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AMELIORATIVE EFFECT OF PACLOBUTRAZOL AND CHLORMEQUAT ON DROUGHT STRESSED PLANTS OF *VETIVERIA ZIZANIOIDES*

A.H.A. FAROOQI*, A. KHAN AND A.K. SRIVASTAVA

Plant Physiology and Biochemistry Division, Central Institute of Medicinal and Aromatic Plants, P.O. CIMAP, Lucknow-226 015, India

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SUMMARY

Vetiver is important source of essential oil production in drought region. Responses of *Vetiveria zizanioides* (vetiver) to drought stress and ameliorative effect of chlormequat chloride, paclobutrazol and ethrel application to drought stressed plants were studied. In the first experiment, relative water content, root yield, oil concentration, khusimol content and oil yield decreased under drought while khusinol and proline concentration increased under drought stress. Ameliorative effects of paclobutrazol and chlormequat chloride were observed in drought stressed plants as root yield, oil concentration and oil yield increased significantly in paclobutrazol treated stressed plants, while oil concentration and khusimol content increased significantly in chlormequat chloride treated stressed plants. In the second experiment oil concentration, and oil yield increased under drought stress and ameliorative effect of chlormequat chloride was observed on oil concentration in drought stressed plants. Changes in physiological traits indicated that paclobutrazol and chlormequat chloride can partially alleviate the detrimental effect of drought.

Key words*: Chlormequat chloride, drought, paclobutrazol, *Vetiveria zizanioides

INTRODUCTION

Vetiver has a wide root system consisting of long, fibrous root and forming a sort of facilitate mass extending two to three meter deep. Root tissues contain oil producing cells responsible for its characteristic odour. The secretory cells are localized in the first cortical layer outside the endodermis of mature roots. The essential oil of vetiver is used mainly as flavours (in spices and herbs) and fragrances (e.g. perfumery, cosmetics, soaps). Besides Thailand, Java, Indonesia, China and other Asian countries vetiver is cultivated in India also. It can grow in wet land as well as saline and drought stress conditions. The growth of vetiver and biosynthesis of essential oil is influenced by both environmental and plant factors. Changes in primary metabolite processes due to nutrient and external growth conditions may play an important role in the regulation of secondary metabolism (Singh-Sangwan *et al*. 2001). The commercial and social utility of vetiver (*Vetiveria zizanioides*) was first realized on account of its aromatic root and lately overwhelmed by environmental application of this plant as such, as well as diverse industrial uses of above ground plant parts (Lavania 2003).

Limited water supply is a major constraint of crop productivity. Drought stress induces various physiological and metabolic responses like stomatal closure, decline in growth rate, antioxidant accumulation and expression of stress induced genes. The biosynthesis of secondary metabolite although controlled genetically but also affected by environmental conditions (Farooqi *et al*. 1998). For the biosynthesis of secondary metabolites drought stress is not always detrimental. It may increase

*Corresponding author, E-mail: ahafarooqi@yahoo.com

or decrease the biosynthesis of secondary metabolites according to the conditions and the species of the plants. Effect of drought stress on secondary metabolites production has been studied in aromatic grasses and Japanese mint. In aromatic grasses (*Cymbopogon* species) essential oil, gereniol and citral content increased 13-30% under drought stress. In palmarosa (*Cymbopogon martinii*), PEP carboxylase, NR activity, protein content, proline and abscisic acid contents were high under drought conditions (Fatima *et al.* 2002). Geraniol dehydrogenase increased under drought stress in lemongrass (Farooqi *et al.* 1998), while, menthol content increased in Japanese mint under drought stress (Mathur *et al.* 2005).

