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Short communication

Ameliorative effect of chlormequat chloride and IAA on drought stressed plants of *Cymbopogon martinii* and *C. winterianus*

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Abstract

Responses of *Cymbopogon martinii* and *C. winterianus* to drought stress and chlormequat chloride and IAA application are compared. These two species are important source of essential oil production in drought regions. For both species and their cultivars relative water content (RWC), herbage yield and oil amount decreased under drought, while oil biosynthesis increased. Oil concentration increased significantly under drought in *C. winterianus* while peroxidase activity increased in *C. martinii*. Amount of geraniol increased under drought stress in *C. martinii* while citronellal and geraniol accumulation decreased in *C. winterianus*. Ameliorative effects of chlormequat chloride and IAA were observed in drought stressed plants of both species. Herbage yield increased significantly in chlormequat chloride and IAA treated stressed plants of *C. winterianus*, while oil concentration increased in *C. martinii*. Ameliorative effect of IAA in increasing oil yield was significant in drought stressed plants of both the species. Changes in various morpho-physiological traits indicated that chlormequat chloride and IAA can partially alleviate the detrimental effect of drought in these aromatic grasses.

Introduction

Aromatic grasses (Poaceae) are hardy plants of sub-tropical and tropical regions of the world. There are several important aromatic grasses of genus *Cymbopogon*, which are cultivated for their essential oils, i.e. lemongrass, palmarosa and citronella Java. Aromatic grasses have great potential as agro and social forestry plants and for wasteland reclamation with their proven soil binding properties (Farooqi et al. 2000). The growth of aromatic grasses and biosynthesis of essential oil is influenced by both environmental and plant factors. Changes in primary metabolic processes due to nutrient or external growth conditions may play an important role in the regulation of secondary metabolism (Singh-Sangwan et al. 2001). Limited water supply is a major environmental constraint of crop productivity. Cultivation of aromatic grasses in India as an irrigated crop has spread over several different agro climatic zones. *C. winterianus* (citronella Java) is a moisture-loving plant and loss of herbage yield is high under drought stress, while *C. martinii* (Palmarosa) is drought resistant (Fatima et al. 2002).

Growth regulators can improve plant growth, development and yield and quality of essential oil (Singh-Sangwan et al. 2001). Foliar application of triacontanol and mixtalol have been shown to significantly increase yield attributes of rose-scented

geranium (Bhatacharya and Rao 1996). An increase in oil content and the isomenthone and neoisomenthol proportion in the oil with application of diaminozide, cycocel and phosphon-D has been observed in *Mentha piperita* (El-Keltawi and Croteau 1986). We have previously noted that chlormequat chloride (CCC) improved monoterpene oil content in *Mentha arvensis* (Farooqi and Sharma 1988). Similarly, in *C. flexuosus*, an increase in oil concentration has been achieved with IAA, IBA and GA_3 application under normal conditions (Farooqi et al. 2001). Plant growth regulators can also confer plant resistance to abiotic stresses such as drought and osmotic stress (Chatterjee 1995; Zhao and Ooserhuis 1997; Vardhini and Rao 2003). The responses of some aromatic grasses to plant growth regulators have been studied (Farooqi et al. 2000; Singh-Sangwan et al. 2001), but not comparatively when different species are under drought stress. Also, the effects of cellular dehydration on biosynthesis of essential oils has not been studied in detail (Singh-Sangwan et al. 1994; Fatima et al. 2002). Our aim is to understand how drought stress alters oil biosynthesis and what ameliorating effects plant growth regulators can have on oil production. Water stress increases peroxidase activity and drought tolerant varieties are characterized by higher peroxidase concentration compared with susceptible varieties (Reddy et al. 2003). We have therefore also examined the effects of drought on peroxidase activity.

The present study investigates the effect of drought on plant growth, essential oil concentration, oil biosynthesis and peroxidase activity in the leaves of *C. martinii* and *C. winterianus*. We also determine if chlormequat chloride and indole acetic acid (IAA) could partially alleviate the detrimental effect of drought on these species. It has been observed that plants treated with various growth retardants were less susceptible to external stress conditions such as drought (Nickle 1982). Application of chlormequat chloride and IAA have been reported to increase oil concentration and yield in normal plants whereas endogenous IAA content decreases under drought stress in the plants.

