Effects of Drought on the Activity of Phenylalanine Ammonia Lyase in the Leaves and Roots of Maize Inbreds

Ashraf Gholizadeh

Research Institute for Fundamental Sciences (RIFS), University of Tabriz, Iran.

Abstract: In a basic investigation, the antioxidative responses of two maize inbreds, A-180 and A-619 were studied. The results revealed that during drought period the total antioxidant capacity is quickly increased in leaf and root tissues of both plants, while it is slowly reverted back to the normal level during recovery. The activity of Phenylalanine ammonia lyase (PAL), a key enzyme involved in the biosynthesis of isoperonoid antioxidative compounds, was found to be sharply increased in the leaves of plants during stress period, while it is remained constant in the roots of both plants. The overall results suggested that isoperonoid compounds may be more responsible antioxidants in drought-challenged antioxidative system of maize.

Key words: antioxidant, drought, stress, maize, FRAP, enzyme activity

INTRODUCTION

Oxidative burst “a rapid transient production of excessive amounts of reactive oxygen species (ROS) Such as superoxide anion $O_2^-$, hydrogen peroxide $H_2O_2$, singlet oxygen $O_2^*$ and hydroxyl radical OH” is one of the earliest observable aspects of a plant response to different types of environmental stresses (Baker, 1995. Wojtaszek, 1997). ROS toxicity in plant system may eventually cause membrane damages through the oxidation of polyunsaturated fatty acids or proteins that often lead to the cell dead (Blokhina, 2003). Alternatively, ROS may be followed by multiple responses in transcription, translation, protein activity, metabolic changes and possibly programmed cell death (Desikan, 2001. Lee, 2007; Rentel, 2004; Yoda, 2006; Van Breusegem, 2006).

In contrast, plants have evolved defense antioxidative mechanisms to combat the danger posed by ROS (Asada, 2006; Halliwell, 2006). Increased levels of antioxidants, involved in the detoxification process of ROS, have been found in resistant plants against various types of environmental stresses (Shao, 2007; Mittler, 2002).

Antioxidative system in plant system is grouped into two classes consisting of enzymatic and non-enzymatic types (Adam, 1995; Bestwick, 2001). The enzymatic antioxidation is carried out by a series of the redox enzymes generally catalyzing electron transfer to ROS using low molecular weight antioxidant compounds; e.g. ascorbate, tocopherol, glutathione, phenols and flavonoids as electron and proton donors (Shao, 2007; Mittler, 2002). Non-enzymatic antioxidation has been found to be carried out by some of the high molecular weight compounds mostly including a number of proteins that avidly scavenge ROS and protect the structures and functions of plants against oxidative damages (Okada, 1998). Usually, oxidative system is controlled / balanced by antioxidative system during the normal growth and developments in plant system. Alternatively, it is well controlled for the better cope of plants with their different environmental stress situations (Asada, 2006; Halliwell, 2006).

Drought stress is a major abiotic factor that limits agricultural crop production. Like to other environmental stresses, it gives rise to the formation of reactive oxygen species which is often followed by the activation of antioxidative system in plants (Zhang, 1994). Plant cells were found to be protected following the activation of antioxidative system. A close relationship between antioxidant activity and drought stress tolerance have been already identified in various plants such as cotton, wheat, pea, oak tree, pine tree, sunflower and very recently it has been reported in garden cress pepper weed plant (Fischer, 1976, Moran, 1994, Schwanz, 1996, Habibi, 2004, Ratayaka, 2003, Saleh, 2009). Although, plants commonly activate their total antioxidation machinery in response to drought stress situations, but it has been well shown that each plant exhibit a specific antioxidative pattern. For examples, in cotton and wheat plants, the activity of glutathione reductase activity has been found to be increased during drought stress (Fischer, 1976); in pea plant, drought stress leads to pronounced decreases in the activities of catalase, dehydroascorbate reductase and glutathio reductase enzyme activities, but it results in the increase of peroxidase, superoxide dismutase and monodehydroascorbate reductase activities (Moran, 1994); in pendunculate oak and maritime pine trees, the activities of catalase and peroxidase enzymes have been found to be reduced during drought period under ambient CO2, but their activities are increased under drought period at elevated CO2 concentrations (Schwanz, 1996); in sunflower the activities of superoxide dismutase, catalase and glutathione peroxidase is increased in plants subjected to drought stress (Habibi, 2004); in garden cress pepper weed, the activities of catalase and
peroxidase have been found to be reduced during drought stress period while superoxide dismutase and glutathione reductase activities have been shown to be increased (Saleh, 2009).

