Screening Drought Tolerance in Caprifig Varieties in Accordance to Responses of Antioxidant Enzymes

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Abstract: This study was conducted to evaluate antioxidant enzyme activities in four caprifig varieties under drought stress and subsequent rewatering in order to screen drought tolerance. Leaf relative water content and membrane stability index significantly reduced under drought stress. ‘Shah Anjir’ and ‘Khormaei’ were able to preserve the highest relative water content and membrane stability index level during the drought period. Soluble proteins concentration significantly increased under drought stress and the increasing rate was higher in the leaves of ‘Pouz Donbali’, ‘Shah Anjir’ and ‘Khormaei’. Drought stress significantly reduced superoxide dismutase and catalase activities in the leaves of the caprifigs. Ascorbate peroxidase and guaiacol peroxidase activities enhanced in the leaves of ‘Pouz Donbali’, ‘Shah Anjir’ and ‘Khormaei’ under drought stress; however, their activities were significantly higher in ‘Shah Anjir’ and ‘Khormaei’. The results showed the significant effects of peroxidase enzymes in drought tolerance of caprifigs. ‘Khormaei’ and ‘Shah Anjir’ with the highest level of ascorbate peroxidase and guaiacol peroxidase activities under water stress were the most drought tolerant cultivars. The results suggested the possibility of evaluating antioxidant enzymes activities as a physiological marker in drought tolerance screening of caprifig and related species.

Key words: Ascorbate peroxidase • Catalase • Caprifig • Membrane stability index • Soluble proteins • superoxide dismutase

INTRODUCTION

Fig is one of the most important horticultural crops grown under Mediterranean climate conditions. Most of world’s fig productions come from rainfed orchards. However, recent extensive droughts significantly affected fig production. Using drought tolerant fig cultivars in drought prone conditions can reduce drought stress pressure and improve plant yield under water deficit conditions. Studies on identification and characterization of relatively drought tolerant fig cultivars have been done and some drought tolerant figs have been introduced. Although the selected figs show improved yield and performance under drought stress, they have limited capacity of drought tolerance and may not meet the future expectations. Hybridization can be used to produce new cultivars with improved drought tolerance which can be used as rootstock or scion. There are many wild varieties known as caprifigs which can be widely found in the mountains of the Middle East. Such varieties usually produce low quality fruits which usually are used in fertilization of some fig cultivars. Some caprifig varieties show good tolerance to drought stress and hence may be involved in breeding programs to produce new drought tolerant rootstocks or fig cultivars. However, there need a reliable tool to screen drought tolerant caprifigs.

The relation between drought stress and antioxidant systems has been studied in some plant species [1]. Drought stress induces production of reactive oxygen species (ROS) such as superoxide (O_{2}^{-}), hydrogen peroxide (H_{2}O_{2}), hydroxyl radicals (•OH) and singlet oxygen (O_{2}) in the leaves of plants [2]. ROS may cause oxidative damages to cells and internal organelles such as lipid peroxidation, chlorophyll bleaching, protein oxidation and nucleic acids [3]. Antioxidant defensive system of plants involving antioxidant enzymes such as superoxide dismutase (SOD), glutathione reductase (GR), catalase and peroxidase and low-molecular antioxidants

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such as ascorbic acid, glutathione, á-tocopherol, flavonoids and carotenoids play a key role in scavenging the ROS [4]. Such antioxidants may be an important factor in the tolerance of various plants to environmental stress [5].

To our knowledge, no studies have been done yet on evaluating drought tolerance of caprifig varieties. Hence, this study was conducted to evaluate the antioxidant enzymes of four widely used caprifigs in south west of Iran, under water stress and subsequent rewatering conditions to screen drought tolerance.

MATERIALS AND METHODS

This study was conducted at the experimental greenhouse of the Department of Horticultural Science of Shiraz University, Iran during March to September, 2012. Plant material used in this study was involved cuttings of four Iranian male figs namely ‘Daneh Sephid’, ‘Pouz Donbali’, ‘Shah Anjir’ and ‘Khormaei’. These genotypes generally are distributed in the southern mountains of Iran. Cuttings of the genotypes were collected at the end of winter 2011 and rooted in sand medium. At the end of winter 2012 the rooted cuttings were transplanted into pots containing 12 kg of sand, leafmould and loamy soil (1:1:1, v/v/v). Three months later, drought stress applied to the trees during their growth period.

