Original Article

Journal of Phytology 2013, 5: 19-24
http://scienceflora.org/journals/index.php/jp/

Study of antioxidant enzymes activity during rooting in *Adhatoda vasica* under different triazole compounds

M. Gnana Mani, R. Panneerselvam*

Department of Botany, Annamalai University, Annamalainagar, Chidambaram, Tamil Nadu, India

Received: 06.05.2013
Revised: 18.06.2013
Accepted: 18.06.2013
Published: 21.06.2013

***Address for correspondence:**
Dr. R. Panneerselvam, Department of Botany, Annamalai University, Annamalainagar - 608 002, Chidambaram, Tamil Nadu, India. E-mail: rpselvam9@hotmail.com

ABSTRACT

Medicinally important plant species, *Adhatoda vasica* belonging to the family Acanthaceae was selected for the present investigation to study the comparative effects of traditional as well as non-traditional growth regulators. The traditional growth regulator selected was indole butyric acid (IBA) and non-traditional one was hexaconazole (HEX) and triadimefon (TDM). Antioxidant enzymes activities, such as superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT), were estimated from control as well as treated plants. The activities of antioxidant enzymes, such as SOD, APX, and CAT, were increased with TDM, HEX, and IBA treatments in *A. vasica* plants. The enhancement was significant on all sampling days. These preliminary results prove TDM and HEX as potential growth regulators which can be used to enhance the antioxidant properties of *A. vasica,* thereby make it an economically valuable medicinal plant.

KEY WORDS: *Adhatoda vasica,* hexaconazole, triadimefon, triazole fungicides

INTRODUCTION

There are a large number of synthetic organic chemicals possessing growth regulating properties, and new ones are being added to the list periodically (Ramanayake *et al*., 2008). Triazole compounds, such as triademefon, paclobutrazol, uniconazole, propiconazole, and hexaconazole (HEX), also have growth regulating properties, induce many morphological and metabolic changes such as reduction in shoot elongation, stimulation of rooting, inhibition of gibberellin biosynthesis, increased chlorophyll content, altered carbohydrate status, and increased cytokinin synthesis (Morab *et al*., 2003). Triazoles interfere with the first three steps in the pathway of ent-kaurene oxidation and thus the formation of ent-kaurenal and ent-kaurenoic acids are inhibited, whereas steps from mevalonic acid to kaurene and form kaurenoic acid to GA12 appears to be affected by the triazoles (Fletcher *et al*., 2000). Plants have developed antioxidant enzymes such as ascorbate peroxidase (APX), dehydroascorbate reductase, glutathione reductase, catalase (CAT), superoxide dismutase (SOD) for scavenging the reactive oxygen species (Vranova *et al*., 2002). APX and CAT represent the major enzymes of H_2O_2 degradation (Vanacker *et al*., 1998). SOD, CAT, APX and glutathione reductase are the present in isoforms with specific subcellular localization. The enzymes of glutathione-ascorbate cycle have been implicated in mitigating the effect of reactive oxygen species (Porcel *et al*., 2003).

SOD is ubiquitous, widely distributed multimeric metalloprotein which CAT the dismutation of into H_2O_2. SOD are classified based on the metal ion in their active site, and they are copper and zinc (Cu/Zn. SOD) containing SOD are the most efficient scavengers of the superoxide anion and as an essential component of the ascorbate-glutathione cycle for the detoxification of toxic oxygen species (Bannister *et al*., 1991). APX and CAT represent the major enzymes of H_2O_2 degradation (Vanacker *et al*., 1998). APX isoenzymes have high specificity for ascorbic acid as an electron donor especially in the case of chloroplastic APX and mitochondrial APX isoenzymes (Leonardis *et al*., 2000). CATs are tetrameric heme containing enzymes that catalyse the dismutation of hydrogen peroxide into water and oxygen (Fornazier

et al., 2002). This is also due to the fact that there is a proliferation of peroxisomes during stress, which might help in scavenging of H_2O_2, which can diffuse the cytosol (Lopez-Huertas *et al*., 2000). The third class of CAT is located in vascular tissues and may be involved in protection against environmental stress (Willekens *et al*., 1994).

