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EFFECT OF TRIAZOLE COMPOUNDS ON THE ANATOMICAL CHARACTERISTICS OF *AMORPHOPHALLUS CAMPANULATUS* BLUME

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The effect of three triazole compounds namely as triadimefon (TDM), paclobutrazol (PBZ) and propiconazole (PCZ) on the leaf anatomical characteristics were studied in the *Amorphophallus campanulatus* plants. Compared to the leaves of untreated control plants, the leaves of triazole treated plants showed several variations like leaf thickness, number of stomata, pore length, and width of stomata, number of spongy cells, palisade cells and number of chloroplast per palisade and spongy cells. From these observations, it can be concluded that, the triazoles has an impact on anatomical characteristics of *A. campanulatus*.

Keywords: *Amorphophallus,* Triazoles, Leaf anatomy.

Elephant foot yam (*Amorphophallus campanulatus* Blume.) is one of the high yielding tuber crops. The corms (tubers) are rich source of carbohydrate and used as vegetable. They are also used in certain ayurvedic medicinal preparations recommended for the cure of piles and dysentery (Sambamurty and Subramanyam, 1989).

Plant growth regulators play a regulatory role in many physiological processes associated with growth and development of plants (Thakur and Thakur, 1993). The use of plant growth regulators resulted in a significant increase in growth and yield of vegetable crops (Singh *et al.,* 1995). They may be natural or synthetic compounds. Substances like triadimefon (TDM), paclobutrazol (PBZ) and uniconazole (UNZ) were shown to have strong plant growth regulating properties and are waiting to be listed as PGR's (Raghava and Raghava, 1998).

Triazole compounds such as TDM, propiconazole (PCZ) and PBZ etc. are widely used as fungicides and they also posses varying degrees of plant growth regulating properties (Fletcher *et al.,* 2000). Triazoles have been called "plant multiprotectants", because of their ability to induce stress tolerance in plants (Kraus and Fletcher, 1994). Protection of plants from apparently unrelated stress by triazoles is mediated by a reduction in free radical damage and increase in antioxidant potential (Kraus *et al.,* 1995). Triazoles affect the isoprenoid pathway. They alter the levels of certain plant hormones by inhibiting gibberellin synthesis, reducing ethylene evolution and increasing cytokinin levels (Kamountsis and Sereli, 1999). Triazole treated plants have a more efficient free-radical scavenging system that enables them to detoxify active oxygen species (Kopyra and Gwozdz, 2003). Some of the earlier works carried out in our lab revealed the morphological and physiological changes associated with triazole treatment in various plants, include the inhibition of plant growth, decreased internodal elongation, increased chlorophyll levels, enlarged chloroplasts, thicker leaf tissue, increased root to shoot ratio, increased antioxidant potentials and an enhancement in alkaloid production (Muthukumarasamy and Panneerselvam, 1997; Panneerselvam *et al.,* 1997; 1998; Gopi *et al.,* 1999; Muthukumarasamy *et al.,* 2000; Gopi *et al.,* 2005; Jaleel *et al.,* 2006a; 2006b). The information available so for about the effect of triazoles on the leaf anatomy in *Amorphophallus* plants is scanty. Therefore, the present investigations was carried out with an objective of evaluating the effect of 3 triazole compounds on the anatomical characters in *A. campanulatus*.