Drought stress applied by withholding irrigation for 15 days. The plants in the control treatment were irrigated every day to keep water content of the pots at field capacity (FC) level. After the experimental period, the drought-stressed plants irrigated to FC level and recovery rate of the genotypes was evaluated after 10 days. The experiment repeated twice. The following observations were made at three steps involving first day of the experiment, at the end of the water stress and after the recovery period.

Leaf membrane stability index (MSI) was determined according to the method of Sairam et al. [6]. Ten leaf discs of each treatment were weighed (FW). They were then hydrated until saturation (constant weight) for 48 h at 5°C in darkness (TW). Leaf discs were dried in an oven at 105 °C for 24 h (DW). Relative water content was calculated according to the following expression [7]:

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\text{RWC} = \frac{(\text{FW}-\text{DW})}{(\text{TW}-\text{DW})} 
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Protein concentration was determined spectrophotometrically at 595 nm using the Bio-Rad Protein Assay Dye Reagent Concentrate (Bio-Rad Laboratories, Hercules, Calif.; catalogue no. 500-0006) using a method based on Bradford [8]. Bovine gamma-globulin (0.25 to 1.4 mg•mL\(^{-1}\)) was used as a standard reference.

For enzyme extraction, frozen (-70 °C) leaves (0.5 g) were ground to fine powder in liquid nitrogen with mortar and pestle and then homogenized in 5 ml extraction buffer of 50 mM PBS, pH 7.8, 0.1 mM EDTA, 0.3% TritonX-100, 4% polyvinylpolypyrroidone (PVP). After centrifugation (4C, 10,500g, 20 min), the supernatant was collected and used for antioxidant enzymes activities analysis.

SOD activity was determined according to Beauchamp and Fridovich [9]. The reaction mixture contained 50 mM K-phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 0.1 µM EDTA, 0.1% TritonX-100, 4% polyvinylpolypyrroidone (PVP). The reaction was started with adding riboflavin and placing the tubes under two 15 W fluorescent lamps for 15 min. The reaction mixture with no enzyme developed maximum color due to maximum reduction of NBT. A non-radiated reaction mixture did not develop color and served as the control. The reduction of NBT was inversely proportional to the SOD activity. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT. A non-radiated reaction mixture did not develop color and served as the control. The reduction of NBT was inversely proportional to the SOD activity. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT.

ASPX was determined using a method described by Nakano and Asada [10] by recording the decrease in absorbance at 290 nm, as ascorbate was oxidized. The assay mixture contained 90 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.65 mM riboflavin and enzyme extract. The reaction was initiated with the addition of approximately 40 µgL enzyme extract. The reaction was started with the addition of 1.0 mM H\(_2\)O\(_2\). The reaction was recorded.
Guaiacol peroxidase (GPX) activity was assayed according to the method of Hemeda and Klein [11]. A 100 ml of reaction mixture contained 10 ml of 1% guaiacol (v/v), 10 ml of 0.3% H₂O₂ and 80 ml of 50 mM phosphate buffer (pH 6.6). Enzyme extract was added and the increase in absorbance due to oxidation of guaiacol (extinction coefficient 26.6 mM⁻¹ cm⁻¹) was monitored at 470 nm.

CAT activity was assayed by monitoring the decomposition H₂O₂ at 240 nm according to the method of Aebi [12]. Reaction mixture was contained 100 mM potassium phosphate buffer (pH 6.5), 1.0 mM EDTA, 60.0 mM H₂O₂ and enzyme extract.

Statistical differences between measurements were analyzed following the analysis of variance ANOVA using SPSS 16.0 software. Differences were considered significant at a probability level of $P < 0.05$.

**RESULTS**

Leaf relative water content (RWC) was significantly lower after water stress and the lowest value found in ‘Dane Sephid’ (Fig. 1). RWC was significantly higher in the leaves of ‘Khormaei’, after water stress. Rewatering increased RWC of the stressed plants. RWC was significantly higher in the leaves of ‘Khormaei’ and ‘Shah Anjir’ after rewatering.

