Effect of abscisic acid and hydrogen peroxide on antioxidant enzymes in *Syzygium cumini* plant

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Abstract The present study was undertaken to study the effect of abscisic acid and hydrogen peroxide on the activities of antioxidant enzymes namely superoxide dismutase (SOD; E.C. 1.15.1.1), catalase (CAT; E.C. 1.11.1.6) and ascorbate peroxidase (APX; E.C. 1.11.1.11) in *Syzygium cumini* plant. The varying concentrations of ABA (2–8 mM/l) and H₂O₂ (2–8 mM/l) modulated enzyme activities differently. In general, some concentrations of the ABA and H₂O₂ stimulated the activities of all the three enzymes except that there was a dose dependent reduction in catalase activity in the plants treated with ABA.

Keywords Antioxidant enzymes · Absciscic acid · Hydrogen peroxide · Reactive oxygen species

Introduction

Antioxidants play an important role in preventing the stress-induced accumulation of reactive oxygen species (Foyer and Noctor 2005). The ROS cause rapid cell damage under stress conditions by reacting on membrane lipids, proteins and nucleic acids (Hariyadi and Parkin 1993; O’Kane et al. 1996; Prasad 1996) but also perform useful functions in cell signalling through interrelating different stimuli and respective responses (Desikan et al. 2004, Gomez et al. 2004, Rubio et al. 2009). The increased ROS content is controlled by an antioxidant system including

low molecular weight antioxidant metabolites (ascorbate, tocopherol, glutathione etc.) and antioxidative enzymes such as superoxide dismutase, ascorbate peroxidase, catalase and guaiacol peroxidase (Suzuki and Mittler 2006, Turhan et al. 2008). The antioxidants thus contribute to improved stress tolerance through detoxification of ROS. Modulation of these antioxidants under various stress conditions is therefore an important adaptive response to withstand adverse condition. Indeed, maintenance of a high antioxidant capacity in cells has been related to increased tolerance against different kinds of environmental stress (Dat et al. 1998, Thomas et al. 1999).

Absciscic acid exerts prominent role in plants in response to environmental stress (Seo and Koshiba 2002). ABA induced the synthesis of hydrogen peroxide and superoxide under stressful conditions and caused oxidative stress (Guan et al. 2000). On the other hand, ABA also stimulates the antioxidant defense system in plants to yield more antioxidants that can resist the oxidative stress (Jiang and Zhang 2001). Absciscic acid is a natural growth regulator which accumulates in plants under stress conditions. However, exogenous ABA significantly decreased the yields in plants indicating a trade-off between new growth of plant and phytochemical synthesis (Zheng et al. 2010). H₂O₂ is a versatile molecule that may be involved in several cell processes under normal and stress conditions (Quan et al. 2008). Pretreatment with low concentrations of H₂O₂ molecule increases the accumulation of glutathione (Murphy et al. 2002) and antioxidant enzyme activity (Azevedo Neto et al. 2005) in plants, thereby alleviating harm from ROS (Murphy et al. 2002; Wahida et al. 2007). *Syzygium cumini* plant belongs to Myrtaceae family and grows naturally in clayey loamy soil in tropical as well as in sub-tropical zones of Indo-gangetic plains. These species

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are reported to be very rich in tannins, flavonoids, essential oils, anthocyanins and other phenolic constituents as antioxidant compounds (Deepak et al. 2011). The present investigation was under taken to study the effects of different concentrations of ABA and H₂O₂ on antioxidant enzymes in *Syzygium cumini* plant.

Materials and methods

Plant material

One month old seedlings of *Syzygium cumini* obtained from Forest Training Institute, Jaipur were grown in pots containing soil and manure under natural photoperiod in the Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India. The plants of uniform height were selected and watered regularly. Two month old plants were irrigated with various concentrations of ABA (2–8 mM/l) and H₂O₂ (2–8 mM/l). After 20 days of treatment, newly growing leaves were taken and analyzed for activities of antioxidant enzyme.

