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Response of mentha (*Mentha spicata* L.) cultivars to low iron nutrition

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Abstract

Response to low iron supply was studied in efficient and inefficient genotypes of spearmint (*Mentha spicata* L.), *viz,* MSS 5, Arka and Neera. Dry matter yield, plant height, total essential oil, carvone content, chlorophyll content, photosynthetic rate and transpiration rate were 12-13% in MSS 5 cultivar than Arka and Neera. Significant positive association of dry matter yield and essential oil and net photosynthesis rate were obtained in MSS 5 variety, whereas, no significant positive correlation was obtained in Neera and Arka.

Key word: *Mentha spicata* L., spearmint, iron, dry matter yield, essential oil, net photosynthesis rate, carvone, Limonene, chlorophyll

Introduction

Mentha spicata L. is indigenous to northern England and is known as spearmint, garden mint and lamb mint *etc*. It is extensively grown in India for production of spearmint (Ellis *et al.*, 1950). Available reports demostrate that Fe deficiency affects the productivity and essential monoterpene oil(s) (Misra and Sharma, 1991, Misra, 1992). The present study was therefore undertaken to study the effects of Fe deficiency on the growth, photosynthesis and oil content of mentha cultivars.

Materials and methods

The experiment was conducted in controlled glass house from December to March at an ambient temperature of 27$\pm$ 3^{o}C and with 11h day length. Uniform suckers of spearmint cultivars *viz*., MSS 5, Arka and Neera were grown in full strength Hoagland's solution as control, were transferred to 5,000 cm^3 plastic containers conaining nutrient solution (Hoagland and Arnon, 1950). Each treatment was replicated three times in completely randomized block design with complete full-strength > 5.6 Fe mg/ml nutrient solution for each variety as a control were taken under the existing studies.

The composition of nutrient solution was (as mg/L):102K, 100 Ca, 70 N- NO_3, 16 S, 12 Mg, 9 Cl, 5P, 0.52 B, 0.33 Mn, 0.33 Mo, 0.10 Zn, 0.02 Cu; and Fe was supplied as Fe-EDTA (ferric ethylene diamine tetra acetate) at 0, 0.056, 2.8 and 5.6 mg Fe/ litre. Initial pH of the nutrient solution was 6.2-6.8, which was monitored and adjusted periodically with 1.0 M KOH or 2.0 M H_2SO_4 to maintain a value of 7.2. The treated plants of 0 and 0.056 mg Fe/litre did not survive after 10 days of treatment. However, deficient (0.56 mg Fe/litre) ones survived with less height and poor growth. Survival with complete chlorosis was obtained at 2.8 mg Fe/litre whereas, 5.6 mg Fe/ml treated plants were healthy with sufficient luxuriant growth. Later on for screening of efficient genotype of spearmint only 2.8 and 5.6 mg Fe/litre were considered. Plants were harvested, 90 days after transplanting. At harvest the tops of both the plants per container were separated immediately above the fibrous root zone. Observations were taken on plant height, branches/plant and number of nodes. Shoot and root samples were washed in 1% non-ionic detergent solution to remove dust and avoid contamination and then rinsed in deionized water.

Dry matter was determined after samples were air-dried at 60-70^{o}C upto constant weight in a forced air oven. The dried samples were milled and then passed through a 40 mesh seive before storing in polythene bags. Samples of the fresh leaves were used for the determination of total oil, carvone, limonene, total chlorophyll, and chlorophyll 'a' and 'b'. Estimation of essential oil was done after steam distillation of 100 g freshly chopped leaves in Clevenger apparatus (Clevenger, 1928). The oil constituent, carvone and limonene were determined by gas liquid chromatography (Model Perkin Elmer 3920 B). The column was TCD stainless steel, packed with 10% carbowax (20 mesh) on chromosorb WNAW. Injector and detector temperatures were maintained at 25^{o}C. The flow of H_2 gas was 28 cm^3 min^{-1}. Chlorophyll in nearly fully expanded apical leaves was determined in 80% acetone extracts of fresh tissue. Chlorophyll `a' and `b' were assayed in the extracts at 645 and 663 nm respectively in a spectrophotometer using the method of Arnon (1954).

Photosynthesis of leaves was measured in a closed system using a portable photosynthesis system Licor model Li-6000, with certain modifications, as described by by Misra and Srivastava (1991) at an irradiance 300-450 mmol m^{-2}sec^{-1}.

Results and discussion

Severity of Fe deficiency symptoms was greater at low Fe level in Arka and Neera than in MSS 5. The Fe deficiency was observed by complete chlorosis of the younger leaves and with green colouration of older leaves of the plant. Severe symptoms of Fe deficiency appeared on young leaves of plants grown at 0 and 0.56 mg Fe/ml. Severe deficiency symptoms occurred in Arka and Neera. Whereas, MSS 5 showed less chlorosis in 0.56 mg Fe/ ml. Margins of severely deficient leaves were completely yellow with marginal browning and necrosis, and the tips died completely. Similar typical acute Fe-deficiency symptoms have been reported by other workers (Wallace and Mueller, 1980, Mengel *et al.*, 1984, Misra, 1992). Plants grown at or below 1.4 mg/ml Fe showed less dry matter yield and higher fresh weight. The severity of Fe deficiency was greater in Arka and Neera than in MSS 5. Enhanced Fe supply increased the plant height, number of branches, fresh and dry matter yields in MSS 5 (Table 1). It perhaps showed Fe becomes sufficient for this genotype and thereby affecting the physiology of the plant and the difference caused was due to the genotypic effect between the three cultivars (Blaylock *et al.*, 1985, 1986, Brown and Ambler, 1970).

