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Differential effects of hexaconazole and paclobutrazol on the foliage characteristics of Chinese potato (*Solenostemon rotundifolius* Poir., J.K. Morton)

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ABSTRACT In the present investigation, the impact of hexaconazole (HEX) and paclobutrazol (PBZ), triazole fungicides, on the leaf anatomy of Chinese potato (*Solenostemon rotundifolius* Poir., J.K. Morton) was studied. The thickness of leaf, upper and lower epidermis, number of palisade and spongy cells per unit area, number of chloroplast per palisade and spongy cells, number of stomata in upper and lower epidermis, stomatal pore length and width were observed in both control and treatments. Leaves treated with HEX and PBZ showed several variations in the anatomical characteristics.

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KEY WORDS

Solenostemon rotundifolius
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Solenostemon rotundifolius Poir., Morton is one of the important vegetable crops belongs to the family Labiatae and cultivated in many parts of the world for its edible tubers. In the phylogenetic and taxonomic studies of the higher plants, anatomical characteristics have a profound role. The structure and ontogeny of stomata in different plants will vary with the application of different growth regulators (Gupta et al. 2004). Leaf anatomy is an important feature for internal water balance of the plants. The anatomical characteristics were found changed due to the application of growth regulators. Triadimefon treated mulberry plants showed great variations in the stomatal structure and functions (Sreethar 1991). Triazole compounds are systemic fungicides, which have plant growth regulating properties (Fletcher and Hofstra 1990).

The impact of triazole plant growth regulators on hormonal changes (Ye et al. 1995; Fletcher et al. 2000), photosynthetic rates (Panneerselvam et al. 1997) and enzyme activities (Muthukumarasamy and Panneerselvam 1997) have been reported. The plant growth regulating properties of triazoles are mediated by their inference with isoprenoid pathway and shift in the balance of plant hormones (Fletcher et al. 2000). Paclobutrazol (PBZ) increased the leaf thickness in rape plant due to elongated palisade cells (Zhou et al. 1993) and wheat leaves (Sopher et al. 1999). The triazole compounds protect plants from chilling stress (Feng et al. 2003), salt stress (Muthukumarasamy et al. 2000) as well as exhibit powerful fungicidal properties (Davis and Curry 1991). Previous works proved the ability of triazole compounds such as triadimefon (TDM) in enhancing the antioxidant potential in plants like *Catharanthus roseus*

(Jaleel et al. 2006). The information available so far about the effect of triazole on leaf anatomy in plants is less. Hence it is aimed to understand the effect of triazole compounds such as hexaconazole (HEX) and PBZ in *S. rotundifolius*. The objectives of the present investigation were to study the impact of HEX and PBZ on leaf thickness, thickness of upper and lower epidermis, number of palisade and spongy cells per unit area, number of chloroplast per palisade and spongy cells and number of stomata and stomatal pore length in *S. rotundifolius* plants.

Materials and Methods

The tubers of *S. rotundifolius* were obtained from Central Tuber Crop Research Institute (CTCRI), Kerala and planted in the Botanical Garden of Annamalai University, Tamil Nadu. In the present investigation, a field experiment was conducted in Randomized Block Design (RBD) with 7 replicates in *S. rotundifolius* during 2004-2005. Each plant was treated with 10 mg l⁻¹ (active principles) of HEX and PBZ on vegetative stages like 80, 100 and 140 days after planting (DAP). The treatments were given by soil drenching. The fully expended mature leaves of plants, which emerged after the treatments were collected randomly on 90, 120 and 150 DAP from each concentration and control.

The leaves were washed thoroughly with water and fixed them in formalin: acetic acid: ethyl alcohol (5:5:90 v/v/v). Thin transverse-sections were taken, stained and observed under calibrated light microscope and the thickness of leaf was measured by precalibrated ocular micrometer. Epidermal peels were taken out from the basal, middle and apical regions by adopting direct peel method. The epidermal peels were

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stained with 1% Delafield's haematoxylin and mounted in 50% glycerin (Dwivedi and Singh 1990). The observations were taken on 90, 120 and 150 DAP in seven replicate peels in each treatments. The leaf, upper and lower epidermis thickness expressed in micrometers and number of stomata in upper and lower epidermal cells per unit area were calculated by using the generally followed formula of Metcalfe and Chalk (1979). The length and width of stomatal pores were measured randomly in each treatment on the lower surface. The number of spongy cells per unit area and chloroplast per palisade and spongy cells were also calculated separately to find out the relative effect of triazole compounds.

Statistical analysis

The data were analysed using the analysis of variance (ANOVA) as described by the method outlined by Ridgman (1975). Means were compared between treatments from the error mean square by Least Significant Difference (LSD) at the $P \leq 0.05$ and $P \leq 0.01$ confidence level using Tuckeys (1953) test.

Results and Discussion

Thickness of leaves treated with triazoles was increased to a level higher than that of control leaves in *S. rotundifolius* plants. Among the triazole treatments there is no significant variation in thickness of leaf, upper and lower epidermis (Table 1). TDM treatments increased the thickness of leaf in plants (Asami et al. 2000).