Drought stress significantly decreased membrane stability index (MSI) in the leaves of the caprifig varieties. MSI was significantly lower in ‘Dane Sephid’ and ‘Pouzdonbali’ (Fig. 2). Rewatering significantly increased MSI in the leaves of the stressed plants; however, MSI did not fully recovered to the control level in ‘Dane Sephid’ and ‘Pouzdonbali’.

Protein concentration significantly increased in the leaves of the caprifigs under drought stress (Fig. 3). The highest protein concentration was found in the drought stressed leaves of ‘Khormaei’ and ‘Pouz Donbali’. Protein concentration reduced to the control level after rewatering.
Fig. 4: Effects of drought stress (WS) and subsequent rewatering (RW) on superoxide dismutase activity (SOD) in the leaves of four caprifig varieties. Means denoted by the same letter did not significantly differ at $P < 0.05$ according to Duncan's multiple range test.

The highest level of superoxid dismutase (SOD) activities were found in the leaves of the control plants and the highest level was belong to ‘Dane Sephid’ (Fig. 4). SOD activity significantly reduced under drought stress and the lowest values were found in the leaves of ‘Shah Anjir’ and ‘Khormaei’. SOD activity increased in the leaves of drought stressed plants after rewatering and it recovered to the control level in the leaves of ‘Shah Anjir’ and ‘Khormaei’.

Catalase (CAT) activity significantly reduced in the leaves of the caprifigs under drought stress; however, drought stress did not affect CAT activity in the leaves of ‘Dane Sephid’ (Fig. 5). CAT activity increased in the leaves of ‘Pouz Donbali’, ‘Shah Anjir’ and ‘Khormaei’ after rewatering and it recovered to the control level in the leaves of ‘Shah Anjir’ and ‘Pouz Donbali’.

Drought stress significantly increased ascorbate peroxidase (ASX) activity in the leaves of ‘Pouz Donbali’, ‘Shah Anjir’ and ‘Khormaei’ and the highest
ASPX activity was found in the leaves of ‘Shah Anjir’ and ‘Khormaei’ (Fig. 6). ASPX activity was reduced in the leaves of the stressed plants after the rewatering period. However, ‘Dane Sephid’ was an exception and reduced ASPX activity under drought stress was increased after the rewatering period.

Drought stress significantly increased guaiacol peroxidase (GPX) activity in the leaves of the caprifigs and the highest GPX activity was found in the leaves of drought stressed ‘Shah Anjir’ and ‘Khormaei’ (Fig. 7). However, GPX remained at the control level in the leaves of ‘Dane Sephid’. With the exception of ‘Pouz Donbali’, GPX activity significantly reduced in the leaves of drought stressed plants.

**DISCUSSION**

Water stress caused a clear reduction in relative water content (RWC) of the leaves. Relative water content (RWC) under drought stress usually has been considered as a good indicator of drought stress tolerance [13]. Reduced RWC in the leaves of fig genotypes under drought stress is in accordance to results of Karimi et al. [14]. ‘Khormaei’ and ‘Shah Anjir’ was able to preserve RWC level during the water stress period. Preserving RWC has been reported to play a role in the stress tolerance of fig [14, 15]. Stomatal closure and roots ability to continue water absorption under lower soil water potential helps plant to save RWC under drought condition. Preserving higher RWC in ‘Khormaei’ and ‘Shah Anjir’ suggests the possibility of a tolerance strategy by reducing water loss and evading water stress pressure on plant.

Lower MSI was associated with reduced water content in the leaves. MSI is an index to estimate membrane dysfunction under stress. In this study, water stress reduced MSI and it was significantly lower in the leaves of ‘Dane Sephid’ and ‘Pouzdonbali’. Excessive generation of reactive oxygen species formation (ROS) under water stress is one of the major causes of loss of cell membrane stability [16-18]. In accordance to Gholami et al. [15] higher MSI in the leaves of ‘Shah Anjir’ and ‘Khormaei’ might be attributed to increases in antioxidant enzymes activities and inhibition of lipid peroxidation via ROS scavenging.

