Indian J. Plant Physiol., Vol. 10, No. 1, (N.S.) pp. 41-47 (Jan.-Mar., 2005)

AMELIORATIVE EFFECT OF CHLORMEQUAT CHLORIDE ON WATER STRESSED CULTIVARS OF JAPANESE MINT (*MENTHA ARVENSIS*)

PRITI MATHUR, A.H.A. FAROOQI* AND SRIKANT SHARMA

Central Institute of Medicinal and Aromatic Plants (CIMAP) P.O. CIMAP, Lucknow – 226015, India

Received on 16 April, 2004, Revised on 29 March, 2005

SUMMARY

A comparison of responses of five cultivars of Japanese mint (*Mentha arvensis* L. var. Piperascens Mal.) to water stress and chlormequat chloride application has brought out considerable intervarietal variation. Relative water content, water potential, herbage and oil yield decreased under water stress, while abscisic acid, sugar content, peroxidase activity, oil and menthol content increased significantly. Ameliorative effect of chlormequat chloride was observed in stressed plants of different varieties. RWC, herbage and oil concentration increased and ABA and peroxidase activity decreased in chlormequat chloride treated stressed plants, as compared with untreated stressed plants. Observations suggest that chlormequat chloride can partially alleviate the detrimental effect of water stress in Japanese mint.

***Key words*: ABA, chlormequat chloride, *Mentha arvensis*, NR activity, peroxidase, water stress**

INTRODUCTION

Japanese mint (*Mentha arvensis*) (Lamiaceae) is an important crop grown in the subtropical region of the world for essential oil. The oil, a complex mixture of many monoterpenoids and its constituents, is used in the pharmaceutical, flavour and perfumery industries. The biosynthesis and metabolism of monoterpenoids in *M. arvensis* is strongly influenced by different internal and environmental factors (Farooqi *et al*. 1999, Sangwan *et al*. 2001). Japanese mint cultivation in India is spread over different agroclimatic zones as an irrigated crop. The crop is drought sensitive and loss of herbage yield is high under water stress, particularly in the north Indian plains during summer where harvesting time coincides with hot-humid climate.

Plant growth regulators improve development and yield under stress (Shukla *et al*. 1989, Chatterjee 1995, Zhao and Oosterhuis 1997). The responses of *Mentha* sp. to plant growth regulators have been studied extensively, but not under water stress, especially when different cultivars are compared. Application of chlormequat chloride has been shown to increase significantly the essential oil content of Japanese mint with slight inhibition in the herbage (Farooqi and Sharma 1988). The present study investigates the effect of water stress on essential oil and metabolic responses in the leaves of different genotypes of *M. arvensis* and determines if chlormequat chloride could partially alleviate the detrimental effect of water stress on growth, oil yield and metabolic responses of the species.

MATERIALS AND METHODS

Field experiments were carried out at the research farm of Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow in north Indian plains. Suckers

---

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

of different cultivars of Japanese mint (*Mentha arvensis* var. *Piperascens Mal.*) were obtained from the farm nursery. A half dose of inorganic fertilizer (nitrogen @ 120 kg/hectare, potassium @ 60 kg/hectare and phosphours @ 60 kg/hectare) was mixed with the soil, and a half dose was applied to one-month old plants. The soil of the experimental plots was sandy loam in texture and alkaline in reaction (pH 8.2, EC 0.42 ds/m). Two experiments were conducted to establish the effect of chlormequat chloride on water stressed plants. The first experiment was conducted following the layout of a randomized block design with three replications to evaluate the effect of two levels of chlormequat chloride on a single cultivar, Gomti, under water stress and unstressed conditions. The second (final) experiment was conducted following the layout of two factor factorial design with three replications to evaluate the effect of chlormequat chloride in the presence and absence of water stress in four popular cultivars – Kalka, Shivalik, Himalaya and Kosi. Cultivars Gomti, Kalka, Himalaya and Kosi have been released by CIMAP from time to time to sustain a higher menthol mint oil production in India. Shivalik is a standard variety cultivated by farmers. The average value of the treatments (stress, chlormequat chloride) and treatment combinations, along with the control – unstressed and untreated, were compared statistically using the critical differences (CD) at 5% and 1% level of significance in all the studied traits. The cultivar x treatment interaction effect was computed in the final experiment. Simple linear correlation coefficients (r value) were calculated to estimate the interrelationship between characters under study (Das and Giri 1974, Gupta and Kapoor 1983).