The objectives of this study are to evaluate the effect of triadimefon (TDM), hexaconazole, and indole butyric acid (IBA) on the activity of antioxidant enzymes such as APX, SOD and CAT of *Adhatoda vasica* plants under field conditions.

MATERIALS AND METHODS

A. vasica L. belongs to the family Acanthaceae was selected for the present investigation. The cuttings were collected from Sivapuri in Chidambaram, Cuddalore District, Tamil Nadu, India. The stem cuttings of uniform thickness having three nodes were used for planting. Each stems cutting were 18 cm height and planted in 5 cm inside the polythene bags. The stem cuttings are dipped for 10 min in 1% bavestin before planting.

The treatments of 15 mg/L TDM and 15 mg/L HEX concentrations were found to increase the dry weight significantly and in a higher concentrations they slightly decreased the growth and dry weight. Hence, 15 mg/L TDM and 15 mg/L HEX concentrations were used to determine the effect of these chemicals on the antioxidant enzyme activities of *A. vasica*. 1 L of 15 mg/L TDM and 15 mg/L HEX solution per plant was used for the treatment and control was treated with 1 L of irrigation water. 1 L of 0.3 mg/L IBA solution per plant was used for the treatment and control was treated with 1 L of irrigation water. The treatment was given on 15, 30, 45, and 60 DAP. The average temperature was 32/26°C (maximum and minimum) and relative humidity varied between 60% and 75% during the experimental period. TDM [1-(4-chlorophenoxy)–3,3-dimethyl-1-(1H-1, 2, 4- triazole–1–Y1)–2 butanone] [C_{14} H_{16} Cl N_3 O_2] M.W. 293.75 has been obtained from Bayer India Ltd., Mumbai and HEX (2- (2, 4-dichlorophenyl)-1- (2 H-1, 2, 4-triazole-1-Y1) hexan – 2-01) [C14 H17 Cl_2 N3 O] M.W. 314.2 has been obtained from Rallis India Ltd., Mumbai, Maharashtra, India. Indole-3-butyric acid (C6H4. NH.CH: C(CH2)3.COOH) M.W.203.24) was obtained from Sigma Chemicals, Bangalore used for this study. The experimental part of this work was carried out in Botanical Garden and Stress Physiology Lab, Department of Botany, Annamalai University, Tamil Nadu.

ANTIOXIDANT ENZYMES

SOD (SOD, EC: 1.15.1.1)

Crude enzyme extract was prepared, for the assay of SOD by the method of Hwang *et al*. (1999). The enzyme protein was determined by Bradford (1976) method. SOD activity was assayed as described by Beauchamp and Fridovich (1971). The reaction medium was prepared and to 3 ml reaction medium, 1 ml of enzyme extract was added. The reaction mixture contained 1.17×10^{-6} M riboflavin, 0.1 M methionine, 2×10^{-5} potassium cyanide and 5.6×10^{-5} M nitroblue tetrazodium salt (NBT), dissolved in 0.05 M sodium phosphate buffer (pH 7.8). The mixture was illuminated in glass test tubes by two sets of Philips 40 W fluorescent tubes. Illumination started to initiate the reaction at 30°C for 1 h. Those without illumination saved as blank and kept in dark. The absorbance was read at 560 nm in the spectrophotometer against blank. SOD activity was expressed in units. One unit is defined as the amount of change in the absorbance by 0.1 per h per milligram protein under the assay condition (Cherry, 1963).

APX (APX, EC: 1.11.1.11)

APX was extracted and estimated by the method of Asada and Takahashi (1987). 1 ml of reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 200 µL of enzyme extract. The absorbance was read as a decrease at 290 nm against the blank, correction was done for the low, non-enzymatic oxidation of ascorbic acid by H_2O_2 (extinction coefficient 2.9/mM/cm). The enzyme activity was expressed in units/mg protein (U = change in 0.1 absorbance/min/mg protein).

CAT (CAT, EC: 1.11.1.6)

CAT activity was assayed as described by Chandlee and Scandalios (1984). The activity of enzyme CAT was measured using the method of Chandlee and Scandalios (1984) with modification. The assay mixture contained 2.6 ml of 50 ml of 50 mM potassium phosphate buffer (pH 7.0) 0.4 ml, 15 mM H_2O_2 and 0.04 ml of enzyme extract. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm. The enzyme activity was expressed in units 1 mM of H_2O_2 reduction per minute per mg protein.