MATERIALS AND METHODS

Field experiments were conducted during the

crop growth seasons in the every year month of September to February by the Completely Randomized Block Design (CRBD) methods. The plants were treated with three different triazole compounds like TDM, PBZ and PCZ @ 20 mg l^{-1} $plant^{-1}$ (active principle) by soil drenching method on 30, 70 and 110 days after planting (DAP). The fully mature leaves of the plants were collected on 40, 120 and 200 DAP from treated and untreated plants. The leaves were washed thoroughly with water and fixed in formalin acetic acid (FAA) and alcohol. For studying the internal structure of leaf was studied by taking thin transverse sections with microtome. They were stained and observed under a calibrated light microscope. The thickness of leaf was measured by means of precalibrated ocular micrometer. Epidermal peels were taken out from the basal, middle and apical regions by adopting direct peal method. The epidermal peels were stained with 1% Delafieds hematoxylin and mounted in 50% glycerin. The observations were taken at 40, 120 and 200 DAP in three replicates for each treatments. The data was analysed by ANOVA (Ridgman, 1975) and means were compared between treatments from error means square by LSD at $p = 0.05$ and 0.01 confidence levels using Tukey's (1953) test. The calculations were done by applying the generally followed formula of Metcalfe and Chalk (1975).

RESULTS AND DISCUSSION

In *Amorphophallus* plants, the thickness of leaf increased gradually in treated plants than the control. Among the 3 triazole treatments, there were no significant differences variation in the leaf thickness (Table 1). TDM treatment increased the leaf thickness in wheat and barley (Sopher *et al.*, 1999; Asami *et al.*, 2000), PBZ treated sugarbeet (Dalziel and Lawrence, 1984) and *Aechemea faciata* (Zin *et al.*, 1986).

Triazole treatments caused increased number of cells per unit area in the palisade and spongy layer of the leaf. It also increased the number of chloroplast per cell. Among the treated plants there was no significant differences (Table 2). Increased mesophyll thickness, chloroplast size reported in wheat with TDM treatment (Gao *et al.*, 1988) and PBZ treated *Chrysanthemum* (Burrows *et al.*, 1992). The mesophyll cells are densely packed in soybean leaves treated with PBZ (Hawkins *et al.*, 1985). TDM and other triazole compounds increased the levels of cytokinins in cucumber (Fletcher and Arnold, 1986) and rice (Izumi *et al.*, 1988). The increased cytokinin levels might induce the cell division and thereby increase the number of cells in the palisade and spongy cells. The increased cytokinin levels also accelerates chlorophyll differentiation and chlorophyll production (Harvey *et al.*, 1974).

Triazole compounds caused increased number of stomata per unit area than the control plants. TDM treatment increased stomatal number per unit area in wheat leaves (Gao *et al.*, 1988). Increase in the number of stomata could be due to the effect of the triazoles on the hormone balance. The increased number of stomata in the triazole treated plants can be attributed to cell divisions induced through the increased cytokinin content as reported by Fletcher and Arnold (1986).

In the present study triazole treatments decreased the stomatal pore length and width in the leaves of *Amorphophallus* (Table 3). TDM treatment decreased stomatal width but produced no effect on stomatal length in wheat leaves (Gao *et al.*, 1988). TDM induced ABA content in bean (Asare-Boamah *et al.*, 1986). The increased ABA content might have induced the stomatal closure as observed in uniconazole treated *Phaseolus vulgaris* (Mackay *et al.*, 1990).

The leaf thickness, towards upper and lower epidermis and number of cells in the palisade and spongy layers significantly increased with triazole treatments. The number of chloroplasts per cell and number of stomata per unit area increased while decreasing the length and width of stomatal pore. Since most of the work has been aimed at controlling stomatal aperture is to maintain a favourable internal

Table 1: Effect of triazole compounds on anatomical characteristics of *A. campanulatus* on 40 DAP