Materials and 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, was applied basally at the time of planting. Rooted slips of *C. martinii* cultivars RRL (B0-77, RLB (B)-77E, RRL (B)-69 and IW-31245E and *C. winterianus* cultivars RRL (B)-18, RRL (B)-15, Jor.Lab-2 and Banglore were transplanted into the experimental plots of 5 m^2 (at 0.45 m $\times$ 0.45 m distance between each plant) in July. The plants were allowed to grow for 60 days until the beds were randomly categorized into six treatment sets as outlined below. In the unstressed plants (control), water was supplied to maintain plants at 12–14% soil moisture content. About 1500 l (6 $\times$ 250 l) water was provided per plot during the period of growth. 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 and IAA were sprayed three times at 20 days interval with Tween 80 (0.01%) as the dispersant.

- Treatment 1: control – plants maintained at 12–14% soil moisture (unstressed and untreated with IAA or chlormequat chloride)
- Treatment 2: CCC – unstressed plants treated with chlormequat chloride (1000 mg l^{-1})
- Treatment 3: IAA – unstressed plants treated with IAA (50 mg l^{-1})
- Treatment 4: drought stress – plant subjected to drought stress (soil moisture 3–4%) but not treated with IAA or chlormequat chloride
- Treatment 5: drought stress + CCC – plant subjected to drought stress (soil moisture 3–4%) that were treated also with chlormequat chloride (1000 mg l^{-1})
- Treatment 6: drought stress + IAA – plant subjected to drought stress (soil moisture 3–4%) that were treated also with IAA (50 mg l^{-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, tiller number, leaf area and herbage yield. Leaf area was measured using a Li-Cor LI-3100 leaf area meter. Relative water content (RWC) was measured using leaf discs as described (Singh-Sangwan et al. 1994). Peroxidase activity was determined in fresh leaves (0.5 g) as described by Pulter (1974). The catalytic activity was expressed as the increase in absorbance min^{-1} mg^{-1} protein. Protein was estimated according to Lowry et al. (1951) using bovine serum albumin as a standard. Oil biosynthesis was studied using [2-^{14}C] acetate as described (Fatima et al. 2002). After harvesting the essential oil concentration was determined by hydro-distillation using a Clevenger-type apparatus. The oil composition was determined by GC using a Perkin–Elmer GC model 3920B equipped with TCD detector (Fatima et al. 2002). The radiolabel incorporation in the oil was computed according to Singh et al. (1991):

$$\% \text{ incorporation} = \frac{\text{Radioactivity detected in oil}}{\text{Total radioactivity provided}} \times 100$$

$$[2\text{-}^{14}\text{C}] \text{ acetate incorporated (pmol) into essential oil} = \frac{\text{DPM } [^{14}\text{C}] \text{ in essential oil}}{2.22} \times \frac{1}{61.54}$$

The experiment was conducted following the layout of a two factor factorial randomized block design with three replications. The treatment means in each trait were compared statistically using the critical difference (CD) test at 5 and 1% levels of significance for the cultivar × treatment interaction effect. Simple linear correlation coefficients were calculated to estimate the interrelationship between characters under study, see (Fatima et al. 2002).

Results and discussion

In general, the response of both species to long-term drought stress was a significant reduction in growth and herbage yield (Tables 1 and 2). Reduction in tiller number and herbage yield was greater for *C. winterianus* than *C. martinii*. Chlormequat chloride decreased plant height significantly in stressed plants of *C. martinii*. The decrease in plant height was more evident in *C. martinii*, as it can attain a height up to 3 m, while *C. winterianus* has a condensed stem and produces large number of leaves (Fatima et al. 2002). Leaf area in general increased significantly by the application of chlormequat chloride and IAA irrespective of cultivar or species under drought stress, but the increase was greater in *C. winterianus* than in *C. martinii*. Tiller number also increased in stressed plants of *C. winterianus* due to chlormequat chloride application; the increase was between 24–61%. The application of chlormequat chloride and IAA significantly increased herbage yield in stressed plants of *C. winterianus* with the increase being greater in IAA treated plants. Herbage yield increased in stressed plants by 20–42% in *C. martinii* and 33–85% in *C. winterianus* due to IAA treatment.

Relative water content decreased significantly under drought stress in both the species (Tables 1 and 2); the decrease was greater with *C. winterianus* (23–25% compared to the control) than in *C. martinii* (14–18% over control). The smaller decrease in RWC in *C. martinii* suggests greater drought tolerance, which is supported by the growth responses to drought.

