Phytochemistry, Vol. 26, No. 5, pp. 1333–1334, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00
Pergamon Journals Ltd.

SALINITY DEPRESSION OF GROWTH AND ESSENTIAL OIL FORMATION IN SPEARMINT AND MARJORAM AND ITS REVERSAL BY FOLIAR APPLIED CYTOKININ*

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(*Received* 1 *September* 1986)

Key Word Index—*Mentha spicata*; spearmint; *Majorana hortensis*; marjoram; Lamiaceae; metabolism; essential oils; monoterpenes; salinity; cytokinins.

Abstract—Irrigation of spearmint (*Mentha spicata*) and marjoram (*Majorana hortensis*) with a saline solution consisting of $CaCl_2$ and NaCl reduces overall growth, suppresses essential oil formation and alters the monoterpene composition of the resulting oil. Simultaneous foliar application of the cytokinin diphenylurea (at 10 ppm) or kinetin (at 4 ppm) largely reverses the adverse effects of salinity on both growth and essential oil production.

INTRODUCTION

Recent studies have demonstrated that foliar applications of low levels of the cytokinins kinetin and diphenylurea increase essential oil yield in several members of the Lamiaceae (*Mentha, Salvia, Lavandula*) [1–3]. Conversely, salinity reduces essential oil yield in lamiaceous plants [4], presumably by inhibiting supply of cytokinin from roots to shoots and thus altering the ratio between leaf cytokinin and abscisic acid [5–8]. Since the application of exogenous cytokinin has been shown to be partially effective in reversing stress-induced symptoms of cytokinin deficiency in plants [9, 10], it was of interest to determine if foliar application of kinetin and diphenylurea was effective in overcoming salinity-induced suppression of essential oil formation. Spearmint (*Mentha spicata*) and marjoram (*Majorana hortensis*) were employed as models in this work since preliminary studies had shown these species to have a fairly low salt tolerance level [4 and unpublished observations].

RESULTS

Alternate daily irrigation of spearmint and marjoram with distilled water and with a salt solution consisting of 0.25 M $CaCl_2$ plus 0.5 M NaCl (1 M in Cl^-) resulted in characteristic reduction of growth [4] compared to controls irrigated with distilled water only (Table 1). Fresh weight of the tissue, plant height and leaf area were significantly depressed by the saline solution for both species. Fresh weight reduction was about equally distributed between leaves and stems, and both leaf and internode number were unchanged. Leaf colouration was considerably darker in the saline-treated plants and the root systems on these plants were noticeably reduced in size (*ca* 30%) when compared to the controls raised under identical environmental conditions. One set of treated and control plants was maintained beyond the normal harvest date and was irrigated thenceforth with distilled water only. The previously treated plants flowered 10–12 days earlier than controls irrigated with distilled water for the whole duration.

Identical salinity trials were also carried out with solutions of 0.5 M and 2 M Cl^- (with proportions of Ca^{2+} and Na^+ as before). At the lower level of salinity, growth suppression was also significant, but less pronounced. The higher salt level was toxic toward both species. Downward cupping of the leaves and necrosis of the margins were pronounced, with young leaves turning chlorotic before dying.

Irrigation with the 1 M salt solution resulted in a 20% decrease in essential oil yield on a fresh weight basis in both species (Table 1), while on a per plant basis oil yield was reduced by 40% in spearmint and 50% in marjoram. Possible changes in the proportions of the two major monoterpene components of each oil were also monitored under the influence of salinity; in spearmint the level of limonene was increased and the level of carvone was concomitantly decreased relative to controls irrigated with water only (Table 1). In the case of marjoram, salt stress afforded an increase in the level of sabinene which was accompanied by a decrease in the proportion of sabinene hydrate.

When the plants that were subjected to saline irrigation were simultaneously treated by weekly foliar application of low levels of cytokinin, the saline-induced suppression of growth was largely reversed (Table 1). Diphenylurea (at 10 ppm) was slightly more effective than was kinetin (at

*This is Scientific Paper No. 7551, Project 0268, College of Agriculture and Home Economics Research Center, Washington State University, Pullman, WA 99164–6340, U.S.A. This investigation was supported in part by Department of Energy Contract DE-AMO6-76RL02221, Agreement DE-AT06-82ER12027, and by grants from the Washington Mint Commission and Mint Industry Research Council.