Preparation of enzyme extract

Leaf tissues (0.5 g) was ground to a fine powder in 2 ml of 50 mM potassium phosphate buffer (pH 7.0), 1 mM ethylenediamine tetraacetic acid (EDTA), 1 mM ascorbic acid (AA), 2% (w/v) polyvinylpyrrolidone (PVP) and 0.05% (w/v) Triton X-100 using a chilled pestle and mortar. The homogenate was centrifuged at 10,000 g for 20 min at 4°C and the supernatants thus collected was used for the assays of catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (GPX). Protein concentrations in the enzyme extract were determined by the method of Bradford (1976) using bovine serum albumin (BSA; Sigma, fraction V) as a standard.

Catalase (EC 1.11.1.6)

Catalase (CAT) activity was determined spectrophotometrically by measuring the rate of H₂O₂ disappearance at 240 nm (Aebi 1984). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0) and 10.5 mM H₂O₂. The reaction was run at 25°C for 2 min, after adding the enzyme extract and rate of decrease in absorbance at 240 nm ($E=39.4 \text{ mM}^{-1} \text{ cm}^{-1}$) was used to calculate the enzyme activity.

Ascorbate peroxidase (EC 1.11.1.11)

Ascorbate peroxidase (APX) was assayed by the method as described by Nakano and Asada (1981). The reaction

mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.2 mM EDTA, 0.5 mM ascorbic acid and 0.25 mM H₂O₂. The reaction was started at 25°C by the addition of H₂O₂ after adding the enzyme extract. The decrease in absorbance at 290 nm for 1 min was recorded and the amount of APX was calculated from the extinction coefficient $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$.

Superoxide dismutase (EC 1.15.1.1)

For assay of superoxide dismutase (SOD), fresh leaves (1 g) were homogenized in 8 ml potassium phosphate buffer (50 mM, pH 7.8) containing 0.1 mM Na₂-EDTA and 1% insoluble PVP with a chilled pestle and mortar. The homogenate was centrifuged at 20,000 g for 20 min. The supernatant was collected and used for the assay of SOD following the method of Beauchamp and Fridovich (1971). Reaction mixture was prepared by mixing 27 ml of 50 mM potassium phosphate, pH 7.8, 1.5 ml of L-methionine (300 mg/10 ml) and 1 ml of nitroblue tetrazolium salt (NBT) (14.4 mg/10 ml). Aliquots (2 ml) of this mixture were delivered into small glass tubes, followed by 20 µl of enzyme extract and 10 µl of riboflavin (4.4 mg/100 ml). The mixture was illuminated for 15 min in an aluminum foil-lined box, containing two 20 W fluorescent tubes. A control tube in which the sample was replaced by 20 µl of buffer was run in parallel and the absorbance at 560 nm was measured in all tubes. Under the described conditions, the increase in absorbance without the enzyme extract was taken as 100% and the enzyme activity was calculated by determining the percent inhibition per minute. 50% inhibition was taken as equivalent to 1 unit of SOD activity.

Statistical analysis

Each experiment was repeated three times. Values were expressed as means ± SE. One-way analysis of variance (ANOVA) was used for comparisons between the means. In all cases the confidence coefficient was set at 0.05.

Results and discussion

Diverse environmental stresses differentially affect plant processes that lead to loss of cellular homeostasis accompanied by ROS, which causes oxidative damage to membranes, lipids, proteins and nucleic acids (Srivalli et al. 2003). The response of ascorbate peroxidase activity was more significant at the minimum concentration of ABA (2 mM/l) as compared to control plant shown in Fig. 1(a). However no significant differences were observed with higher concentration of ABA treatment. Previous study supported the increasing activity of APX enzyme (Bellaire

Fig. 1 **a** ABA effect on APX enzyme **b** H_2O_2 effect on APX enzyme **c** ABA effect on CAT enzyme **d** H_2O_2 effect on CAT enzyme **e** ABA effect on SOD enzyme **f** H_2O_2 effect on SOD enzyme

et al. 2000). Among the four concentrations of hydrogen peroxide tested, the 6 mM/l was most effective in increasing activity of ascorbate peroxidase enzyme as shown in Fig. 1(b).