Statistical Analysis

Each treatment was analyzed with at least four replicates, and a standard deviation (SD) was calculated and data are expressed ± SD of four replicates.

RESULTS

Antioxidant Enzymes

SOD

Leaf

The activity of SOD of the leaves increased with the age of the plant. Triazole treatment increased the higher level of SOD activity significantly when compared to control. Among the triazoles, HEX and IBA treated plants showed a higher level of SOD activity than that of TDM and it was 132.6 156.6 and 168.4% over control, respectively, on 60 DAS (Table 1).

Stem

The activity of SOD of the stem increased with the age of the plant. Triazole treatment increased the higher level of SOD activity significantly when compared to control. Among the triazoles, HEX and IBA treated plants showed the higher level of SOD activity than that of TDM and it was 126.8%, 142.3%, and 159.3% over control, respectively, on 60 DAS (Table 2).

Root

The SOD activity in the root increased with the age of the plant. Triazole treatment showed the higher level of SOD activity in the root when compared with control, and it was 123.44% over control for TDM and 120.63% over control for HEX and 120.1% over control for respectively IBA on 45 and 60 DAS. Among the organs, leaf showed higher level of SOD activity when compared to stem and root (Table 3).

Table 1: Effect of IBA, TDM and HEX SOD-leaf in *Adhatoda vasica*

Growth stages (DAP)	Control	IBA	HEX (15 mg/L)	TDM (15 mg/L)
15	3.375±0.121	4.144±0.148	4.870±0.174	5.240±0.175
30	3.688±0.127	4.571±0.158	5.567±0.186	5.923±0.204
45	3.902±0.135	5.069±0.169	5.995±0.214	6.450±0.222
60	4.229±0.141	5.610±0.200	6.578±0.235	7.091±0.236

Values are mean±SD of four samples expressed in unit/mg protein. IBA: Indole butyric acid, TDM: Triadimefon, HEX: Hexaconazole, SOD: Superoxide dismutase, DAP: Days after planting, SD: Standard deviation

Table 2: Effect of IBA, TDM and HEX SOD-stem in *Adhatoda vasica*

Growth stages (DAP)	Control	IBA	HEX (15 mg/L)	TDM (15 mg/L)
15	3.119±0.111	3.431±0.118	4.215±0.145	4.400±0.157
30	3.361±0.112	4.086±0.146	4.471±0.160	5.083±0.175
45	3.574±0.128	4.215±0.145	4.998±0.172	5.496±0.183
60	3.973±0.142	4.955±0.165	5.610±0.187	5.710±0.184

Values are mean±SD of four samples expressed in unit/mg protein. IBA: Indole butyric acid, TDM: Triadimefon, HEX: Hexaconazole, SOD: Superoxide dismutase, DAP: Days after planting, SD: Standard deviation

APX

Leaf

The APX activity increased with the age in the leaves of *A. vasica*. Triazole treatment significantly increased the APX activity to an appreciable level. Among the triazole treatment, HEX treatment increased it to the higher level than that of TDM and IBA (Figure 1).

Stem

The APX activity increased with the age in the stem of *A. vasica*. Triazole treatment significantly increased the APX activity to an appreciable level. Among the triazole treatment, HEX treatment increased it to a higher level than that of TDM and IBA (Figure 2).

Root

Triazole treatments significantly increased the APX activity to an appreciable level in the root. The APX activity increased at the time of root maturation. There is some significant variation in the APX activity between these two triazole treatments and IBA (Figure 3).

CAT

Leaf

In the leaf tissue, the CAT activity increased with the age in the control and treated plants. Triazole treatments significantly increased the activity of CAT to a higher extent when compared to control. Among the triazoles, HEX and IBA treatment increased the CAT activity to a larger extent when compared to TDM and it was 158.6%, 141.5%, and 110.5% over control on 60DAS, respectively, (Table 4).