The first experiment was conducted in plots of 2m x 3m, each plot consisting of three rows with inter-row distance of 60 cm. Suckers of *M. arvensis* var. Gomti were soaked with solution of chlormequat chloride (500 ppm and 1000 ppm) for 24 h in the presence of Tween 80 (0.01%). Suckers (5-7 cm long) were planted continuously in furrows about 2 cm below the surface in each row in January. The plants were allowed to grow for 60 days under identical conditions. Subsequently the beds were randomly categorized into two sets. In the first set (control), water was supplied to maintain plants at 12-14% soil moisture content. In the second set (stressed), plants were subjected to mild water stress by regulating the quantity of water so that soil moisture content ranged between 3-4% (Fatima *et. al.* 2002). Stressed plants ultimately developed symptoms of wilting of leaves. Observations were taken on unit plots of 1m^2. Effect of chlormequat chloride was studied on herbage yield, leaf/ stem ratio, water relations, nitrate reductase activity, abscisic acid (ABA) content, oil content and yield in stressed as well as unstressed plants.

In the second experiment, suckers of four cultivars, namely Himalaya, Kosi, Kalka and Shivalik, were soaked with solutions (1000 ppm) of chlormequat chloride for 24 hour in the presence of Tween 80 (0.01%) and planted in plots of 4 m x 4.9 m, with two rows of each variety. The plants were allowed to grow for two months under identical conditions. Subsequently the beds were randomly categorized into two sets. In the first set (control), plants were maintained at 12-14 % soil moisture content, while in the second set (stressed), plants were maintained at 3-4 % moisture content. The observations were taken on a sample plot of 1m^2 after two months of plant growth when stressed plants had developed symptoms of wilting and the lower leaves started falling after senescence.

Essential oil

Essential oil was estimated from the fresh herb by Clevenger type apparatus (Guenther 1955) and the major oil constituents, *i.e.* menthol, menthone etc., were determined by gas liquid chromatography (Perkin Elmer Model 3920 B) equipped with TCD (thermal conductivity detector). The stainless steel column (200 cm x 0.3 cm) of the GLC was packed with 10% carbowax coated on 80\100 chromosorb WAW (20 mesh). The column was temperature programmed from 100-200^{o}C at the rate of 4^{o}C/min. Injector and detector temperature were maintained at 200^{o}C. The flow of H_2 was 0.47 cm/sec. Data processing for area percentage was done on a Hewlet Packard integrator model HP–3390. Component identification was done by comparing the retention times with those of authentic standards of compounds known to be present in the *M. arvensis* oil.

Physiological parameters

Water potential (Ψ) of leaves was measured by a thermocouple psychrometer. For this, leaf discs (5mm) from top-most (65% expanded) leaves were punched from the middle of leaves and placed in psychrometer chambers for equilibration for one hour at ambient temperature. Following the equilibration, water potential was assessed as described by Fatima *et al.* (2002). Relative water content (RWC) was measured in leaf discs (Mathur *et al.* 2001). Total sugar was estimated by anthrone reagent according to the method of Yem and Willis (1954).

In vivo nitrate reductase activity was determined following the method of Hageman and Hucklesby (1971). Optical density was measured at 540 nm using a spectrophotometer (Spectronic 21D, Milton Roy). The catalytic activity is expressed as mμmol nitrite formed per g fw per hr.

For the assay of ABA, fresh material (40 g) was homogenized and extracted with methanol (1:4 w/v). Methanol was evaporated *in vacuo* at 40°C and ABA was purified with TLC. The purified samples were further analyzed by HPLC (Farooqi *et al.* 1989).