The CAT activity decreased gradually with increasing concentration of ABA, the maximum decrease being 70% under the influence of 8 mM/l of ABA concentration (Fig. 1(c)). The maximum activity was observed in control plant (9.78 unit/g fr.wt.). Unlike the ABA, the increasing concentrations of hydrogen peroxide consistently increased activity of CAT shown in Fig. 1(d). The SOD activity increased with increasing ABA, the maximum being at 8 mM/l (Fig. 1(e)). On the other hand, H_2O_2 treated plants showed significant increase in SOD enzyme activity reaching the maximum at 4 mM/l of H_2O_2 (Fig. 1(f)).

It has been well documented that ABA can result in the increased generation of ROS, increased expression of antioxidant genes, and enhanced capacity of antioxidant defense systems in plants (Jiang and Zhang 2004). Previous

studies showed that ABA induced the apoplastic H_2O_2 accumulation in maize leaves and increased the activities of antioxidant enzymes such as SOD and APX (Hu et al. 2005, 2006; Zhang et al. 2006). Agarwal et al. (2005) reported a decrease in oxidative stress in ABA treated in wheat cultivars.

The present study confirms that there was a drastic decrease in CAT activity but initial and overall increase in APX and SOD activity respectively in ABA treated plants. Hydrogen peroxide treatment was very effective in increasing APX, CAT and SOD enzyme activity. Previous studies suggested that coordinate function of antioxidant enzymes such as APX, CAT, SOD and glutathione reductase helps in processing of ROS and regeneration of redox ascorbate and glutathione metabolites (Halliwell 1974; Wise 1995; Asada 1996; Foyer and Nector 2000). A considerable and significant increase in the activity of SOD enzyme was recorded in both ABA and H_2O_2 treated plants.