Table 3: Effect of IBA, TDM and HEX SOD-root in *Adhatoda vasica*

Growth stages (DAP)	Control	IBA	HEX (15 mg/L)	TDM (15 mg/L)
15	2.549±0.088	2.799±0.096	3.147±0.112	3.503±0.121
30	2.734±0.091	3.047±0.105	3.560±0.123	4.115±0.147
45	3.076±0.109	3.374±0.116	4.001±0.144	4.656±0.161
60	3.617±0.117	3.859±0.138	4.571±0.152	5.354±0.185

Values are mean±SD of four samples expressed in unit/mg protein. IBA: Indole butyric acid, TDM: Triadimefon, HEX: Hexaconazole, SOD: Superoxide dismutase, DAP: Days after planting, SD: Standard deviation

Table 4: Effect of IBA, TDM and HEX CAT-leaf in *Adhatoda vasica*

Growth stages (DAP)	Control	IBA	HEX (15 mg/L)	TDM (15 mg/L)
15	4.390±0.156	5.106±0.182	5.459±0.194	5.046±0.181
30	4.556±0.160	5.522±0.197	6.030±0.215	5.252±0.187
45	4.868±0.173	6.113±0.218	6.445±0.230	5.646±0.201
60	5.044±0.180	6.560±0.234	7.006±0.250	5.875±0.211

Values are mean±SD of four samples expressed in unit/mg protein. IBA: Indole butyric acid, TDM: Triadimefon, HEX: Hexaconazole, SOD: Superoxide dismutase, DAP: Days after planting, SD: Standard deviation, CAT: Catalase

Stem

In the stem tissue, the CAT activity increased with age of the plant. Triazole treatments significantly increased the CAT activity to a level of higher than that of control. Among the triazole TDM treated plants showed higher level of CAT activity when compared to HEX and IBA in stem tissue (Table 5).

Root

In the root tissue, the CAT activity increased with age of the plant. Triazole treatments significantly increased the CAT activity to a level of higher than that of control. Among the triazole, TDM treated plants showed higher level of CAT activity when compared to HEX and IBA in root tissue (Table 6).

DISCUSSION

SOD

In *A. vasica* TDM, HEX and IBA increased the activity of SOD to a larger extent and this level was very high in the leaves when compared to stem and root. SOD is a major scavenger of reactive oxygen species and it catalyses the dismutation of superoxide anion radical (O_2-) with great efficiency resulting in the production of H_2O_2 and O_2 (Chen and Asada, 1989; Smirnoff, 1993).

Uniconazole treatment protected corn seedlings from damage, and the stress protection is mediated by an increased activity of antioxidant enzymes (Pinhero and Fletcher, 1994). Similar observations were observed that paclobutrazol treatment increased the activity of SOD, glutathione reductase and APX in the leaves and roots of bean (Gehlot *et al.,* 1989), wheat (Kraus *et al.,* 1995) banana (Biyan *et al.,* 1995) and maize seedlings (Pinhero *et al.,* 1997).

APX

The APX activity increased in the triazole treated *A. vasica* plants when compared to control. Similar observation was made in paclobutrazol treated *Echinochola framentacea* (Sankhla *et al.,* 1992) and TDM treated cucumber seedlings (Feng *et al.,* 2003). The enzyme of glutathione-ascorbate cycle has been implicated in mitigating the effect of reactive oxygen species (Gara *et al.,* 2000; Porcel *et al.,* 2003). Antioxidant enzymes such as APX, SOD and antioxidant metabolites like ascorbate, glutathione, carotenoids are involved in scavenging reactive oxygen species (Vranova *et al.,* 2002). The triazole compounds enhanced the free radical scavenging capacity in treated plants including the levels of carotenoids, ascorbate SOD and APX (Senaratna *et al.,* 1988; Kraus *et al.,* 1995).

Treatment with IBA increased the APX activity in *A. vasica*. Similar results were previously reported in *C. roseus* plants in both soil drench and foliar applications of GA_3 (Jaleel *et al.,* 2007).

CAT

Triazoles showed higher influence in increasing the CAT activity both in the shoot and tuber of Chinese potato.