S.No.	Parameters	Treatments				F-ratio	LSD
		Control	TDM (20 mg l^{-1})	PBZ (20 mg l^{-1})	PCZ (20 mg l^{-1})		
1.	Leaf thickness (µm)	58.54	60.37	62.19	60.11	NS	3.448
2.	Total number of upper epidermal cells	9.21	9.64	9.68	9.32	NS	0.733
3.	Total number of lower epidermal cells	5.18	5.45	5.30	5.41	NS	0.146
4.	Number of palisade cells per unit area (mm^2)	19.74	22.76	22.65	21.81	**	1.398
5.	Number of spongy cells per unit area (mm^2)	35.44	39.06	38.72	38.37	**	1.592
6.	Number of chloroplast per palisade cell (per cell)	10.11	11.16	11.07	11.04	NS	0.726
7.	Number of chloroplast per spongy cell (per cell)	10.76	12.50	12.25	12.02	*	1.169
8.	Number of stomata in lower epidermis (per mm^2)	9.64	10.35	10.25	10.12	*	0.303
9.	Stomatal pore length (µm)	11.62	9.54	9.75	9.33	**	0.844
10.	Stomatal pore width (µm)	2.90	2.31	2.41	2.10	*	0.316

TDM–Triadimefon; PBZ–Paclobutrazol; PCZ–Propiconazole; LSD–Least significance difference; *-0.05% level; **-0.01% level; NS–Not significant.

Table 2: Effect of triazole compounds on anatomical characteristics of *A. campanulatus* on 120 DAP

S.No.	Parameters	Treatments				F-ratio	LSD
		Control	TDM (20 mg l^{-1})	PBZ (20 mg l^{-1})	PCZ (20 mg l^{-1})		
1.	Leaf thickness (µm)	68.38	77.35	78.32	75.45	**	4.786
2.	Total number of upper epidermal cells	11.21	12.04	11.91	11.79	NS	0.801
3.	Total number of lower epidermal cells	7.86	8.66	8.39	8.63	NS	0.407
4.	Number of palisade cells per unit area (mm^2)	21.22	26.57	26.13	25.50	**	1.539
5.	Number of spongy cells per unit area (mm^2)	39.66	46.04	45.56	44.57	**	1.245
6.	Number of chloroplast per palisade cell (per cell)	12.21	14.30	14.25	14.42	**	1.187
7.	Number of chloroplast per spongy cell (per cell)	12.11	14.49	14.34	14.07	*	1.445
8.	Number of stomata in lower epidermis (per mm^2)	13.96	15.34	15.50	15.58	*	0.800
9.	Stomatal pore length (µm)	13.60	12.13	11.75	11.59	**	1.065
10.	Stomatal pore width (µm)	3.61	2.93	3.00	2.64	**	0.189

TDM – Triadimefon; PBZ – Paclobutrazol; PCZ – Propiconazole; LSD – Least significance difference; * - 0.05% level; ** - 0.01% level; NS – Not significant

Table 3: Effect of triazole compounds on anatomical characteristics of *A. campanulatus* on 200 DAP

S.No.	Parameters	Treatments				F-ratio	LSD
		Control	TDM (20 mg l^{-1})	PBZ (20 mg l^{-1})	PCZ (20 mg l^{-1})		
1.	Leaf thickness (µm)	82.31	100.51	99.02	97.72	**	8.285
2.	Total number of upper epidermal cells	12.81	14.11	14.44	14.02	NS	0.989
3.	Total number of lower epidermal cells	9.81	11.45	11.03	11.27	NS	0.912
4.	Number of palisade cells per unit area (mm^2)	23.42	31.18	31.51	30.22	**	1.248
5.	Number of spongy cells per unit area (mm^2)	44.39	57.70	53.81	52.33	**	4.037
6.	Number of chloroplast per palisade cell (per cell)	12.76	14.56	14.51	14.31	*	1.386
7.	Number of chloroplast per spongy cell (per cell)	14.14	16.26	16.43	16.24	*	1.492
8.	Number of stomata in lower epidermis (per mm^2)	16.25	17.78	17.70	17.57	**	1.026
9.	Stomatal pore length (µm)	14.91	13.30	13.71	13.50	*	0.988
10.	Stomatal pore width (µm)	4.31	3.55	3.86	3.30	**	0.189

TDM–Triadimefon; PBZ–Paclobutrazol; PCZ–Propiconazole; LSD–Least significance difference; *-0.05% level; **-0.01% level; NS–Not significant

water balance and water use efficiency (Waisel *et al.*, 1969). The applications of triazoles like TDM, PBZ and PCZ may prove to be useful for inducing drought avoidance mechanisms and inturn enable plants to yield more under drought or water stressed conditions (Gupta *et al.*, 2004). It can be concluded that different triazole compounds may be useful to trigger drought avoidance mechanisms in plants like *Amorphophallus* and make them more suitable for dryland agriculture. Some of the studies made earlier support the findings of the present work.