For peroxidase assay, fresh leaves (0.5 g) were ground in liquid nitrogen with 5 ml of 50 mM potassium phosphate buffer (pH 7.0). The homogenates were centrifuged at 8000 g for 30 min at 4^0C. The supernatants were assayed for determination of peroxidase activity as described by Pulter (1974) with some modifications. The assay mixture consisted of guaiacol (1 mM) and hydrogen peroxide (1.3 mM) in sodium acetate buffer (100 mM, pH 5.5) at 30^0C. The reaction was recorded at 470 nm. Peroxidase activity was expressed as the increase in absorbance/min/mg protein. One enzyme unit was defined as the change of one OD /min/mg protein. Protein was estimated according to Lowry *et al.*(1951) using bovine serum albumin as standard.

RESULTS AND DISCUSSION

Herbage yield decreased in all cultivars under water stress, the reduction being maximum in Shivalik (Table 1). Leaf-stem ratio and RWC decreased under water stress in all the cultivars in the range of 8-37% and 29-36%, respectively. The values of water potential (Ψ) decreased over control in all the cultivars. As such, *Mentha arvensis* is a water loving plant. The relationship between RWC and Ψ has often been used to quantify the dehydration tolerance of tissues. Tissues which maintain a high RWC as Ψ decreases, are more tolerant to dehydration (Iannucci *et al*. 2002). The observed significant decrease in RWC under drought indicates water sensitive nature of *M. arvensis*. Soluble sugar content increased significantly under stress and an increase of 87% over the control was observed in cv. Gomti (Table 2). However, small decrease in Ψ under stress indicated that accumulation of sugar did not play a significant role in osmotic adjustment.

In vivo nitrate reductase activity decreased under water stress in cv. Gomti (Table 2). The decrease in activity of this and other enzymes of nitrogen metabolism, under water stress, was reported earlier (Gupta and Sheoran 1979). ABA content increased significantly in stressed plants of Gomti *i.e.* by 12 fold over the control (Table 2). Increase in ABA content in aromatic grasses under stress has been reported earlier (Fatima *et al.* 2002). The role of ABA in signal transduction has been studied to show that it is associated with induction of certain stress inducible genes under stress, which protect plant against dehydration (Shinozaki and Shinozaki 1997). ABA might be triggering biosynthesis of secondary metabolites which increase under stress in medicinal and aromatic plants. Estimation of linear correlation showed that ABA content was negatively associated with water potential ($r=-0.92, P<0.01$), RWC ($r=-0.81, P<0.05$), plant height ($r=-0.86, P<0.05$), and herb yield ($r=-0.83, P<0.05$) (Table 3).

A significant increase in peroxidase activity was observed in untreated stressed plants (Table 1). The increase was 62% in cv. Kalka and 55% in Kosi, over the control. Peroxidase activity increases under abiotic stress in plants and scavenges free radicals generated under stress (Grassman *et al*. 2002). Oil content increased significantly in Gomti and Kalka cultivars under water stress by 17 and 33%, respectively (Table 1 and 2). However, other cultivars were not affected. Menthol increased significantly in stressed plants, in all the cultivars. The increase in oil content and menthol content in *M.*

Table 1. Effect of water stress and chlormequat chloride on different cultivars of *Mentha arvensis*.