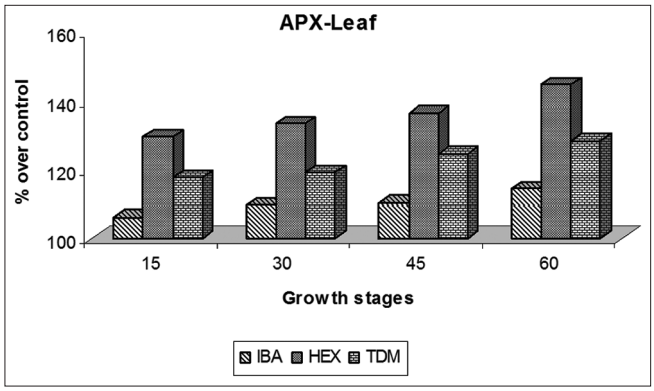

Figure 1: Indole butyric acid, hexaconazole and triadimefon induced changes in ascorbate peroxidase-leaf of *Adhatoda vasica*

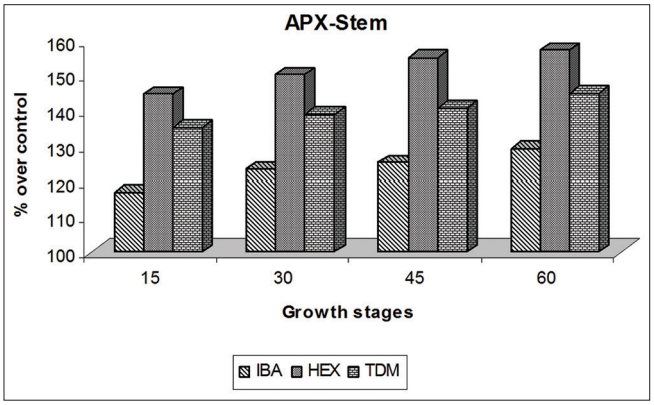

Figure 2: Indole butyric acid, hexaconazole and triadimefon induced changes in ascorbate peroxidase-stem of *Adhatoda vasica*

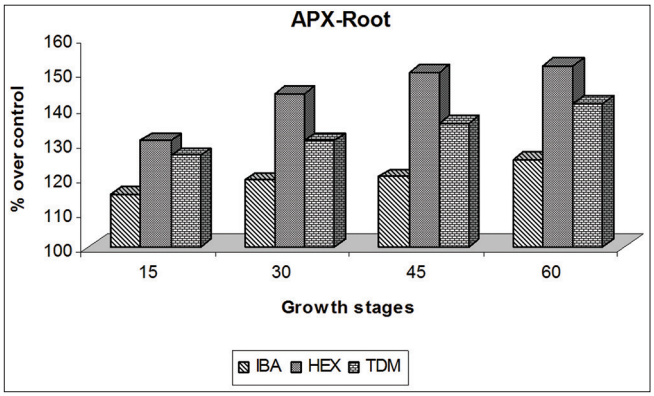

Figure 3: Indole butyric acid, hexaconazole and triadimefon induced changes in ascorbate peroxidase-root of *Adhatoda vasica*

Table 5: Effect of IBA, TDM and HEX CAT-stem in *Adhatoda vasica*

Growth stages (DAP)	Control	IBA	HEX (15 mg/L)	TDM (15 mg/L)
15	3.850±0.132	4.141±0.143	4.432±0.152	4.733±0.163
30	4.162±0.143	4.577±0.157	4.961±0.171	5.366±0.185
45	4.369±0.150	4.930±0.170	5.241±0.180	5.823±0.201
60	4.515±0.155	5.169±0.178	5.615±0.193	6.248±0.215

Values are mean±SD of four samples expressed in unit/mg protein. IBA: Indole butyric acid, TDM: Triadimefon, HEX: Hexaconazole, SOD: Superoxide dismutase, DAP: Days after planting, SD: Standard deviation, CAT: Catalase

Table 6: Effect of IBA, TDM and HEX CAT-root in *Adhatoda vasica*

Growth stages (DAP)	Control	IBA	HEX (15 mg/L)	TDM (15 mg/L)
15	4.888±0.188	5.200±0.192	5.459±0.210	6.529±0.251
30	5.106±0.196	5.511±0.211	6.093±0.234	7.182±0.276
45	5.439±0.209	5.958±0.229	6.788±0.276	7.691±0.295
60	6.373±0.245	7.048±0.271	7.276±0.280	10.110±0.348