Peroxidase activity increased significantly under drought in *C. martinii* with an increase between 40–151% compared to the control (Table 3). The increase in peroxidase activity was greater for *C. martinii* under drought stress compared to *C. winterianus*. Again the greater peroxidase activity in *C. martinii* can be linked to its drought tolerant nature. Higher peroxidase activity has been reported in drought stress tolerant genotypes compared to susceptible genotypes (Jha and Singh 1997; Reddy et al. 2003). Peroxidases are believed to prevent the degradation of membrane integrity due to free radicals induced under drought stress (Mandal and Singh 2000). Effects of chlormequat chloride and IAA on peroxidase activity irrespective of species were not consistent.

Oil concentration was higher in stressed plants of *C. winterianus* ranging from a 30 to 55% increase compared to control plants (Table 4). Oil concentration was negatively correlated with RWC ($r = -0.443$; $p < 0.05$). The application of chlormequat chloride and IAA increased oil

Table 1. Effects of drought stress and the application of chlormequat chloride and IAA on the relative water content, plant height, tiller number, leaf area and herbage yield of *C. martinii* after 120 days of growth.

Cultivar	Treatment	RWC (%)	Plant height (cm)	Tillers number	Leaf area (cm^2)	Herbage yield (kg $plant^{-1}$)
RRL(B)-77	Control	88	128	51	20.3	0.40
	CCC	85	123	56	21.1	0.35
	IAA	83	141	42	24.6	0.70
	Stress	75	122	41	19.6	0.30
	Stress + CCC	74	111	44	27.2	0.35
	Stress + IAA	71	126	35	25.7	0.37
RRL(B)-77E	Control	91	137	98	23.4	0.55
	CCC	88	127	109	25.7	0.45
	IAA	86	142	73	27.7	0.65
	Stress	75	127	69	22.6	0.41
	Stress + CCC	77	110	73	25.7	0.5
	Stress + IAA	77	131	56	25.6	0.55
RRL(B)-69	Control	90	227	65	38.6	1.6
	CCC	88	188	89	40.6	0.8
	IAA	87	247	52	44.6	0.95
	Stress	76	195	52	36.9	0.95
	Stress + CCC	85	162	63	44.6	1.15
	Stress + IAA	83	204	47	40.3	1.35
IW-31245E	Control	93	162	170	20.2	1.00
	CCC	92	150	205	23.8	0.80
	IAA	90	172	137	31.9	1.20
	Stress	80	157	135	20.0	0.75
	Stress + CCC	83	137	162	22.8	0.8
	Stress + IAA	81	161	125	22.7	0.90
CD int. 5%		5.1	9.7	14.2	4.4	0.07
CD int. 1%		6.8	13.0	19.0	6.0	0.10

CD int. – Interaction CD between varieties × treatments.

concentration significantly in drought stressed plants of *C. martinii*; the increase was 16–52 and 33–56%, respectively, over untreated stressed plants. An ameliorative effect of plant growth regulators on secondary metabolite concentration during drought stress has been reported (Abdin et al. 2001). Application of IAA and chlormequat chloride have been shown to increase the essential oil concentration of *Cymbopogon flexuosus*, *C. jwarancusa*, *Mentha piperita*, *M. arvensis*, *Pelargonium* spp. and *Salvia officinalis* (Farooqi et al. 2001; Singh-Sangwan et al. 2001).

The amount of oil per plant decreased under drought stress in *C. martinii*. Among the cultivars of *C. martinii* the decrease in amount of oil ranged from 18 to 46% compared to the control plants (Table 3). Stress induced changes in the yield of oil per plant are likely due to changes in biomass production under drought stress, rather than any direct effect on oil synthesis (Simon et al. 1992). Although, net amount of oil per plant under stress decreases and attracts an agronomic disadvantage, it suggests that under moisture deficiency cultivation conditions, the plants may be closely planted for improved harvests without significant plant to plant competition (due to lower tillering and retarded growth). Further, the biological significance of stress response could be attributing a chemoecological advantage through secondary metabolites. Drought induces oxidative stress at the cellular and intracellular level. Since, secondary metabolites possess strong antioxidant activities, their enhanced concentrations may help in controlling stress induced damage(s). The ameliorative effect of IAA on oil yield was significant in stressed plants of both species (except for RRL(B)-77 cultivar of *C. martinii*), the increase was between 29 and 162% compared to drought stressed cultivars of *C. winterianus* and between 54 and 85% in *C. martinii*.

Table 2. Effects of drought stress and the application of chlormequat chloride and IAA on the relative water content, plant height, tiller number, leaf area and herbage yield of *C. winterianus* after 120 days of growth.