Soluble proteins concentration significantly increased in the leaves of the caprifigs under drought stress. Gholami et al. [15] also reported increase in soluble proteins concentration in the leaves of common fig during drought stress. It could be related to increase in synthesis of the proteins which are necessary for acclimation to water deficit condition [19]. Increased soluble proteins synthesis beside accumulation of free soluble carbohydrates and amino acids play a major role in the osmotic adjustment under drought stress [20]. Although increase in soluble proteins was observed in the all caprifigs, concentration of soluble proteins in the leaves of ‘Dane Sephid’ was significantly lower than the others. Hence it could be suggested that ‘Pouz Donbali’, ‘Shah Anjir’ and ‘Khormaei’ possessed a better osmotic adjustment and higher drought tolerance than ‘Dane Sephid’. On the other hand, increased soluble proteins concentration under drought stress may show reprogramming of the cells via increasing drought tolerance related enzymes.

Oxidative stress caused by ROS formation during drought stress damages proteins, membrane lipids and other cellular components [21]. An efficient enzymatic antioxidant system can protect plants against oxidative injury [22]. Severity and duration of stress treatment and also the species and age of the plant cause variation in antioxidant enzymes activities. SOD activity usually increases under drought stress [23]; however, In the present study, drought stress reduced SOD activity in the leaves of caprifigs and the lowest activity was found in the leaves of ‘Shah Anjir’ and ‘Khormaei’. Quartacci and Navaro [24] and Gholami et al. [15] also reported reduced SOD activity under drought stress. As SOD processing is substrateinducible [25], reduced SOD activity is probably due to the reduced formation of ROS as substrate which leads to reduced expression of SOD encoding genes. Increased ASPX and GPX activity under drought stress may be correlated to reduced SOD activity via enhanced ROS decomposition capacity.

It has been shown that peroxidase enzymes activity is higher in the plants with enhanced oxidative stress tolerance [26]. In the current study ASPX and GPX activity increased under drought stress and their activity was significantly higher in the leaves of ‘Shah Anjir’ and ‘Khormaei’. Sofo et al. [27] also reported higher ASPX activity in the leaves of drought tolerant Pronus hybrids. Increased ASPX activity under drought stress is essential for protection of chloroplasts against ROS damage [27]. The increased activity of ASPX and GPX may be responsible for the higher MSI. However, increase in GPX activity showed a better correlation to drought stress tolerance in the caprifigs than ASPX.
Increase in peroxidase enzymes under water deficit condition was in coincidence with reduced CAT activity in the leaves. These results are in agreement with Khan et al. [28], Pan et al. [29] and Gholami et al. [15]. The reduction of CAT activity is supposedly due to the inhibition of enzyme synthesis or change in the assembly of enzyme subunits under stress conditions. It may also be associated with degradation caused by high concentration of H$_2$O$_2$. It can be concluded that peroxidase enzymes play the major role in eliminating H$_2$O$_2$ in the leaves of drought tolerant caprifigs and CAT probably is not involved in drought tolerance mechanisms of caprifig.

CONCLUSION

According to the results, drought tolerance of the caprifig varieties was found as the following order ‘Khormaei’ > ‘Shah Anjir’ > ‘Pouz Donbali’ > ‘Dane Sephid’. Drought tolerant caprifigs ‘Khormaei’ and ‘Shah Anjir’ always had higher rate in the activities of APXD and GPX than ‘Pouz Donbali’ and ‘Dane Sephid’, under water deficit condition. The results suggest that caprifigs ‘Khormaei’ and ‘Shah Anjir’ are able to remove ROS and other free radicals more easily than ‘Khormaei’ and ‘Shah Anjir’. Therefore, ‘Khormaei’ and ‘Shah Anjir’ possesses a higher antioxidant capacity under drought stress. As a consequence, it can be concluded that higher activity of peroxidase enzymes probably play a key role in limitation of cellular damages under drought stress and can be considered as an efficient drought protection mechanism in caprifig. On the other hand, SOD and CAT probably are not involved in antioxidant defense system of caprifig.

REFERENCES