Values are mean±SD of four samples expressed in unit/mg protein. IBA: Indole butyric acid, TDM: Triadimefon, HEX: Hexaconazole, SOD: Superoxide dismutase, DAP: Days after planting, SD: Standard deviation, CAT: Catalase

Increased CAT activity has been reported in different stress conditions in different plants viz., rice (Shaoyun, 1997), tomato (Kerdnaimongkol *et al.,* 1997) and radish (Sankari *et al.,* 2006).

There was an increase in the CAT isoforms during water stress which declined on rewatering in upland rice, and a new CAT isoform was also induced again on water stress, showing the importance of CAT in the scavenging of ROS (Srivalli *et al.,* 2003). CAT is tetrametric heme-containing enzymes that catalyze the dismutation of H_2O_2 into water and oxygen. They are localized mainly in the peroxisomes. There is a proliferation of peroxisomes during stress, which might help in scavenging of H_2O_2, and diffuse from the cytosol (Lopez-Huertas *et al.,* 2000).

The CAT could protect the plants during stress from oxidative damage caused by the ROS (Bunkelmann and Trelease, 1995). The action of SOD helps in conversion of superoxide radical into H_2O_2. Hence, all the antioxidant enzymes, with their interactive mechanism of action protect the plants from oxidative stress. The triazoles helped in increasing the activity of these antioxidant enzymes, and thereby increased the scavenging of the ROS and enhance the antioxidant potential.

REFERENCES

Asada K, Takahashi M. Production and scavenging of active oxygen in photosynthesis. In: Kyle DJ, Osmond B, Arntzen CJ, editors. Photoinhibition, Amsterdam: Elsevier; 1987. p. 227-87.

Bannister WH, Bannister JV, Barra D, Bond J, Bossa J. Evolutionary aspects of superoxide dismutase: The copper/zinc enzyme. Free Radic Res Commun 1991;12:349-61.

Beauchamp C, Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Ann Biochem 1971;44:276-87.

Biyan Z, Lifeng L, Huibai H, Zehuai W. Effects of low temperature and paclobutrazol on superoxide dismutase and abscisic acid of bananas (Musa sp.). Acta Hortic Sin 1995;22:331-5.

Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Ann Biochem 1976;72:248-53.

Bunkelmann J, Trelease RN. Molecular cloning and characterization of ascorbate peroxidase localized to the glyoxysome membrane of cotton cotyledons. Plant Physiol 1995;108:567.

Chandlee JM, Scandalios JG. Analysis of variance affecting the catalase development programme in maize scutellum. Theor Appl Genet 1984;69:71-7.

Chen G, Asada K. Ascorbate peroxidase in tea leaves occurrence of two isoenzymes and the differences in their enzymatic and molecular properties. Plant Cell Physiol 1989;30:987-8.

Cherry JH. Nucleic acid in mitochondria and enzyme changes in cotyledon of peanut seeds during germination. Plant Physiol 1963;38:440-6.

Feng Z, Guo A, Feng Z. Amelioration of chilling stress by triadimefon in cucumber seedlings. Plant Growth Regul 2003;39:277-83.

Fletcher RA, Gilley A, Sankhla N, Davis TM. Triazoles as plant growth regulators and stress protectants. Hortic Rev 2000;24:56-138.

Fornazier RF, Ferreira RR, Pereira GJ, Molina SM, Smith RJ, Lea PJ, *et al*. Cadmium stress in sugar cane in callus culture: Effects on antioxidant enzymes. Plant Cell Tissue Organ Cult 2002;71:121-31.

Gara L, Paciolla C, Tullio MC, Motto C, Arrigoni O. Ascorbate-dependent hydrogen peroxide detoxification and ascorbate regeneration during germination of highly productive maize hybrids: Evidence of an improved detoxification mechanism against reactive oxygen species. Physiol Plant 2000;109:7-13.

Gehlot HS, Upadhaya A, Davis TD, Sankhla N. Growth and organogenesis is moth bean callus was affected by paclobutrazol. Plant Cell Physiol 1989;30:933-6.