Cultivar	Treatment	RWC (%)	Plant height (cm)	Tillers number	Leaf area (cm^2)	Herbage yield (kg $plant^{-1}$)
RRL(B)-18	Control	82	102	47	53.7	0.57
	CCC	80	97	60	98.6	0.42
	IAA	79	110	40	86.0	0.62
	Stress	60	97	31	50.1	0.38
	Stress + CCC	61	85	50	60.5	0.45
	Stress + IAA	58	102	52	69.6	0.65
RRL(B)-15	Control	88	95	38	91.0	0.60
	CCC	85	92	54	130.0	0.40
	IAA	85	102	33	93.6	0.60
	Stress	68	67	24	78.1	0.35
	Stress + CCC	70	59	34	100.0	0.50
	Stress + IAA	66	73	32	92.0	0.65
Jor Lab-2	Control	81	107	72	65.6	0.85
	CCC	78	95	76	109.0	0.60
	IAA	77	112	42	96.7	0.90
	Stress	62	97	50	54.3	0.60
	Stress + CCC	61	85	78	115.0	0.80
	Stress + IAA	57	102	68	104.0	0.80
Banglore	Control	84	102	58	81.7	0.45
	CCC	83	82	65	107.0	0.35
	IAA	81	107	40	92.3	0.52
	Stress	67	85	41	74.0	0.26
	Stress + CCC	68	81	51	89.3	0.35
	Stress + IAA	67	102	46	84.6	0.40
CD int. 5%		4.6	9.4	9.7	14.4	0.04
CD int. 1%		6.2	12.5	12.9	19.3	0.06

CD int. – Interaction CD between varieties × treatments.

In *C. martinii* the amount of essential oil geraniol increased under drought conditions but it decreased in all *C. winterianus* cultivars (Tables 3 and 4). A decline in citronellal concentration in drought stressed plants of *C. winterianus* was also apparent (although the decrease was only significant in the cultivar RRL(B)-18). Other stress mediated changes in oil composition have been reported for aromatic plants (Singh-Sangwan et al. 2001; Fatima et al. 2002).

The two species differed considerably in the oil biosynthesis under control conditions. The incorporation of ^{14}C into essential oil was greater in *C. winterianus* than in *C. martinii* and it was linked to oil concentration (Tables 3 and 4). It has been reported that monoterpene oil concentration was related to biogenetic rates and activities of key enzymes involved in the pathway in aromatic grasses e.g. geraniol dehydrogenase (Singh-Sangwan et al. 1994; Singh-Sangwan et al. 2001). Application of chlormequat chloride and IAA increased ^{14}C-acetate incorporation into essential oils relative to control plants of *C. winterianus*. The incorporation of ^{14}C-acetate into essential oil significantly increased under drought stress in both the species; the increase was higher (32–60%) in *C. martinii* than in *C. winterianus* (20–36%) (Tables 3 and 4). Again the greater increase in ^{14}C incorporation into the essential oil in *C. martinii* as compared to *C. winterianus* can be linked to the resistant nature of *C. martinii*. An increase in oil biosynthesis under drought stress has been reported earlier in *Cymbopogon* species (Singh-Sangwan et al. 1994; Fatima et al. 2002). Oil biosynthesis increased under drought stress in *C. martinii*, while it declined due to the application of chlormequat chloride and IAA under drought conditions.

Changes in growth, metabolism and essential oil concentration and oil yield (amount per plant) reflect that chlormequat chloride and IAA can partially alleviate the detrimental effect of drought.

Table 3. Effects of drought stress and the application of chlormequat chloride and IAA on peroxidase activity, oil concentration, oil amount per plant, gerniol and geranyl acetate percentage and oil biosynthesis of *C. martinii* after 120 days of growth.