Plant growth regulators play a critical role in regulation of plant growth and secondary metabolites biosynthesis in aromatic and medicinal plants (Singh-Sangwan *et al*. 2001). Studies have indicated that chlormequat chloride, paclobutrazol, GA_3, IAA and ethrel are effective in stimulating production of secondary metabolites like essential oil components, artemisinin and pyrethrins (Shukla *et al.* 1992, Haque *et al.* 2007). Ameliorating effect of IAA and chlormequat chloride on drought stressed plants of aromatic grasses and Japanese mint have been reported (Farooqi *et al.* 2005, Mathur *et al.* 2005). It has also been observed that plants treated with various growth retardants were less susceptible to external stress conditions such as drought (Nickle 1982).

The present study investigates the effect of drought on plant growth, essential oil concentration, oil amount, peroxidase activity and proline content in *Vetiveria zizanioides* and to elucidate whether paclobutrazol, chlormequat chloride and ethrel could partially alleviate the detrimental effect of drought in vetiver.

MATERIALS & METHODS

Field experiments were conducted at the research farm of the Central Institute of Medicinal and Aromatic Plants, Lucknow. The climate of Lucknow is characterized as semi-arid subtropical with 760 mm mean annual rainfall. The soil of the experimental plot was sandy loam having pH 8.2 and EC 0.42 ds m^{-1}, low in available nitrogen (0.01%) with medium level of phosphate (0.0014%). Inorganic fertilizers at the rate of 120 kg per ha nitrogen and 60 kg each of P_2O_5 and K_2O as single super phosphate and muriate of potash, respectively, were applied basally at the time of planting. Rooted slips of *Vetiveria zizanioides* cultivars KS-1 were transplanted into the experimental plots of 5 m^2 (at 0.45m x 0.45m distance between each plant) in March. The plants were allowed to grow for eight months until the beds were randomly categorized into eight treatment sets as outlined below. In the unstressed plants (control), water was supplied to maintain plants at 12-14% soil moisture content. For the drought stress treatments, plants were subjected to mild drought stress by regulating the quantity of irrigation water so that soil moisture content ranged between 3-4% (Fatima *et al*. 2002). Plant growth regulators were applied when the drought stress treatment was started. Chlormequat chloride paclobutrazol and ethrel were sprayed three times at 20 days interval with tween 80 (0.01%) as the dispersant. Concentration of plant growth retardants was selected on the basis of earlier studies (Haque *et al.* 2007).

- Treatment 1 : Control - plants maintained at 12-14% soil moisture (unstressed and untreated with chlormequat chloride, paclobutrazol and ethrel).
- Treatment 2 : CCC- unstressed plants treated with chlormequat chloride (1000 mgl^{-1}).
- Treatment 3 : PBZ - unstressed plants treated with paclobutrazol (80 mgl^{-1}).
- Treatment 4 : Ethrel - unstressed plants treated with ethrel (50 mgl^{-1}).
- Treatment 5 : Drought stress – plants subjected to drought stress (soil moisture 3- 4%) but not treated with chlormequat chloride, paclobutrazol and ethrel.
- Treatment 6 : Drought stress+CCC - plant subjected to drought stress (soil moisture 3- 4%) that were treated also with chlormequat chloride (1000 mgl^{-1})
- Treatment 7 : Drought stress+PBZ - plant subjected to drought stress (soil moisture 3-4%) that were treated also with paclobutrazol (80 mgl^{-1}).

1 Treatment 8 : Drought stress+ethrel - plant subjected to drought stress (soil moisture 3-4%) that were treated also with ethrel (80 mgl^{-1}).

Observations were taken from four randomly selected plants after 120 days of growth when plants showed symptoms of drought stress, such as rolling of first leaf and wilting of other leaves. The effects of drought stress were observed on plant height, plant diameter, and root yield. Relative water content (RWC) was measured using leaf discs as described (Singh-Sangwan *et al*. 1994). Peroxidase activity was determined in fresh leaves as described by Pulter (1974). The catalytic activity was expressed as the increase in absorbance mt^{-1} g^{-1} fw. Proline was estimated by the method as described by Bates *et al*. 1965). Water potential (Ψ) was determined by pressure chamber technique (Scholander *et al.* 1965). The essential oil concentration was determined by hydro-distillation using a Clevenger-type apparatus (Guenther 1955). The oil composition was determined by GC using a Perkin Elmer GC model 3920B equipped with TCD detector.