Hwang SY, Lin HW, Chern RH, Lo HF, Li L. Reduced susceptibility to water logging together with highlight stress is related to increases in superoxide dismutase and

catalase activity in sweet potato. Plant Growth Regul 1999;27:167-72.

Jaleel CA, Gopi R, Manivannan P, Sankar B, Kishorekumar A, Panneerselvam R. Antioxidant potentials and ajmalicine accumulation in *Catharanthus roseus* after treatment with giberellic acid. Colloid Surf B Biointerfaces 2007;60:195-200.

Kerdnaimongkol K, Bhatia A, Jaly RJ, Woodson WR. Oxidative stress and diurnal variation in Chilling sensitivity of tomato seedlings. Am Soc Hortic Sci 1997;122:485-90.

Kraus TE, Mc Kersie BD, Fletcher RA. Paclobutrazole-induced tolerance of wheat leaves to paraquat may involve increased antioxidant enzyme activity. J Plant Physiol 1995;145:570-6.

Leonardis SD, Dipierro N, Dipierro S. Purification and characterization of an ascorbate peroxidase from potato tuber mitochondria. Plant Physiol Biochem 2000;38:773 9.

Lopez-Huertas E, Charlton WL, Johnson B, Graham IA, Baker A. Stress induces peroxisome biogenesis genes. EMBO J 2000;19:6770-7.

Morab H, Koti RV, Chetti MB, Patil PV, Nalini AS. Role of nutrients in inducing rust resistance in soybean. Indian J Plant Physiol 2003;8:85-8.

Pinhero RG, Fletcher RA. Paclobutrazol and ancymidol protect corn seedlings from high and low temperature stresses. Plant Growth Regul 1994;15:47-53.

Pinhero RG, Rao MP, Paliyath G, Murr DP, Fletcher RA. Changes in activities of antioxidant enzymes and their relationship to genetic and paclobutrazol induced chilling tolerance of maize seedlings. Plant Physiol 1997;114:695-704.

Porcel R, Barea JM, Ruiz-Lozano JM. Antioxidant activities in mycorhizal soybean plants under drought stress and their possible relationship to the process of nodule senescence. N Phytol 2003;157:135-43.

Ramanayake SM, Maddegoda KM, Vitharana MC, Chaturani GD. Root induction in three species of bamboo with different rooting abilities. Sci Hortic 2008;118:270-3.

Sankari S, Gopi R, Gomathinayagam M, Sridharan R, Somasundaram R, Panneerselvam R. Responses of triazoles on growth and antioxidant levels in white radish. Indian J Appl Pure Biol 2006;21:77-80.

Sankhla D, Sankhla N, Upadhyaya A, Kachnwaha S, Mali S, Dinesh R, Joshi S. Effect of uniconazole on growth and activities of active oxygen processing enzymes in *Zizyphus* seedlings. Proc Plant Growth Regul Soc Am 1997;24:208-12.

Senaratna T, Mackay CE, Mc Kersie BD, Fletcher RA. Triazole-induced chilling tolerance in tomato and its relationship to antioxidant content. J Plant Physiol 1988;133:56-61.

Shaoyun L. The activities of protective enzymes of rice seedlings subjected to drought and their relationship to drought tolerance. J S China Agric Univ 1997;18:21-5.

Smirnoff N. The role of active oxygen in the response of plants to water deficit and desiccation. N Phytol 1993;125:27-58.

Srivalli B, Viswanathan C, Chopta RK. Antioxidant defense in response to abiotic stresses in plants. J Plant Biol 2003;30:121-39.

Vanacker H, Carver TL, Foyer CH. Pathogen-induced changes in the antioxidant status of the apoplast in barley leaves. Plant Physiol 1998;117:1103-14.

Vranova E, Inze D, Breusegem F, Van Breusegem F, Smirnoff N. Signal transduction during oxidative stress. J Exp Bot 2002;53:1227-36.

Willekens H, Langebartels C, Tire C, Vanmontagu M, Inze D, Vancamp W. Differential expression of catalase gene in *Nicotiana plumbagini* folia L. Natl Acad Sci USA 1994;91:10450-4.