REFERENCES

Asami T Y K M, N Nagata, K Yamagishi, S Takatsuto, S Fujioka, N Muroushi, I Yamaguchi & S Yoshida 2000 Characterization of brassinazole, a triazole-type brassinosteroid, a biosynthesis inhibitor. *Plant Physiol* **123** 93-100.

Asare-Boamah N K, G Hofstra, R A Fletcher & E B Dumbroff 1986 Triadimefon protects bean plants from water stress through its effects on ABA. *Plant Cell Physiol* **27** 383-390.

Burrows G E, T S Boag & W P Stewartt 1992 Changes in leaf, stem and root anatomy of *Chrysanthemum* cv. *Lillian* Hock. following paclobutrazol application. *J Plant Growth Regul* **11** 189-194.

Dalziel J & D K Lawrence 1984 Biochemical and biological effects of kaurene oxidase inhibitors such as paclobutrazol. In Biochemical aspects of synthetic and natural occurring plant growth retardants (eds Menhennett R & D K Lawrence). *British Plant Growth Regul* **11** 47-57.

Fletcher R A & V Arnold 1986 Stimulation of cytokinins and chlorophyll synthesis in cucumber cotyledons by triadimefon. *Physiol Plant* **66** 197-201.

Fletcher R A, A Gilley, T D Davis & N Sankhla 2000 Triazoles as plant growth regulators and stress protectants. *Hort Rev* **24** 55-138.

Gao J, G Hofstra & R A Fletcher 1988 Anatomical changes induced by triazoles in wheat seedlings. *Can J Bot* **66** 1178-1185.

Gopi R, B M Sujatha, S N Rajan, L Karikalan & R Panneerselvam 1999 Effect of triadimefon in the NaCl stressed cowpea (*Vigna unguiculata*) seedlings. Indian J *Agri Sci* **69** 743-745.

Gopi R, R Sridharan, R Somasundaram, G M Alagu Lakshmanan & R Panneerselvam, 2005 Growth and photosynthetic characteristics induced by triazoles in *Amorphophallus campanulatus* Blume. *Gen Appl Plant Physiol* **31:** 171-180.

Gupta S K, R P Raghava & N Raghava 2004 Stomatal studies of cowpea (*Vigna unguiculata* (L.) Walp.) cultivars in relation to bromiconazole. *J Ind Bot Soc* **83** 116-119.

Harvey B M R, B C Lu & R A Fletcher 1974 Benzyladenine accelerates chloroplast differentiation and stimulates photosynthetic enzyme activity in cucumber cotyledons. *Can J Bot* **52** 2581-2586.

Hawkins A F, H F Hughes & C A Hart 1985 Effect of the growth regulator, paclobutrazol on structure and photosynthesis of soybean leaves. *Plant Growth Regul* **12** 127-142.

Izumi K, S Nakaguwa, M Kobayashi, H Oshio, A Sakurai & N Takahashi 1988 Levels of IAA, cytokinins, ABA and ethylene in rice plants as affected by a gibberellin biosynthesis inhibitor uniconazole-P. *Plant Cell Physiol* **29** 97-104.

Jaleel C A, R Gopi, G M Alagu Lakshmanan & R Panneerselvam 2006a Triadimefon induced changes in the antioxidant metabolism and ajmalicine production in *Catharanthus roseus* (L.) G. Don. *Plant Sci* **171** 271-276.