Data analyzed statistically and analysis of variance (ANOVA) for split plot design wass work out using software GenStat Release 7.22 TE (PC/Windows).

RESULTS AND DISCUSSION

In the first experiment, plant height decreased significantly under drought stress and the decrease was 20% compared to control (Table 1). Paclobutrazol and ethrel decreased plant height significantly in stressed plants. Fresh root yield significantly under drought stress and the decrease was 29% over the control but decrease in root yield was not significant in the second experiment (Table 3). The application of paclobutrazol significantly increased root yield in stressed plants and the increase was 32% over untreated stressed plants but it decreased in the second experiment (Table 3). Plant diameter increased in stressed plants due to paclobutrazol and chlormequat chloride application and the increase was 12 and 16%, respectively. Decrease in herbage yield under water stress have also been reported in other medicinal and aromatic plants (Singh-Sangwan *et al*.2001, Fatima *et al*.2002).

Relative water content decreased significantly under drought stress and the decrease was 9-19 %. The less decrease in RWC under drought stress in vetiver suggests greater drought tolerance nature as observed in *Cymbopogon martinii* (Farooqi *et al*. 2005). Peroxidase activity values were high in vetiver plants

Table 1. Effects of drought stress and the application of chlormequat chloride, paclobutrazol and ethrel on growth, peroxidase activity, relative water content and water potential of *Vetiveria zizanioides*

Treatment	Plant height (cm)	Plant diameter (cm)	Fresh root yield (g $plant^{-1}$)	Peroxidase (ΔOD mg^{-1} protein)	Relative water content (%)	Water potential (Ψ) (-MPa)
Control	93.7	28.3	311.0	9.46	74	1.8
CCC	88.3	28.7	380.0	15.9	79	1.7
PBZ	91.7	29.3	323.3	16.6	78	1.6
Ethrel	96.0	29.00	355.0	13.0	74	1.7
Stress	75.3	22.3	220.0	7.6	67	2.8
Stress+ CCC	90.0	26.7	221.0	14.4	71	2.4
Stress+ PBZ	74.0	25.00	291.3	15.5	72	2.25
Stress+ Ethrel	81.7	23.7	222.0	13.8	7.3	2.7
l.s.d. int.5%	4.63	2.53	30.63	2.80	5.92	0.14

l.s.d. int.- Interaction l.s.d. between conditions x treatments.

compared to other aromatic plants (Farooqi *et al*. 2005, Mathur *et al.* 2005). The greater peroxidase activity can be linked to its drought tolerant nature. Higher peroxidase activity has been reported in drought stress tolerant genotypes compared to susceptible genotypes (Reddy *et al*. 2003). Peroxidase activity increased significantly by the application of chlormequat chloride, paclobutrazol and ethrel irrespective of drought or unstressed conditions, but the increase was greater under drought conditions (80-103%). Increase in peroxidase activity by the application of plant growth regulators is also reported in other plants (Farooqi *et al*. 2005, Mathur *et al.* 2005).

Proline content was higher in stressed plants ranging from 47-218% compared to control (Table 2). Proline content increased significantly by the application of chlormequat chloride, paclobutrazol and ethrel, irrespective of drought or unstressed conditions. Proline content increased by 66-154% due to ethrel, chlormequat chloride and paclobutrazol treatment in stressed plants. In a similar study in aromatic grasses, high proline accumulation has been reported under drought stress (Fatima *et al.* 2002).