Maize is an important crop in the world and it is well characterized by the multiplicity of its agro-industrial uses. In many areas, the productivity of this crop is limited due to drought stress. The improvement of its productivity in such areas requires understanding the mechanisms of its drought tolerance.

The aim of this study was to determine the antioxidative status of the maize leaf and root tissues during drought stress shifting and evaluate their relative activity of phenylalanine ammonia lyase under drought stress.

**MATERIALS AND METHODS**

**Plant Growth and Treatments:**

The seeds of maize (inbred lines A-180 and A-619) were randomly (with no information about their drought resistance) provided by Dr. B. Baghban Kohnehrouz (plant genetic engineering laboratory, Department of Plant Breeding and Biotechnology, University of Tabriz, Iran). Seeds were surface sterilized using 1% NaOCl for 15 min and then rinsed three times in distilled water. Surface sterilized seeds were germinated and grown till seedling stage in petri plates. The seedlings with uniform sizes were transferred into experimental tubes containing 20 ml of modified Hoagland nutrient solution (Gholizadeh, 2007). They were allowed to grow under laboratory sun light condition (day to night period of 12h: 12h and humidity of about 65%). For drought stress treatment of the test plants, the nutrient medium was completely withdrawn for 24 h. The experimental materials were taken as leaf or root blades and processed for experimental assessments. After stress period, plants were reverted back to the normal non-stressed conditions and allowed for recovery. The leaf and root materials were harvested as blades from the same plant and processed for further experiments.

**Total Antioxidant Power Assay:**

The total antioxidant ability of the plant leaf and root materials was determined using ferric reducing antioxidant power (FRAP) assay of Benzie and Strain (1996). To 1 ml of plant extract in 0.1 M phosphate buffer (pH 7.0), 3 ml of FRAP reagent (10 mM TPTZ: tripyridyl triazine, 20 mM FeCl₃·6H₂O and 300 mM sodium acetate buffer (pH 3.6) in the ratio of 1: 1: 10) was added and the reaction mixture was incubated at 37°C for 4 min. The assessment was carried out spectrophotometrically at A 593. Antioxidant potential of samples was determined against the standard curve of ferrous sulphate (Fe, 100-1000 μM). Ascorbic acid (100 μM) served as a positive control and BSA considered as negative control. FRAP values were calculated as follows: FRAP value (μmol 100mg⁻¹) = A593 of test sample / A593 of standard) × FRAP value (μmol 100mg⁻¹) of standard. FRAP values of all test samples were presented as (μmol Fe⁰ per 100 mg tissue fresh weight).

**Enzyme Extraction:**

200 mg of leaf and root tissues were separately homogenized in 1 ml of 0.1 M phosphate buffer, pH 7.0 containing 0.5 μl of β-mercaptoethanol and a pinch of polyvinyl polypyrrolidone (PVP). The homogenate was centrifuged at 12,000 × g for 10 min and the supernatant was used for redox enzymes activity assay.

**Assay of Phenylalanine Ammonia Lyase:**

200 mg of leaf material was homogenized in 2 ml of 25 mm borat buffer, pH 8.8 containing 2 μl β-mercaptoethanol and a pinch of polyvinyl polypyrrolidone (PVP). The homogenate was filtered through the cheese cloth, centrifuged at 12,000 × g for 10 min and the supernatant was used for enzyme activity assay according to Sadisivam and Manickam (1992). One unit of the enzyme was defined as increase in absorbance of one unit per min. The activity of the enzyme was expressed as units per mg of soluble protein.

**Statistics:**

Data points in the figures represent the means ± SE of three individual treatments with two replicants per treatment. Variance analysis was analyzed by ANOVA at P≤0.05. Values followed by different Latin letters in each assay on the graphs are statistically different (P≤0.05).

**RESULTS AND DISCUSSION**

**Total Antioxidative Ability:**

Earlier studies have been shown that drought stress in plants induces the “oxidative burst” that is obviously followed by the activation of their antioxidative system (Zhang, 1994, Fischer, 1976, Moran, 1994, Schwanz, 1996, Habibi, 2004, Ratnayaka, 2003, Saleh, 2009). We conducted our studies on the antioxidative responses of maize plant maize plant, known as a rich source of redox potentials under drought condition. In order to determine the total antioxidative status of test tissues, the ferric reducing antioxidant power (FRAP) method was used (Benzie, 1996). FRAP test is known as a simple and reproducible method used for the assessment of the total antioxidative capacities from different sources.
The results showed that the total antioxidative capacity of A-180 leaves is sharply increased during stress period, so that the FRAP value is reached to 1.8 during stress period (Fig. 1). Data showed that during recovery period this ability is slowly fell down to the level of before stress stage. The total antioxidative status of the leaf tissues is reached to the level of before stress stage (~ 0.6 μmol Fe^{II} / 100 mg) after four days of recovering. Variation in the total antioxidative status of the maize A-619 leaves was also showed similar pattern to A-180 (Fig. 1, left panel). The FRAP value was sharply increased to 1.1, but it was slowly declined to the level of before stress stage (~ 4.4 μmol Fe^{II} / 100 mg) after four days of recovering. These results are in confirmation of already reported results in the case of gardencress plant (Saleh, 2009).