Jaleel C A, R Gopi, P Manivannan & R Panneerselvam 2007 Responses of antioxidant defense system of *Catharanthus roseus* (L.) G. Don. to paclobutrazol treatment under salinity. *Acta*

Physiol Plant **29**: 205-209.

Kamountsis A P & A G Sereli 1999 Paclobutrazol affects growth and flower bud production in gardenia under different light regimes. *Hort Sci* **34** 674-675.

Kopyra M & E A Gwozdz 2003 Antioxidant enzymes in paraquat and cadmium resistant cell lines of horseradish. *Biol Lett* **40** 61-69.

Kraus T E, R C Evans, R A Fletcher & K P Pauls 1995 Paclobutrazol enhances tolerance to increased levels of UV-B radiation in soybean (*Glycine max*) seedlings. *Can J Bot* **73** 797-806.

Kraus T E & 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.

Mackay C E, J C Hall, G Hofstra & R A Fletcher 1990 Uniconazole induced changes in abscisic acid, total amino acid and proline in *Phaseolus vulgaris*. *Pest Biochem Physiol* **37** 71-82.

Metcalfe C R & L Chalk 1979 *Anatomy of the dicotyledons*, 2nd eds., Vol. 1, Clerendon Press, Oxford.

Muthukumarasamy M & R Panneerselvam 1997 Amelioration of NaCl stress by triadimefon in peanut seedlings. *Plant Growth Regul* **22** 157-162.

Muthukumarasamy M, S Dutta Gupta & R Panneerselvam 2000 Enhancement of peroxidase, polyphenol oxidase and superoxide dismutase activities by triadimefon in NaCl stressed *Raphanus sativus* L. *Biol Plant* **43** 317-320.

Panneerselvam R, M Muthukumarasamy & L Karikalan 1997 Triadimefon enhances growth and net photosynthetic rate in NaCl stressed plants of *Raphanus sativus* L. *Photosynthetica* **34** 605-609.

Panneerselvam R, M Muthukumarasamy & S N Rajan 1998 Amelioration of NaCl stress by triadimefon in soybean seedlings. *Biol Plant* **41** 133-137.

Raghava R P & N Raghava 1998 Triazoles: a new group of promising synthetic plant growth regulators – an overview. *Advances in plant physiology* (ed Hemantaranjan A), Scientific Publ. (Jodhpur), India, Vol. 1, pp 291-320.

Ridgeman W J 1975 Experimentation in biology: *An introduction to design and analysis*. Thompson Litho Ltd., East Cambridge, Scotland. pp. 81-100.

Sambamurty A V S S & N Subramanyam 1989 *A text book of economic botany*, Willey Eastern Ltd., New Delhi, India pp 294-296.

Singh S, K Singh & S P Singh 1995 Effect of hormones on growth and yield characters of seed crop kharif onion (*Allium cepa* L.). *Indian J Plant Physiol* **38** 193-196.

Sopher R C, M Krol, N P A Huner & R A Fletcher 1999 Chloroplastic changes associated with paclobutrazol induced stress protection in maize seedling. *Can J Bot* **77** 1-12.

Thakur P S & A Thakkur 1993 Influence of triacontanol and mixtalol during plant moisture stress in *Lycopersicon esculentum* cultivars. *Plant Physiol Biochem* **31** 433-439.

Tuckey J W 1953 The problem of multiple comparison. Priceton University Press, New Jersey.

Waisel Y, G A Borger & T T Koztowaski 1969 Effect of phenyl mercuric acetate on the stomatal movement and transpiration of excised *Betula papyrifera* Marsh leaves. *Plant Physiol* **44** 685-690.

Ziv M, T Yogev & O Krebs 1986 Effect of paclobutrazol and chlormequat on growth pattern and shoot proliferation of normal and variant *Aechemea faciata* Baker plant regenerated *in vivo*. *Israel J Bot* **35** 175-185.