Global Journal of Molecular Sciences 4 (2): 63-67, 2009
ISSN 1990-9241

Responses of Non-Enzymatic Antioxidant Potentials in Radish by Triazole Compounds

R. Sridharan, P. Manivannan, P.V. Murali, R. Somasundaram and R. Panneerselvam

Stress Physiology Lab, Department of Botany,
Annamalai University, Annamalainagar 608 002, Tamil Nadu, India

Abstract: A study was undertaken to estimate the effect of triazole viz. triadimefon and hexaconazole on the non-enzymatic antioxidant potential of radish (*Raphanus sativus* L.). Triadimefon (TDM) 10 mg^{l-1} and hexaconazole (HEX) 5 mgL^{-1} who treated to per plant in one pot, on 8, 23,38 and 53 days after sowing (DAS). The non-enzymatic antioxidant contents like ascorbic acid (AA), reduced glutathione (GSH) and α-tocopherol (α-toc) (RBF) were extracted and assayed on 30 and 60 DAS form shoot and tuber of both control and triazole treated plants. Triazole treatment increased the non-enzymatic antioxidants on the plant *Raphanus sativus*.

Key words: Triazole · Antioxidant · *Raphanus sativus*

INTRODUCTION

Free radical is a chemical species with an unpaired electron that can be neutral, positive or negatively charged. Although there alone four common oxygen metabolites in biologic system of free radicals and they are (i) superoxide anion (O^{-2}), (ii) hydrogen peroxide (H_2O_2), (iii) hydroxyl radical (OH) and (iv) single oxygen (O_2) [1]. These free radicals can be formed via enzymatic reactions from non-autooxidiable substances such as halo alkanes, phenols, nitro compounds and aromatic amines [2]. Free radicals one also formed when cell constituents are exposed to ionizing radiation [3]. In higher plants dissipation of excess photochemical energy is an immediate and finely turned response which occurs through heat irradiation, alternate sinks for photosynthetic elements and down regulation to photo system II [4-7]. The photo reduction of oxygen is an important alternative sink for the consumption of excess energy but is associate with an increase in the generation of reactive oxygen intermediates such as hydrogen peroxide (H_2O_2) superoxide anion (O_2) hydroxyl radical (OH) and oxygen (O_2) [8-10] superoxide anion can also be formed during the conditions of photo inhibition whereby an electron from photo system I is accepted by dioxygen, producing superoxide [4-9]. Stress condition also results in an elevated rate of single electron transport chain to molecular oxygen resulting in the formation of superoxide radicals and consequential hydrogen peroxide and hydroxyl radical production [7-10].

Two main classes of plant defenses against oxidatative stress can be classified as non-enzymatic and enzymatic systems. The first class (non-enzymatic) consists of small molecules such as ascorbic acid, glutathione, α-tocopherol, reduced glutathione and riboflavin which can react directly with the reactive oxygen species, second class (enzymatic) defenses have the capacity to elimate superoxides by the enzymes superoxide dismutase, ascorbate peroxidase, peroxidase and catalase [5-7] some non-enzymatic and enzymic antioxidant defenses include enzymes capable of removing, neutralizing or scavenging free radicals and oxyintermediates. Without there defenses, plants could not efficiently convent solar energy to chemical energy [9,10].

Triazole compounds affected the activities of several enzymes, especially those related to detoxification of active oxygen species and antioxidant metabolism [11-13]. They also protect plants from biotic and abiotic stresses including fungal pathogen, drought, salinity, air pollutions and low and high temperature [12-15] and also it affect the isoprenoid pathway and alter the level of certain plant hormones by inhibiting gibberellin synthesis, reducing ethylene evolution and increasing cytokinin, kinetin levels some of the previous works carried out in our lab revealed the morphological and physiologic changes associated with triazole treatment in various plants, include inhibition of plant growth, increased chlorophyll levels, enlarged chloroplasts, thicker leaf tissue, increased root to shoot ratio and increased the

Corresponding Author: Dr. R. Panneerselvam, Department of Botany, Annamalai University,
Annamalainagar 608 002, Tamil Nadu, India

antioxidant potentials [11-17]. Therefore, there is a need to investigate the efficient of this compound in the enhancement of antioxidant potentials in white radish plants in order increase their medicinal properties and making them valuable tuber crop. Hence, this study aims to evaluate the ability of triazole to enhance the antioxidant potentials and membrane integrity, with special emphasis to antioxidant potential and membrane integrity constituents. *Raphanus sativus* (white radish) is an important vegetable crop in India and south east countries. The leaves and tubers of radish are used to prepare salad and also cooked as vegetables. It is rich in vitamin 'C' and minerals like sulphur. It is also used as a medicine in currying liver disorders and jaundice. This tuber crops are rich source of energy for people living under sustenance level since, it is available at a cheaper price for the poor people.