References

- Aebi H (1984) Catalase in vitro. *Methods Enzymol* 105:121–126
- Agarwal S, Sairam RK, Srivastava GC, Meena RC (2005) Changes in antioxidant enzymes activity and oxidative stress by abscisic acid and salicylic acid in wheat genotypes. *Biol. Plant* 49:541–550
- Asada K (1996) Radical production and scavenging in chloroplasts. In: Baker N (ed) *Photosynthesis and the environment*. Kluwer Academic, Dordrecht, pp 123–150
- Azevedo Neto AD, Prisco JT, Enéas-Filho J, Medeiros JVR, Gomes-Filho E (2005) Hydrogen peroxide pre-treatment induces salt-stress acclimation in maize plants. *J Plant Physiol* 162:1114–1122
- Beauchamp C, Fridovich I (1971) Superoxide dismutase improved assays and an assay applicable to acrylamide gels. *Anal Biochem* 44:276–287
- Bellaire BA, Carmody J, Braud J, Gossett DR, Banks SW, Lucas MC, Fowler TE (2000) Involvement of abscisic acid dependent and -independent pathways in the regulation of antioxidant enzyme activity during NaCl stress in cotton callus tissue. *Free Rad Res* 33:531–545
- Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein using the principle of protein-dye binding. *Anal Biochem* 72:248–254
- Dat JF, Foyer CH, Scott I (1998) Changes in salicylic acid and antioxidants during induced thermotolerance in mustard seedlings. *Plant Physiol* 118:1455–1461
- Deepak RR, Sonia C, Maninder PK, Pranita J, Ramabhai TP (2011) Biochemical, microbiological and physiological changes in Jamun (*Syzium cumini* L.) kept for long term storage under modified atmosphere packaging. *J Food Sci Technol* 48(3):357–365
- Desikan R, Cheung M, Clarke A, Golding S, Sagi M, Fluhr R (2004) Hydrogen peroxide is a common signal for darkness- and ABA-induced stomatal closure in *Pisum sativum*. *Funct Plant Biol* 31:913–920
- Foyer CF, Nector G (2000) Oxygen processing in photosynthesis regulation and signaling. *New Phytol* 146:359–388
- Foyer CH, Noctor G (2005) Oxidant and antioxidant signaling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. *Plant Cell Environ* 28:1056–1071
- Gomez JM, Jimenez A, Olmos E, Sevilla F (2004) Localization and effects of long-term NaCl stress on superoxide dismutase and ascorbate peroxidase isoenzymes of pea (*Pisum sativum* cv. Puget) chloroplasts. *J Exp Bot* 55:119–130
- Guan L, Zhao J, Scandalios JG (2000) Cis-elements and trans-factors that regulate expression of the maize cat1 antioxidant gene in response to ABA and osmotic stress: H₂O₂ is the likely intermediary signaling molecule for the response. *Plant J* 22:87–95
- Halliwell B (1974) Superoxide dismutase, catalase and glutathione peroxidase: solutions to the problems of living with oxygen. *New Phytol* 73:1075–1086
- Hariyadi P, Parkin K (1993) Chilling-induced oxidative stress in cucumber (*Cucumis sativus* L. cv. Calipso) seedlings. *J Plant Physiol* 141:733–738
- Hu X, Jiang M, Zhang A, Lu J (2005) Abscisic acid-induced apoplastic H₂O₂ accumulation up-regulates the activities of chloroplastic and cytosolic antioxidant enzymes in maize leaves. *Planta* 223:57–68
- Hu X, Zhang A, Zhang J, Jiang M (2006) Abscisic acid is a key inducer of hydrogen peroxide production in leaves of maize plants exposed to water stress. *Plant Cell Physiol* 47:1484–1495
- Jiang M, Zhang J (2001) Effect of abscisic acid on active oxygen species, antioxidative defence system and oxidative damage in leaves of maize seedlings. *Plant Cell Physiol* 42:1265–1273
- Jiang M-Y, Zhang J-H (2004) Abscisic acid and antioxidant defense in plant cells. *Acta Botanica Sinica* 46:1–9
- Murphy TM, Sung WW, Lin CH (2002) H₂O₂ treatment induces glutathione accumulation and chilling tolerance in mung bean. *Funct Plant Biol* 29:1081–1087
- Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol* 22:867–880
- O’Kane D, Gill V, Boyd P, Burdon R (1996) Chilling, oxidative stress and antioxidant responses in *Arabidopsis thaliana* callus. *Planta* 198:371–377
- Prasad T (1996) Mechanisms of chilling-induced oxidative stress injury and tolerance in developing maize seedlings: changes in antioxidant system, oxidation of proteins and lipids, and protease activities. *Plant J* 10:1017–1026
- Quan LJ, Zhang B, Shi WW, Li HY (2008) Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network. *J Integr Plant Biol* 50:2–18
- Rubio MC, Bustos-Sammamed P, Clemente MR, Becana M (2009) Effects of salt stress on expression of antioxidant genes and proteins in the model legume *Lotus japonicus*. *New Phytologist* 181:851–859
- Seo M, Koshiba T (2002) Complex regulation of ABA biosynthesis in plants. *Trends Plant Sci* 7:41–48
- Srivalli B, Vishanathan C, Renu KC (2003) Antioxidant defense in response to abiotic stresses in plants. *J Plant Biol* 30:121–139
- Suzuki N, Mittler R (2006) Reactive oxygen species and temperature stresses: a delicate balance between signaling and destruction. *Physiol Plant* 126:45–51
- Thomas DJ, Thomas JB, Prier SD, Nasso NE, Herbert SK (1999) Iron superoxide dismutase protects against chilling damage in the cyanobacterium *Synechococcus* species PCC7942. *Plant Physiol* 120:275–282
- Turhan E, Gulen H, Eris A (2008) The activity of antioxidative enzymes in three strawberry cultivars related to salt-stress tolerance. *Acta Physiol Plant* 30:201–208
- Wahida A, Perveen M, Gelania S, Basrab SMA (2007) Pretreatment of seed with H₂O₂ improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. *J Plant Physiol* 164:283–294
- Wise RR (1995) Chilling enhanced photo-oxidation: the production, action and study of reactive oxygen species produced during chilling in the light. *Photosynth Res* 45:79–97
- Zhang A, Jiang M, Zhang J, Tan M, Hu X (2006) Mitogen-activated protein kinase is involved in abscisic acid-induced antioxidant defense and acts downstream of reactive oxygen species production in leaves of maize plants. *Plant Physiol* 141:475–487
- Zheng L, Xin Z, Amandeep KS, Liwei G (2010) Effects of Exogenous Abscisic Acid on Yield, Antioxidant Capacities, and Phytochemical Contents of Greenhouse Grown Lettuces. *J Agric Food Chem* 58:6503–6509