Oil concentration decreased under drought stress in first experiment but increased in second experiment (Table 2 & 3). It is reported that among essential oil bearing plants oil concentration can increase, decrease or remain constant due to stress depending upon the plant species (Fatima *et al.* 1999). However, application of chlormequat chloride and paclobutrazol increased oil concentration significantly in drought stressed plants in the first experiment and the increase was 18.5% and 26% over untreated stressed plants, while in the second experiment application of chlormequat chloride increased oil concentration significantly in drought stressed plants. Increase in the essential oil concentration of Japanese mint due to chlormequat chloride application has been reported earlier (Farooqi and Sharma 1988). Ameliorative effect of growth regulators on secondary metabolites concentration is reported in other medicinal and aromatic plants (Singh-Sangwan *et al*. 2001). In the present study the stess mediated changes in oil composition were predominantly reflected in major oil constituent khusinol, which increased significantly under drought stess. Compositional alteration in essential oil as a consequence of drought stress has also been reported in mint and aromatic grasses (Mathur *et al.* 2005, Fatima *et al*. 2002).

Oil amount (oil yield) per plant significantly decreased under drought stress in the first experiment (Table 2) and the decrease in stressed plants 35% over control. In the second experiment oil amount increased due to drought and the increase was mainly due to increase in oil concentration (Table 2). Stress induced changes in the oil yield are thought to be result of the effect of stress on plant growth and differentiation or due to direct effect on oil synthesis (Charles *et al.* 1990,

Table 2. Effects of drought stress and the application of chlormequat chloride, paclobutrazol and ethrel on oil concentration, oil amount per plant, khusinol and khusimol percentage of *Vetiveria zizanioides*

Treatment	Oil concentration ($g\ 200g^{-1}$ fw)	Oil amount ($g\ plant^{-1}$)	Khusinol (%)	Khusinol (%)	Proline ($\mu g\ g^{-1}$ freeze dw)
Control	02.4	3.6	9.0	3.0	113
CCC	02.4	4.5	38.0	3.3	113
PBZ	02.8	4.8	5.0	12.5	260
Ethrel	03.3	5.9	3.2	16.4	210
Stress	02.2	2.4	23.0	2.1	186
Stress+ CCC	02.6	2.8	26.2	5.7	360
Stress+ PBZ	02.7	4.0	24.7	9.6	473
Stress+ Ethrel	02.3	2.5	14.9	8.2	310
l.s.d. int.5%	0.19	1.08	2.468	1.336	053

l.s.d. int.- Interaction l.s.d. between conditions x treatments.

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Table 3. Effects of drought stress and the application of chlormequat chloride, paclobutrazol and ethrel on growth, relative water content, water potential, oil concentration, and oil amount per plant of *Vetiveria zizanioides*

Treatment	Fresh root yield (g plant^{-1})	Relative water content (%)	Water potential (Ψ) (-MPa)	Oil oncentration (g 200g^{-1} fw)	Oil amount (g plant^{-1})
Control	230	44	2.6	1.9	1.71
CCC	145	53	2.3	2.2	1.5
PBZ	159	53	2.8	1.6	1.3
Ethrel	201	56	3.1	2.0	2.0
Stress	223	37	3.3	2.5	2.5
Stress+ CCC	151	44	3.4	3.0	3.0
Stress+ PBZ	134	60	3.5	1.7	1.7
Stress+ Ethrel	191	53	3.1	1.9	1.9
l.s.d. int.5%	76.3	7.0	0.95	1.04	0.72

l.s.d. int.- Interaction l.s.d. between conditions x treatments.

Simon *et al*. 1992). In the first experiment, the application of paclobutrazol increased oil amount significantly in drought stressed plants and the increase was 67% compared to untreated drought stressed plants, while in the second experiment application of chlormequat chloride increased oil amount significantly and the increase was 23% over untreated stressed plants (Table 2 & 3). Changes in growth, metabolism and essential oil concentration and oil amount per plant reflect that chlormequat chloride and paclobutrazol can partially alleviate the detrimental effect of drought.

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