MATERIALS AND METHODS

The seeds were obtained from mahyco-Maharashtra hybrid seeds co. Ltd. Maharashtra. India and planted at the botanical garden of the Annamalai University. Two seeds were sown in each plastic pot of 30cm diameter and 30cm height containing 3 kg of soil mixture containing red soil, sand and farm yard manure at 1:1:1 ratio. Then the seedling thinned to one per pot on 6th day after sowing. Triadimefon was obtained from Bayer, Germany and hexaconazole was obtained from imperial chemical industries, England.

10mg L^{-1} triadimefon and 5mg L^{-1} hexaconazole were used for this study. The seedlings were treated with deionized water (control), 10mg L^{-1} triadimefon and 5mg L^{-1} hexaconazole solution alone per plant on 8, 23, 38 and 53 days after sowing (DAS). Then the plants were harvested randomly on 30 and 60 DAS and separated into tuber and shoot and used for extraction and assay of antioxidant potentials of radish plant.

Ascorbic Acid Content: Ascorbic acid (AA) content was assayed as described by Omaye *et al*. [18]. The AA content was determined using a standard curve prepared with AA and the results were expressed in mg g^{-1} dry weight (DW).

Reduced Glutathione: The GSH content was assayed as described by Griffith and Meister [19]. GSH contents were expressed in μg g^{-1} fresh weight (FW).

α-Tocopherol Content: α-Tocopherol (α-toc) activity was assayed as described by Backer *et al*. [20]. The α-toc content was calculated using a standard graph made with known amount of α-toc and expressed in mg g^{-1} fresh weight (FW).

Statistical Analysis: Statistical analysis was performed using the one-way analysis of variance (ANOVA) followed by the Duncan's multiple range test (DMRT). The values mean ±SD for six samples in each group p values < 0.05 were considered as significant.

RESULT AND DISCUSSION

Triazole treatment increased the non-enzymatic antioxidant AA, content (Table 1, Fig. 1) in shoot and tuber when compared to control plant. Among the organs tuber had higher AA content when compared to the shoot. α-tocopherorl content also increase in both organs by triazole treatment, when compared to compared to control plant. Like wise reduced glutothione content also. increased in both organ by 15mg triadimefon and 5mg hexaconazole treated plant when compare to control plant among the treatments triadimefon had higher content when compared to hexaconazole treated one. AA has been proposed to have roles in regulation of photosynthesis [21], cell expansion and trans-membrane electron transport [22]. Triazole increased the level of the antioxidant like, AA, α-tocopherol in seedlings and protected membranes by preventing or reducing oxidative damage [12]. AA acts as an antioxidant, protecting cells against oxidative stress. AA has the capacity to eliminate different AOS including signet oxygen, super oxide and hydroxyl radicals.

α-Tocopherol content increased with triazole treatment in radish (Table 2, Fig. 2). It is synthesized in the chloroplasts and closely associated with the thylokoid membrane of the chloroplasts. The thylakoid membrane, which contains substantial unsaturated lipids, is one of the major sites of oxidative damage through lipid peroxidation. [7,8]. Triazole increased the antioxidant such as α-tocopherol and ascorbate levels and enhanced activities of peroxidase and catalase in tomato. The GSH content was found increased under triazole application in radish (Table 3, Fig. 3). The increase in GSH can be correlated with its ability to scavenge single oxygen, peroxides and hydroxyl radicals and is involved in recycling of AA in the ascorbate glutathione pathway in

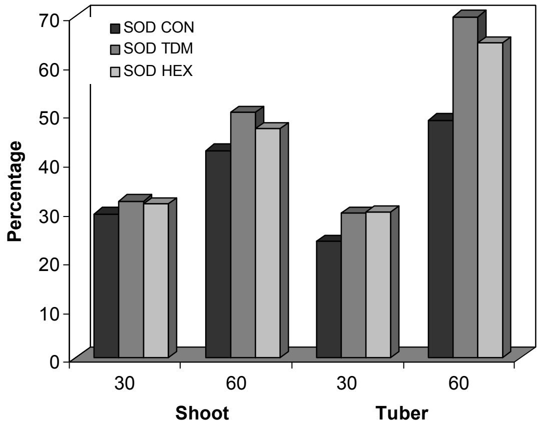

Fig. 1: Effect of 10 mg L-1 TDM and 5mg L-1 HEX on AA content of shoot and tubers of radish plant (values one given as mean ± SD of six replicates expressed in mg g-1 F.W)