Cultivar	Treatment	Leaf: stem ratio	Herbage yield (kg/plot)	Oil content (g/100g fw)	Oil yield (g/plot)	Menthone content (%)	Menthol content (%)	Relative water content (%)	Water potential (-MPa)	Peroxidase activity (OD/min/mg protein)
Himalaya	A	1.4	89.5	1.05	93.66	2.3	77.33	84.33	0.016	1.3
	B	1.6	89.3	1.20	96.66	2.3	80.33	85.33	0.016	1.3
	C	0.94	45.4	1.20	52.66	3.2	82.66	59.33	0.073	1.9
	D	0.89	52.8	1.06	56.00	2.5	80.66	54.00	0.042	2.8
Kosi	A	1.17	66.0	1.20	74.33	1.7	76.33	86.00	0.020	2.0
	B	1.45	68.4	1.33	86.33	2.2	80.00	87.33	0.021	2.5
	C	0.89	49.8	1.53	54.00	2.4	80.66	61.00	0.032	3.2
	D	0.92	45.7	1.00	46.50	2.7	81.33	58.00	0.025	3.1
Kalka	A	1.13	37.1	1.13	41.66	2.3	71.00	88.66	0.021	1.9
	B	1.83	42.1	1.16	48.66	2.7	76.66	88.66	0.022	1.6
	C	0.93	36.8	1.60	31.00	2.3	85.60	75.33	0.035	2.5
	D	1.05	32.7	1.50	48.50	0.9	91.00	63.66	0.034	3.1
Shivalik	A	1.05	46.6	0.90	41.33	1.1	76.00	77.66	0.026	0.7
	B	1.08	48.2	1.06	32.50	1.8	80.33	85.66	0.025	0.6
	C	0.94	21.8	0.90	31.00	2.7	80.00	50.00	0.048	0.7
	D	0.96	25.3	0.86	36.00	2.1	86.00	50.66	0.048	1.6
	CD int. 5%	0.30	19.2	0.37	20.67	1.0	4.16	11.14	0.002	0.6
	CD int. 1%	0.44	26.2	0.51	39.04	1.7	5.60	15.16	0.003	0.7

CD int. = Interaction CD between varieties x treatments, Plot size: 1m^2

A: Control; B: Chlormequat chloride; C: Chlormequat chloride + Stress; D: Water Stress

Table 2. Effect of water stress and chlormequat chloride on growth, oil content and oil yield of *Mentha arvensis* (cultivar Gomti).

Treatment	Leaf: stem ratio	Herb yield (kg/plot)	Oil content (g/100g fw)	Oil yield (g/plot)	Menthol (%)	Menthone (%)	Relative water content (%)	Water potential (-MPa)	NR activity (mµmol NO_2/g fw/h)	ABA (µg/g fw)	Sugar content (mg/100g dry w)
A	1.10	15.33	0.75	114.97	73.08	10.21	82.43	0.020	69.36	0.015	58.40
B	1.25	15.66	0.78	120.00	78.25	10.84	83.93	0.022	79.34	0.015	72.40
C	1.72	17.66	0.80	125.28	76.47	10.97	82.36	0.020	102.10	0.014	78.10
D	0.91	13.33	0.80	97.00	75.51	11.80	74.20	0.033	19.49	0.092	100.40
E	0.94	15.10	0.88	113.25	74.17	10.11	64.70	0.035	29.30	0.055	85.50
F	0.89	11.00	0.88	96.80	73.27	11.70	53.80	0.044	32.18	0.190	109.40
CD 5%	0.41	3.10	0.07	22.40	7.000	3.00	9.10	0.005	33.10	0.012	12.49
CD 1%	0.59	4.40	0.09	31.74	10.000	4.30	12.90	0.008	47.20	0.014	17.78

A: Control; B: Chlormequat chloride 500 ppm; C: Chlormequat chloride 1000 ppm; D: Chlormequat chloride 500 ppm + stress; E: Chlormequat chloride 1000 ppm + stress. F: Water stress; Plot size : 1m^2

Table 3. Correlation coefficient (r) among the various characters of *M. arvensis* (cultivar Gomti).

Character	RWC	Leaf stem ratio	Herb yield	NR activity	Sugar	ABA	Oil content	Menthol
Ψ	0.83*	0.86*	0.92*	0.79*	0.89**	-0.92**	-0.22	-0.49
RWC		0.68*	0.76*	0.34	0.79*	-0.81	-0.58	-0.36
Leaf stem ratio			0.99**	0.68*	-0.77	-0.77	-0.45	-0.67
Herb yield				0.72*	-0.82*	-0.83*	-0.43	-0.60
NR activity					-0.65*	-0.63	-0.30	-0.31
Sugar content						0.98**	0.27	0.18
ABA content							0.32	0.47
Oil content								0.51

* = significant at 5%, ** = significant at 1%

arvensis has been reported to be due to osmotic stress (Mathur *et al.* 2001). Monoterpenoids and oil content increase under water stress in several aromatic plants (Sangwan *et al.* 2001). It appears that increase in secondary metabolites occurs to make the plants withstand the stress. Correlation studies showed that oil content and menthol were positively and significantly related with peroxidase (Table 4).