The number of cells per unit area in the palisade spongy

layers and chloroplast number per cells in the leaves increased by the HEX and PBZ treatments when compared to control leaves (Table 2). Among the triazole treatments, there was no significant difference in these characters. Increased mesophyll thickness, chloroplast size and level were reported in wheat with TDM (Gao et al. 1988). Triazoles increased the cytokinin levels in various plants like cucumber (Fletcher and Arnold 1986). The increased cytokinin level also can accelerate chloroplast differentiation and chlorophyll production and also protect the integrity of chlorophyll molecule (Fletcher et al. 2000).

Several variations like stomatal pore length, width and unequal accessory cells were observed in treated leaves. In the case of untreated leaves all stomata are open and have large stomatal pore length but width of stomata gradually decreased in the leaves of treated plants (Table 2). Triazole treatments caused the closure of stomata in bean (Fletcher and Hofstra 1988). Thiapenthenol reduced stomatal opening and reduced water consumption in mesophyll, a transient raise in the ABA content in bean (Asare-Boamah et al. 1986). This increased ABA content might have induced the stomatal closure as observed in uniconazole treated *Phaseolus vulgaris* (Mackay et al. 1990).

From the above observations it is clear that the triazole compounds affected stomatal pore length and width, stomatal pore size, thickness of upper and lower epidermis and the number of stomata, palisade, spongy cells, chloroplast per palisade and spongy cells. This is in accordance with the previous reports of Bora et al. (2002) and Gupta et al. (2004). It is previously reported that the application of PBZ can in-

Table 1. Effect of triazole fungicides on leaf anatomical characteristics of *S. rotundifolius*.

Growth stages (DAP)	Control	HEX 10 mg l ⁻¹	PBZ 10 mg l ⁻¹	LSD
Leaf thickness (μ meter)				
90	62.18*	74.4*	66.13*	0.87
120	88.48*	89.40*	88.33*	1.24
150	89.61*	89.91*	86.15*	1.31
Thickness of upper epidermis (μ meter)				
90	11.83*	14.36*	14.15*	0.67
120	12.24*	13.04*	14.31*	0.62
150	13.02*	16.03*	13.67*	0.58
Thickness of lower epidermis (μ meter)				
90	8.43 ^{NS}	7.19 ^{NS}	7.11 ^{NS}	0.14
120	9.56*	8.94*	9.94*	0.12
150	9.99*	10.12*	9.99*	0.18
Number of palisade cells per unit area				
90	21.54 ^{NS}	22.71 ^{NS}	21.61 ^{NS}	0.18
120	22.61*	23.41*	24.48*	0.20
150	23.13*	25.67*	25.01*	0.21
Number of spongy cells per unit area				
90	37.04**	42.10**	41.41**	0.86
120	38.99**	45.61*	44.31*	0.94
150	41.67**	49.41*	49.01*	1.02

HEX – hexaconazole; PBZ – paclobutrazol; LSD – least significant difference; NS – non significant; *significant at 0.05 level; **significant at 0.01 level

Table 2. Effect of triazole fungicides on leaf anatomical characteristics of *S. rotundifolius*.

Growth stages (DAP)	Control	HEX 10 mg l ⁻¹	PBZ 10 mg l ⁻¹	LSD
Number of chloroplast per palisade cells				
90	12.14*	13.11*	13.01*	0.18
120	14.15 ^{NS}	14.30 ^{NS}	14.90*	0.26
150	14.17 ^{NS}	14.20 ^{NS}	14.84*	0.22
Number of chloroplast per spongy cells				
90	11.50 ^{NS}	11.36 ^{NS}	12.95*	0.14
120	12.51*	14.49*	14.34*	0.15
150	13.34*	15.42*	15.59*	0.28
Number of stomata in lower epidermis (nos/mm ² leaf area)				
90	10.61*	11.59*	11.65*	0.17
120	12.41**	15.34**	15.50**	0.21
150	14.41**	17.45**	17.20**	0.26
Lower stomatal pore length (μ meter)				
90	12.04**	10.51**	10.35**	0.12
120	13.01*	12.34*	11.75**	0.16
150	13.99*	12.98*	12.90*	0.18
Lower stomatal pore width (μ meter)				
90	3.41*	2.52*	2.63*	0.08
120	3.50*	2.93*	3.00*	0.09
150	3.67*	3.10*	3.21*	0.09

HEX – hexaconazole; PBZ – paclobutrazol; LSD – least significant difference; NS – non significant; * – significant at 0.05 level; ** – significant at 0.01 level

crease the xylem water potentials (Thakur et al. 1998) and can increase the cytokinins under drought conditions (Zhu et al. 2004). The judicious application of triazole like HEX and PBZ may prove to be a useful tool for decreasing transpiration and inturn inducing drought avoidance mechanisms. It can be concluded that triazole such as HEX and PBZ may be useful to trigger drought avoidance mechanisms in plants like *S. rotundifolius*.