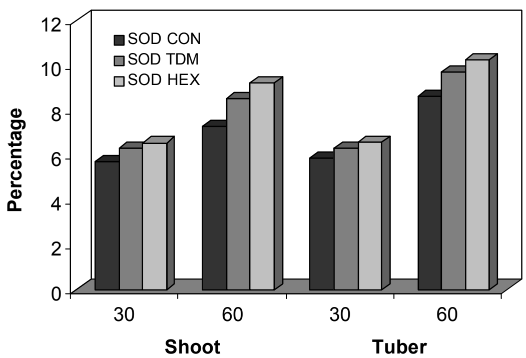

Fig. 2: Effect of TODM and HEX on a - TOC content of shoot and tuber of radish plant (values are given as mean ± SD of six replicates expressed in mg g-1 F.W)

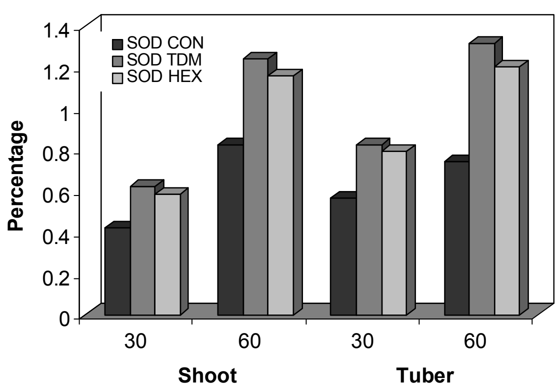

Fig. 3: Effect of TDM and HEX on GSH content of shoot and tubers of radish plant (values one given as mean ± SD of six replicates expressed in mg g-1 F.W)

Table 1: Effect of 10 mg L-1 TDM and 5mg L-1 HEX on AA content of shoot and tubers of radish plant (values one given as mean ± SD of six replicates expressed in μg g-1 F.W)

Das	Control	TDM 10mg L^{-1}	HEX 5-mg L^{-1}
Shoot			
30	29.46	32.09 (108.94)	31.50 (106.58)
60	42.45	50.26 (118.42)	46.91 (110.53)
Tuber			
30	23.78	29.57 (124.36)	29.82 (125.42)
60	48.68	69.84 (143.48)	64.42 (132.34)

Table 2: Effect of TODM and HEX on α-TOC content of shoot and tuber of radish plant (values are given as mean ± SD of six replicates expressed in μg g-1 F.W)

Das	Control	TDM 10mg L^{-1}	HEX 5-mg L^{-1}
Shoot			
30	5.72	6.31 (110.41)	6.54 (114.36)
60	7.27	8.50 (116.92)	9.21 (126.76)
Tuber			
30	5.85	6.28 (107.46)	6.58 (112.64)
60	8.62	9.71 (112.65)	10.22 (118.65)

Table 3: Effect of TDM and HEX on GSH content of shoot and tubers of radish plant (values one given as mean ± SD of six replicates expressed in μg g-1 F.W)

Das	Control	TDM	HEX
Shoot			
30	0.421	0.621	0.585
60	0.821	1.241	1.158
Tuber			
30	0.562	0.821	0.790
60	0.740	1.315	1.202

chloroplasts[23]. One of the triazole of ketoconazole treated plants can increase the riboflavin content can increase the membrane stability and prevent membrane degradation due to oxidation of the lipid component of the membrane by the reactive oxygen species [24-27]. It is involved in lipid peroxidation and oxidized to act as an electron acceptor. From out results, it can be concluded that the TDM, HEX application can enhance largely the non-enzymatic antioxidant quantity, which is great importance in impairing economic values of the plant.

REFERENCE

1. Davis, D.G. and H.R. Swanson, 2001. Activity of stress-related enzymes in the perennial weed leafy spurge (*Euphorbia esula* L.). Env. Exp. Bot., 46: 95-108.