Cultivar	Treatment	Peroxidase (ΔOD mg^{-1} protein)	Oil concentration (mg leaf^{-1})	Oil amount (g plant^{-1})	Geraniol (%)	Geranyl acetate (%)	^{14}C-acetate incorporation into oil (%)	^{14}C-acetate incorporated into oil (Pico moles)
RRL(B)-77	Control	2.4	1.3	4.6	90.3	1.80		
	CCC	2.1	1.2	3.2	93.6	1.20		
	IAA	4.2	1.8	6.6	91.5	6.00		
	Stress	3.5	1.2	3.8	94.3	1.70		
	Stress + CCC	4.1	2.0	4.0	90.5	3.50		
	Stress + IAA	6.3	1.7	4.7	90.6	1.60		
RRL(B)-77E	Control	1.8	1.4	3.9	85.0	5.20		
	CCC	2.2	1.1	3.2	85.4	1.10		
	IAA	1.7	1.5	4.8	83.5	1.60		
	Stress	2.6	1.1	2.1	87.1	3.15		
	Stress + CCC	3.0	1.5	3.9	89.5	0.57		
	Stress + IAA	2.8	1.6	3.9	85.3	0.58		
RRL(B)-69	Control	2.2	1.8	9.9	79.1	8.70	0.022	17.88
	CCC	1.8	1.6	5.8	80.1	9.30	0.023	18.69
	IAA	1.9	2.7	17.1	81.2	14.20	0.020	16.25
	Stress	3.1	1.4	6.1	87.5	8.75	0.029	23.56
	Stress + CCC	2.2	1.8	8.8	85.3	10.30	0.019	15.43
	Stress + IAA	3.2	2.1	9.4	85.6	11.20	0.014	11.37
IW-31245E	Control	1.6	2.0	5.5	77.8	7.75	0.015	12.19
	CCC	2.2	1.2	4.9	81.8	6.70	0.019	15.44
	IAA	2.0	3.2	6.3	79.6	10.40	0.018	14.62
	Stress	4.0	1.6	3.5	88.2	2.45	0.024	19.50
	Stress + CCC	3.3	1.9	4.5	86.3	9.05	0.021	17.06
	Stress + IAA	6.9	2.5	6.4	92.4	2.05	0.020	16.25
CD int. 5%		0.3	0.3	1.5	4.8	2.9	0.0035	
CD int. 1%		0.4	0.4	2.0	6.5	3.8	0.0047	

CD int. – Interaction CD between varieties × treatments.

Table 4. Effects of drought stress and the application of chlormequat chloride and IAA on peroxidase activity, oil concentration, oil amount per plant, citronellal, citronellol, gerniol and oil biosynthesis of *C. winterianus* after 120 days of growth.

Cultivar	Treatment	Peroxidase (ΔOD mg^{-1} protein)	Oil concentration (mg $leaf^{-1}$)	Oil amount (g $plant^{-1}$)	Citronellal (%)	Citronellol (%)	Geraniol (%)	^{14}C-acetate incorporation into oil (%)	^{14}C-acetate incorporated into oil (Pico moles)
RRL(B)-18	Control	6.7	9.3	5.4	14.1	12.9	24.5		
	CCC	10.1	11.8	5.2	12.6	20.1	33.7		
	IAA	10.9	15.3	10.1	11.0	15.1	23.4		
	Stress	11.6	12.6	5.2	10.2	14.8	16.2		
	Stress + CCC	13.0	14.8	6.5	10.0	15.1	15.7		
	Stress + IAA	15.1	16.4	13.8	12.5	14.7	17.8		
RRL(B)-15	Control	8.7	9.1	6.1	12.4	14.4	28.0		
	CCC	9.1	13.0	4.3	17.4	16.9	23.6		
	IAA	10.3	13.5	6.5	11.7	13.7	20.4		
	Stress	11.0	11.9	5.1	11.6	22.6	15.9		
	Stress + CCC	9.4	12.8	5.7	11.2	21.1	16.4		
	Stress + IAA	10.5	14.0	9.9	13.4	16.4	15.2		
Jor Lab-2	Control	3.0	9.8	8.4	15.0	15.9	15.5	0.025	20.31
	CCC	3.9	10.6	6.5	15.1	13.9	15.5	0.028	22.74
	IAA	4.4	12.6	9.9	17.4	15.3	18.1	0.027	21.94
	Stress	3.5	15.2	9.8	12.9	15.4	11.8	0.034	27.62
	Stress + CCC	3.0	19.6	11.7	15.9	13.8	15.5	0.033	26.81
	Stress + IAA	4.5	17.5	12.7	17.1	13.9	14.8	0.027	21.94
Banglore	Control	3.5	12.9	6.1	13.4	16.8	18.0	0.029	23.56
	CCC	4.4	14.8	6.9	13.2	16.0	16.2	0.034	27.62
	IAA	3.8	17.0	8.7	12.8	14.8	12.2	0.033	26.81
	Stress	4.2	17.4	4.4	11.3	14.9	10.2	0.035	28.44
	Stress + CCC	3.9	18.1	5.4	11.3	13.6	11.7	0.037	30.06
	Stress + IAA	4.3	20.3	6.2	13.8	14.2	11.3	0.039	31.69
CD int. 5%		1.8	2.7	1.2	2.3	1.7	2.5	0.0025	
CD int. 1%		2.5	3.6	1.5	3.0	2.3	3.4	0.0035	

CD int. – Interaction CD between varieties × treatments.

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