important to quantify the total protein for evaluated lines, in terms of the specific activity to each enzyme. The values of protein for each treatment are represented in Table 1. All genotypes had significant differences in their content in total protein content. Highest total protein content was detected in MTT2.

The H₂O₂ production is thought to be increased under various abiotic stresses in order to enhance gene expression of active oxygen scavenging enzymes [14]. The Table 1 represents differences in peroxidase activity of different maize lines. Maximum peroxidase activity was detected in MTT1 and lowest in MTT2. Even under normal growth conditions, many metabolic processes produce ROS in plants, such as superoxide (O₂⁻), hydroxyl radical (OH⁻) and hydrogen peroxide [15]. Meanwhile, plants possess efficient antioxidant defense systems for scavenging ROS [16]. Catalase and peroxidase are the major antioxidant enzymes in plant system. Plants with high levels of antioxidants, either constitutive or induced, have been reported to have greater resistance to this oxidative damage [15].

Reactive oxygen species play an important role in oxidative stress related to the heat stress. Antioxidants act as a major defense against radical mediated toxicity by protecting the damages caused by free radicals. Increase in catalase and peroxidase activity is supposed to be an adaptive trait possibly helping to overcome the damage to the tissue metabolism by reducing toxic levels of H₂O₂ produced during cell metabolism and protection against oxidative stress. Plants with high levels of antioxidants have been reported to have greater resistance to oxidative damage, and thus, the results are found to be informative and interesting. Further experiments that explore different biochemical and molecular approaches and agronomic management practices are needed to investigate the actual heat

stress responses and their effects on final crop yield. In the context of global climate change, occurrence of extreme climatic factors and uncertainties are believed to be increasing. Further rise in average temperature would also contribute to changes in cropping pattern. Maize is one of the few crops of high potentiality. Understanding heat stress tolerance mechanism and incorporation of such traits in to maize genotypes would further enhance the possibility of mitigation, adaptation and spread to newer environment in coming decades.

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