2. Gehlot, H.S., A. Upadhaya, T.D. Davis and N. Sankhla, 1989. Growth and organonensis is moth bean callus was affected by paclobutrazol, Plant Cell Physiol., 30: 933-936.
3. Asada, K., 1992. Ascorbate peroxidase-a hydrogen-scavenging enzyme in plants, Physiol. Plant, 85: 235-241.
4. Mehlhorn, H., M. Lelandais, H.G. Korth and C.H. Foyer, 1996. Ascorbate is the natural substrate for plant peroxidases, FEBS. Lett., 378: 203-206.
5. Kraus, T.E., B.D. Mc Kersie and R.A. Fletcher, 1995. Paclobutrazol induced tolerance of wheat leaves to paraquat may involve increased antioxidant enzyme activity, J. Plant Physiol., 145: 570-576.
6. Jaleel, C.A., R. Gopi, G.M.A. Lakshmanan and R. Panneerselvam, 2006. Triadimefon induced changes in the antioxidant metabolism and ajmalicine production in *Catharanthus roseus* (L.) G. Don, Plant Sci., 171: 271-276.
7. Jaleel., C.A., R. Gopi, P. Manivannan and R. Panneerselvam, 2007b. Responses of antioxidant defense system of *Catharanthus roseus* (L.) G. Don. to paclobutrazol treatment under salinity, Acta. Physiol. Plant, 29: 205-209.
8. Jaleel, C.A., R. Gopi, P. Manivannan and R. Panneerselvam, 2007c. Responses of antioxidant defense system of *Catharanthus roseus* (L.) G. Don. to paclobutrazol treatment under salinity, Acta Physiol. Plantarum, 29: 205-209.
9. Jaleel, C.A., P. Manivannan, A. Kishorekumar, B. Sankar, R. Gopi, R. Somasundaram and R. Panneerselvam, 2007d. Alterations in osmoregulation, antioxidant enzymes and indole alkaloid levels in *Catharanthus roseus* exposed to water deficit, Colloids Surf. B: Biointerfaces, 59: 150-157.
10. Manivannan, P., C.A. Jaleel, A. Kishorekumar, B. Sankar, R. Somasundaram, R. Sridharan and R. Panneerselvam, 2007a. Propiconazole induced changes in antioxidant metabolism and drought stress amelioration in *Vigna unguiculata* (L.) Walp, Colloids Surf. B: Biointerfaces, 57: 69-74.
11. McDermott, J.H., 2000. Antioxidant Nutrients: Current dietary recommendations and research update, J. Am. Pharm. Assoc., 40: 785-799.
12. Slater, T.F., 1984. Free radical mechanisms in tissue injury, Biochem. J., 222: 1-15.
13. Foyer, C.H., 1996. Oxygen metabolism and electron transport in photosynthesis. In: Oxidative stress and the molecular biology of antioxidant defenses (ed.). J. Scandalios. Cold spring Harbor Laboratory Press, New York, pp: 587-621.
14. Huner, N.P.A., G. Oquist and F. Sarhan, 1998. Energy balance and acclimation to light and cold, Trends Plant Sci., 3: 224-230.
15. Dodge, A.D., 1971. The mode of action of bipyridylium herbicides, paraquat and diquat, Endeavour, 30: 130-355.
16. Scandalios, J.G., 1993. Oxygen stress and superoxide dismutase, Plant Physiol., 101: 7-12.
17. Steffen, K.L., 1991. Avoidance of photooxidative stress, balancing energy flux within the chloroplast. In active oxygen/oxidative stress and plant metabolism (ed.). E.J. Pell and K.L. Steffen. Current topics in plant physiology, Amer. Soc. Plant Physiol. Rockville., 6: 119-130.
18. Omaye, S.T., J.D. Turn bull and H.E. Sauberilick, 1979. Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Method Enzymol., 62: 3-11. Academic Press: New York.
19. Griffith, O.W. and A. Meister, 1979. Potent and specific inhibition of glutathione synthesis by buthionine sulfoximine (s-n-butylhomocysteine sulfoximine). J. Biol. Chem., 254: 7558-7560.
20. Backer, H., O. Frank, B. De Angells and S. Feingold, 1980. Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol. Nutr. Res. Internat, 21: 531-536.
21. Willekens, H., C. Langebartels, C. Tire, M. Vanmontagu, D. Inze and W. Vancamp, 1994. Differential expression of catalase gene in Nicotiana plumbagini folia L. Proceedings of the National Academy of Sci., USA 91: 10450-10454.
22. Shaaltiel, Y. and J. Gressel, 1986. Multi enzyme oxygen radical detoxifying system correlated with paraquat resistance in Conyza bonariensis, Pesti. Biochem. Physiol., 26: 22-28.
23. Ella, E.S., O. Ito and K. Tanaka, 1998. Role of ascorbate and active oxygen scavenging enzymes in post submergence injury in rice 14. Ann. Sci. Con. Fed. Crop. Sci. Soc. Philippines, 23: 82.

24. Cooper, M., F.A. Van Eeuwijk, S.C. Chapman, D.W. Podlich and C. Löffler, 2006. Genotype by-environment interactions under water-limited conditions. In: Ribaut JM (ed) Drought Adaptation in Cereals. Haworth Press, Inc, pp: 51-96.
25. Kraus, T.E. and R.A. Fletcher, 1994. Paclobutrazol protects wheat seedlings from heat and paraquat injury. Is detoxification of active oxygen involved? Plant Cell Physiol., 35: 45-52.
26. Kraus, T.E., B.D. Mc Kersie and R.A. Fletcher, 1995. Paclobutrazol induced tolerance of wheat leaves to paraquat may involve increased antioxidant enzyme activity, J. Plant Physiol., 145: 570-576.
27. Kopyra, M. And E.A. Gwozdz, 2003. Antioxidant enzymes in paraquat and cadmium resistant cell lines of horseradish, Biol. Lett., 40: 61-69.