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Table 1. Effect of salinity and cytokinins on growth and essential oil of spearmint and marjoram

Treatment	Growth parameters (%)			Oil yield		Oil composition (%)	
	plant wt	plant ht	leaf area	% fr. wt	mg/plant	Limonene	Carvone
Control (*M. spicata*)	100	100	100	0.50	45.5	11.9	74.5
1 N salt	72	72	65	0.40	28.9	14.8	65.0
1 N salt + 10 ppm DPU	84	86	75	0.51	39.1	10.8	77.3
1 N salt + 4 ppm KTN	81	82	69	0.50	37.3	10.4	74.4
LSD (0.05)	3	12	9	0.06	4.0	2.0	2.5
						Sabinene	Sabinene hydrate
Control (*M. hortensis*)	100	100	100	0.20	23.8	14.5	44.2
1 N salt	64	72	61	0.16	12.2	18.3	36.5
1 N salt + 10 ppm DPU	102	92	95	0.21	24.9	13.8	45.6
1 N salt + 4 ppm KTN	87	91	78	0.19	19.4	15.7	42.8
LSD (0.05)	5	10	8	0.02	3.0	2.5	5.9

Growth parameters are reported as a percentage of the controls raised under identical environmental conditions; for details see Experimental. DPU = diphenylurea, KTN = kinetin.

4 ppm) in restoring plant weight and height to more normal values. In general, leaf expansion remained somewhat inhibited, and root development was not markedly improved, by concurrent treatment with cytokinin. In addition to reversing growth suppression, foliar applied cytokinins fully restored oil yields on a percentage fresh weight basis, and significantly improved yields on a per plant basis, for those plants irrigated with saline solution (Table 1). Both spearmint and marjoram responded similarly to cytokinin with regard to oil composition, in that both diphenylurea and kinetin restored the levels of the monoterpenes in the salt-treated plants to values indistinguishable from untreated controls.

Results of this study indicate that spearmint and marjoram are sensitive to salinity which suppresses growth and essential oil formation, perhaps via inhibition of cytokinin metabolism and transport. The simultaneous foliar application of cytokinin, a treatment which in isolation increases growth and oil production [1], largely reverses any effects of salinity.

EXPERIMENTAL

Plant material. M. hortensis (sweet marjoram) was grown from seed and *M. spicata* was propagated from single-node cuttings [11] under defined environmental and nutritional conditions [12]. Plants were thinned and allowed to grow for 4 weeks before saline irrigation and cytokinin treatment.

Treatments. Each expt was conducted in randomized complete block design with 3 replications totalling 60 plants per treatment. Solns of salt ($CaCl_2$ plus NaCl) and cytokinin (diphenylurea and kinetin) at the indicated concns were prepd in dist. H_2O and dist. H_2O containing Tween 20, respectively. Plants were irrigated daily either with dist. H_2O, or on alternating days with dist. H_2O or the salt soln. Cytokinins were applied on a weekly basis with a hand sprayer (to the point of run-off). Four total applications of cytokinin were made and the plants were harvested 1 week after the last treatment. The irrigation regime was maintained throughout the course of the expt. Controls irrigated with dist. H_2O and sprayed without cytokinin were included in each expt. Data on growth characteristics (fr. wt of plant, leaves, stem and total branches; length, width and number of leaves; length of stem; number of internodes and internode length; and root wt) were collected at harvest and subjected to analysis of variance with comparison of the means by least significant differences at $P = 5\%$ (LSD 0.5) [13]. Control plants typically weighed 4–6 g, were 16–21 cm (spearmint) or 10–13 cm (marjoram) in height and bore leaves with an average surface area of 7–8 cm^2 (spearmint) or 5–6 cm^2 (marjoram).

Oil analysis. A minimum of 3 representative 10 g samples of fresh tissue were steam dist. using a simultaneous steam distn–solvent extn apparatus (J&W Scientific). The still was charged with 0.05 N KOH to prevent the isomerization of *cis*-sabinene hydrate to terpinen-4-ol in marjoram oil; fenchone was employed as the int. std in all cases. The details of distn, sample storage and GC analysis of the essential oils are provided in ref. [11]. Fr. wt yield, per plant yield and percentage of major oil constituents were calculated based on the int. std. Data were statistically analysed as before and the LSD (0.05) of the mean values is reported.

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