By the application of chlormequat chloride, herb yield increased in the unstressed plants of cvs Gomti and Himalaya, decreased in Shivalik and remained unaltered in Kalka and Kosi (Tables 1 and 2). Improvement in herb yield was also observed in the stressed plants of Gomti, Shivalik and Kosi. The herb yield increased by 21-37% in Gomti in stressed plants due to chlormequat chloride treatment compared to untreated stressed plants.

Leaf-stem ratio increased significantly in the unstressed plants by the application of chlormequat chloride @1000 ppm. The increase was 56% over the control in cv. Gomti, 24% in Kosi and 14% in Himalaya. Increase in leaf-stem ratio was also observed in stressed plants. Decrease in plant height may be the reason for the increase in leaf-stem ratio (Farooqi and Sharma 1988). Decrease in plant height due to chlormequat chloride application has also been reported in barley (Sanvicente *et al.* 1999).

Effect of chlormequat chloride on Ψ was statistically significant in the stressed plants of cv. Gomti (Table 2). However, significant effect of chlormequat chloride on Ψ was not observed in stressed and unstressed plant of other cultivars (Table 1). Decrease in RWC in chlormequat chloride treated stressed plants was 5-19%

Table 4. Correlation coefficient (r) among the various characters of *M. arvensis*.

Character	RWC	Herb yield	Oil content	Menthol	Peroxidase sp. activity	Oil yield
Ψ	0.94**	0.66*	- 0.08	0.65*	- 0.21	-0.59
RWC		0.65*	-0.14	0.60*	-0.29	0.45
Herb yield			-0.10	0.41	-0.16	0.93**
Oil content				0.38	0.75**	0.22
Menthol					0.52	0.27
Peroxidase sp. activity						0.01

* = significant at 5%, ** = significant at 1%

over stressed plants in cvs. Kalka, Kosi and Himalaya. Ameliorative effect of chlormequat chloride on RWC was observed in stressed plants of cv. Gomti. RWC was increased by 20% and 40% in the stressed plants treated with 500 ppm and 1000 ppm chlormequat chloride, respectively (Table 2). Nitrate reductase activity increased by 14% and 97% over the control in unstressed Gomti plants by the application of 500 ppm and 1000 ppm chlormequat chloride, respectively. A significant decrease (50-70%) in ABA level was noticed in stressed plants over untreated stressed plants due to the treatment. Shukla *et al.* (1990) have reported similar findings with *Artemisia annua*. Sugar content increased in chlormequat chloride treated stressed and unstressed plants significantly and the increase was in the range of 24-72% over control.

Oil content was higher both in chlormequat chloride treated unstressed and stressed plants and the increase was in the range of 4-17% in unstressed plants and 14-41% in stressed plants of Himalaya, Kosi, Gomti and Kalka cultivars, compared to control (Tables 1 and 2). Increase in the oil content of Japanese mint due to chlormequat chloride application has also been reported earlier (Farooqi and Sharma 1988). Ameliorative effect of chlormequat chloride on oil yield was significant in stressed plants of Kosi only. Peroxidase activity in the treated stressed plants of Kosi and Himalaya was significantly less than in the untreated stressed plants. These results substantiate some earlier findings with *Mentha arvensis* (Farooqi *et al*. 2003).

Changes in water relations indicate that water requirement of Japanese mint is high. Changes in growth, metabolism and essential oil content and yield reflect that chlormequat chloride can partially alleviate the detrimental effect of water stress on water relations, nitrate reductase activity, accumulation of ABA and herbage yield in Japanese mint.

ACKNOWLEDGEMENT

The authors are thankful to the Director of the Institute for providing the necessary facilities.

REFERENCES

Candan, N. and Tanhan, K. L. (2003). Relationship among chlorophyll-carotenoid content, antioxidant enzyme activities and lipid peroxidation levels by Mg^{2+} deficiency in the *Mentha pulegium* leaves. *Plants Physiol. Biochem*. **41**: 35-40.