Similar patterns of total antioxidation capacity (increased activity during stress period followed by decreased activity during recovery) were also detected for root tissues of both inbred lines (Fig. 2, right panel). The maximum and minimum FRAP values for A-180 was found to be 1.3 and 0.5, respectively. In A-619, the related values were not significantly differed from those of leaf tissues. Comparison of data in two maize inbreds suggests that the leaf tissues of A-180 are more active than the root tissues.

**Activation of Phenyl Propanoid Biosynthetic Pathway:**

The enzyme phenylalanine ammonia lyase (PAL) catalyzes deaminating reaction of the amino acid phenylalanine at the gateway from the primary metabolism into the important secondary phenylpropanoid metabolism in plants (Legrand, 1976; Hahlbrock, 1989). Phenylpropanoid compounds not only fulfill various essential functions during plant development, but also they act as important protectants against various biotic and abiotic environmental stresses. The biosynthesis of PAL and accumulation of phenylpropanoid structures have been reported up on pathogenic attack including viruses, tissue wounding, UV irradiation, low temperatures, low levels of nitrogen, phosphate and iron (Dixon, 1995; Ritter, 2004; Gholizadeh, 2004).

An increase in the activity of PAL enzyme has been recently reported in the cases of winter triticale and a drought resistant maize genotype (Hura, 2008; Hura, 2007). Herein, we conducted our studies in two other maize genotypes. Our results showed that the activity of PAL has sharp peaks during one day stress period in the leaves of both test plants (Fig. 2, left panel). It is increased from 4.3 to 8.1 and 4.7 to 9.2 units during stress treatment in A-180 and A-619, respectively. Despite this, the variation pattern in PAL activity differs in both inbreds during recovery. In contrast to stress period, the activity of PAL was found to be slowly declined in the leaf tissues during recovery.

**Fig. 1: Total antioxidation analysis by FRAP test:** Leaf and root materials of two maize inbreds (A-180 and A-619) were processed for their total antioxidative ability using ferric reducing antioxidant power (FRAP) test at three different conditions including before stress, during stress and recovery periods. Non-treated plants were also taken as controls. The data were presented as the means of triplicates (p ≤ 0.05). Left panel presents leaf tissues; Right panel presents root tissues.

The overall results furthermore confirmed that drought stress induce the activity of antioxidative system that may contribute to drought resistance in maize. The results also suggest that the root and leaf tissues may equally contribute to resistance phenomenon in maize plant.
Fig. 2: Assessment of the activity of phenylalanine ammonia Lyase: Activation of propanoids biosynthetic pathway was assessed in terms of PAL activity analysis in both inbreds as described in materials and methods section. Experiment was carried out at three different plant conditions including 24 h before stress treatment (plant condition 1), 24 h after stress treatment (plant condition 2) and 24 h after recovery (plant condition 3). Data were presented as the means of triplicates (p ≤ 0.05). Left panel presents leaf tissues; Right panel presents root tissues.

In roots, the activity of PAL was found to be remained constant. No significant changes were observed for PAL activity in the root tissues of both test plants during stress period (Fig. 2, right panel). It seems that PAL is a responsible antioxidative enzyme in the leaves, but not in the roots of maize plants.

The overall pattern in the activity of the PAL enzyme in the leaf tissues showed similarity to the total antioxidation pattern in plants under drought stress conditional shifting. Now these questions can be raised: 1) may phenylpropanoids be more responsible compounds in drought-challenged maize plants?; 2) how much does antioxidation strategy help to drought resistance in maize and 3) may PAL be considered as a candidate for maize genetic engineering in drought resistance strategy in the future?

We predict that PAL can be a good candidate for antioxidative strategy in drought resistance in maize.

ACKNOWLEDGMENTS

The author of this paper is thankful to RIFS (Research Institute for Fundamental Sciences), University of Tabriz, Iran for funding of this work.

REFERENCES