References

- Asami TYKM, Nagata N, Yamagishi K, Takatsuto S, Fujioka S, Muroushi N, Yamaguchi I, Yoshida S (2000) Characterization of brassinazole, a triazole-type brassinosteroid, a biosynthesis inhibitor. *Plant Physiol* 123:93-100.
- Asare-Boamah NK, Hofstra G, Fletcher RA, Dumbroff EB (1986) Triadimefon protects bean plants from water stress through its effects on ABA. *Plant Cell Physiol* 27:383-390.
- Bora KK, Mathur SR, Ganesh R, Bohra SP (2002) Effect of paclobutrazol on water loss of excised groundnut seeds. *Bioregulators and Applied Plant Biotechnology*. Pointer Publishers, India, pp. 58-64.
- Davis TD, Curry EA (1991) Chemical regulation of vegetative growth. *Crit Rev Plant Sci* 10:151-188.
- Dwivedi JN, Singh BB (1990) Stains or Dyes. In Dwivedi JN, Singh BB, eds., *Essentials of Plant Techniques*, Scientific publishers, Jodhpur, India, p. 9.
- Feng Z, Guo A, Feng Z (2003) Amelioration of chilling stress by triadimefon in cucumber seedlings. *Plant Growth Regul* 30:277-283.
- Fletcher RA, Arnold V (1986) Stimulation of cytokinins and chlorophyll synthesis in cucumber cotyledons by triadimefon. *Physiol Plant* 66:197-201.
- Fletcher RA, Hofstra G (1988) Triazoles as potential plant protectants. In Berg D, Plemple M, eds., *Sterol biosynthesis inhibitors*. Ellis Horwood Ltd., Cambridge, England, pp. 321-331.
- Fletcher RA, Hofstra G (1990) Improvement of uniconazole induced protection in wheat seedlings. *J Plant Growth Regul* 9:207-212.
- Fletcher RA, Gilley A, Davis TD, Sankhla N (2000) Triazoles as plant growth regulators and stress protectants. *Hort Rev* 24:55-138
- Gao J, Hofstra G, Fletcher RA (1988) Anatomical changes induced by triazoles in wheat seedlings. *Can J Bot* 66:1178-1185.
- Gupta SK, Raghava RP, Raghava N (2004) Stomatal studies of cowpea (*Vigna unguiculata* (L.) Walp.) cultivars in relation to bromiconazole. *J Ind Bot Soc* 83:116-119.
- Jaleel CA, Gopi R, Alagu Lakshmanan GM, Panneerselvam R (2006) Triadimefon induced changes in the antioxidant metabolism and ajmalicine production in *Catharanthus roseus* (L.) G. Don. *Plant Sci* 171:271-276.
- Mackay CE, Hall JC, Hofstra G, Fletcher RA (1990) Uniconazole induced changes in abscisic acid, total amino acid and proline in *Phaseolus vulgaris*. *Pest Biochem Physiol* 37:71-82.
- Metcalfe CR, Chalk L (1979) *Anatomy of the dicotyledons*, 2nd eds., vol. 1, Clarendon Press, Oxford.
- Muthukumarasamy M, Dutta Gupta S, Panneerselvam R (2000) Enhancement of peroxidase, polyphenol oxidase and superoxide dismutase activities by triadimefon in NaCl stressed *Raphanus sativus* L. *Biol Plant* 43:317-320.
- Muthukumarasamy M, Panneerselvam R (1997) Amelioration of NaCl stress by triadimefon in peanut seedlings. *Plant Growth Regul* 22:157-162.
- Panneerselvam R, Muthukumarasamy M, Karikalan L (1997) Triadimefon enhances growth and net photosynthetic rate in NaCl stressed plants of *Raphanus sativus* L. *Photosynthetica* 34:605-609.
- Ridgman WJ (1975) *Experimentation in biology: An introduction to design and analysis*. Thomson Litho Ltd., East Kilbride, Scotland, pp. 81-100.
- Sopher RC, Krol M, Huner NPA, Fletcher RA (1999) Chloroplastic changes associated with paclobutrazol induced stress protection in maize seedling. *Can J Bot* 77:1-12.
- Sreethar VM (1991) Proline accumulation and reduced transpiration in the leaves of triazole treated mulberry plant. *Indian Bot Report* 101:1-5.
- Thakur A, Thakur PS, Singh RP (1998) Influence of paclobutrazol and triacanthanol on growth and water relations in olive varieties under water stress. *Indian J Plant Physiol* 3:116-120.
- Tuckey JW (1953) *The problem of multiple comparisons*. Princeton University Press, New Jersey.
- Ye QF, Zhou WJ, Xi HF, Fang JY (1995) Effect of S-3307 on levels of endogenous (IAA, ABA and ZT) and some physiological of rape seedlings. *Acta Agri Zhejiang* 7:451-456.
- Zhou WJ, Shen HC, Xi HF, Ye QF (1993) Studies on the regulation mechanism of paclobutrazol to the growth of rape plant. *Acta Agri Zhejiang* 19:316-320.
- Zhu LH, Peppal A, Li XY, Welander M (2004) Changes of leaf water potential and endogenous cytokinins in young apple trees treated with or without paclobutrazol under drought conditions. *Sci Hort* 99:133-141.