Chatterjee, S.K. (1995). Water stress effect on growth and yield of *Cymbopogon* and its alleviation by n-triacantanol. *Acta. Hortic*. **390** : 19-24.

Das, M.N. and Giri, N.C. (1974). Design and Analysis of Experiment. Wiley Eastern Limited, New Delhi

Farooqi, A.H.A. and Sharma, S. (1988). Effect of growth retardants on growth and essential oil content in Japanese mint. *Plant Growth Regul*. **7** : 39-45.

Farooqi, A.H.A., Shukla, Y.N., Sharma, S. and Bangerth, F. (1989). Endogenous inhibitors and seasonal changes in abscisic acid in *Dioscorea floribunda* Mart Gal. *Plant Growth Regul*. **8**: 225-232.

Farooqi, A.H.A., Luthra, R., Mathur, P., Srivastava, N.K. and Bansal, R.P. (1999). Physiology of cultivated mints. *J. Med. Arom. Pl. Sci*. **21** : 431-441.

Fatima, S., Farooqi, A.H.A. and Sharma, S. (2002). Physiological and metabolic responses of different genotypes of *Cymbopogon martinii* and *C. winterianus* to water stress. *Plant Growth Regul*. **37** : 143-149.

Grassmann, J., Hippeli, S. and Elstner, E.F. (2002). Plants defense and its benefits for animals and medicine: role of phenolics and terpenoids in avoiding oxygen stress. *Plant Physiol. Biochem*. **40** : 471-478.

Guenther, E. (1955). The Essential Oils. Vol. 1, Van Nostrand, Princeton.

Gupta, S.C. and Kapoor, B.K. (1983). Fundamental of Mathematical Statistics. Sultan Chand and Sons, New Delhi.

Gupta, P. and Sheoran, I.S. (1979). Effect of water stress on the enzymes of nitrate metabolism in two *Brassica* species. *Phytochemistry* **18** : 1881-1882.

Hageman, R.N. and Hucklesby, D.P. (1971). Nitrate reductase from higher plants. In: A.S. Pietro (ed.), Methods in Enzymology, vol. 23**,** Acadamic Press, New York.

Lowry, O.H., Rusebrough, N.J., Farr, A.L. and Randall, R.J.(1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem*. **193** : 265-275.

Iannucci, A., Russo, M., Arena, L., Difonzo, N. and Martiniello, P. (2002). Water deficit effects on osmotic adjustment and solute accumulation in leaves of annual clovers. *Euro. J. Agron.* **16** : 111-122.

Mathur, P., Farooqi, A.H.A. and Sharma, S. (2001). Effect of osmotic stress on growth and essential oil content in different cultivars of *Mentha arvensis*. *Indian Perfumer* **45 :** 89 - 93.

Sangwan, N.S., Farooqi, A.H.A., Fatima, S.. and Sangwan R.S. (2001). Regulation of essential oil production in plants. *Plant Growth Regul*. **34** : 3-21.

Samvicente, P., Lazarevitch, S., Blouet, A. and Guckert, A. (1999). Morphological and anatomical modifications in winter barley culm after late plant growth regulator treatment. *Europ. J. Agron*. **11**: 45-51.

Shinozaki, K. and Yamaguchi-Shinozaki, K. (1997). Gene expression and signal transduction in water stress response. *Plant Physiol*. **115** : 327-334.

Shukla, D.S., Sairam, R.K. and Deshmukh, P.S. (1989). Effect of abscisic acid and triadimefon on photosynthesis and nitrate reductase activity during water stress in wheat, *Indian J. Plant Physiol*. **32** : 51-56.

Yemm, E.W. and Willis, A.J. (1954). Estimation of carbohydrates in plant extracts by anthrone. *Biochem*. J. **57** : 508 –514.

Zhao, D. and Osterhuis, D. (1997). Physiological response of growth chamber-grown cotton plants to the growth regulator PGR-IV under water-deficit stress. *Environ. Expt. Bot*. **38** : 7-14.