Table 1. Effect of Fe deficiency on growth attributes of *Mentha spicata*

Cultivars	Height (cm)	No. of branches plant^{-1}	Fresh weight plant^{-1} (g)	Dry weight plant^{-1} (g)
MSS 5	23 a^{z*}	25 a$^{*\,**}$	20 a	4 a
Arka	18 b	20 b*	42 b**	9 b
Neera	19 b	15 c	51 c$^{*\,**}$	10 b
Control	20 c	17 d	38 d	7 c

^{z} Mean values in column with similar alphabet combinations as superscript do not differ significantly at 1% level of significance.
* Significantly different at *p=0.05, **p= 0.01

Decrease in plant height resulted at low Fe concentration at deficient level (< 5.6mg Fe/ml) than control, except in MSS 5 cultivar (Table 1). Further the tolerance to Fe deficiency was higher in MSS 5 than in Arka and Neera. Presumably, the Fe tolerance visualised in deficient (~2.8 mg Fe/ml) Fe concentration. Very less effect on the dry weight was observed in MSS 5 clones, followed by Arka and maximum in Neera (10 g/plant). Severity of Fe deficiency symptoms was greater at low Fe level in Arka and Neera than in MSS 5. The Fe deficiency was observed by complete chlorosis of the younger leaves and with green colouration of older leaves of the plant at 0.56 and 1.4 mg/ml Total chlorophyll (3.71 mg/g) chlorophyll 'a' (2.7 mg/g) were observed in MSS 5 cultivar than the control (Table 2). It further revealed that the tolerance of MSS 5 genotype at low iron supply responsed well as in the Fe-efficient genotypes. Blaylock *et al.* (1985) in tomatoes, Brown and Ambler (1970) in corn also reported the less plant height and the higher total chlorophyll responses in the efficient genotypes.

The spearmint cultivars, responded to low availability of Fe which affected the total oil content and percent of essential oil in the 3 cultivars (Table 3). The carvone concentration (87.88% of the total oil) was maximum under Fe deficiency in MSS 5 followed by Arka and Neera, whereas percentage of total oil under Fe deficient condition was 1.06 ml in MSS 5.

Further, to explain the correlated behaviour of the growth and oil characters, linear relationship were worked out. The presence of a significantly positive correlation among these characters, *viz* fresh weight and dry-matter: (r= 0.941, p≤0.01) in MSS 5; (r= 0.742, p≤0.01) in Neera and; (r=0.699, p≤0.01) in Arka. Dry matter yield and oil content (r=0.879, p≤0.05, in MSS 5; r= 0.694, p≤0.05) in Neera and (r= 0.611, p≤0.05 in Arka) and oil % and carvone content (r=0.967, p≤ 0.05 in MSS 5; r=0.901, p≤ 0.05 and r= 0.701, p≤0.05 in Arka and Neera) indicated that the increase in the yield of fresh herb and dry matter resulted in the increase of oil and carvone contents in more efficient genotype, MSS 5. Visual symptoms of Fe deficiency on leaves, plant height, fresh and dry matter yields indicated that Fe requirement for MSS 5 was higher than Arka and Neera. These spearmint cultivars showed differential response to Fe. Cultivar differences for Fe have been noted for many plant species.

Table 3. Effect of Fe deficiency on oil content of *Mentha spicata*

Cultivars	Oil content	Percentage of total oil (%)	
		Carvone	Limonene
MSS 5	1.06 a$^{z*\,**}$	87.88 a$^{*\,**}$	3.97 a
Arka	0.81 b$^{*\,**}$	75.25 b$^{*\,**}$	6.915 b$^{*\,**}$
Neera	0.64 c$^{*\,**}$	77.58 b$^{*\,**}$	7.906 b$^{*\,**}$
Control	0.45 a	67.819 c	8.991 a

^{z} Mean value in column with similar alphabet combination as superscript do not differ significantly at 1% level of significance by t-test. Significantly different at **p*=0.05, ***p*=0.01

The current paper also reports higher photosynthesis and chlorophyll formation with higher oil and carvone percentage was noted in MSS 5. The results are in accordance with the findings of other workers (Brown and Ambler, 1970). The highly significant positive correlation values have further strengthened the observation that MSS 5 is highly efficient spearmint cultivar compared with Arka and Neera. The effect of Fe deficiency on oil concentration was probably due to the effect of Fe on general metabolism and growth of the plants (Agarwal *et al.*, 1964; 1965). Growth and development of *Mentha spicata* were influenced by the concentration of Fe in a nutrient solution. Fe is available in an appreciable amount in soils, generally in an oxidized ferric form. It is largely required for the growth of all plants (Marsh *et al.*, 1963). However, these differ both in their requirement and in their availability to absorb it from the root medium. Plants which are Fe-efficient have a mechanism to convert it to ferrous form. In the present investigation MSS 5 at the onset of recovery from the chlorosis and pH closely associated with fast-greening and pH acidification of the medium. Other workers also reported such a behaviour of the efficient genotypes. Therefore, MSS 5 is more Fe-efficient than Arka and Neera cultivar. Under severe Fe-deficiency, *M. spicata* showed typical visual chlorosis that was prominent in young leaves. The chlorophyll content was significantly low at acute Fe deficiency. Similarly, the chlorophyll a/b ratio was

Table 2. Effect of Fe deficiency (2.8 mg Fe/ml) on chlorophyll content, chlorophyll a+b, transpiration rate (E) and net photosynthetic rate (Pn) in *Mentha spicata*

Cultivars	Chlorophyll mg/g (fresh weight)			Transpiration rate (E)	CO_2 exchange rate (P_n)
	Chl-a	Chl- b	Chl (a+b)	[m mol/m^2/sec]	[mg(CO_2)/m^2/sec]
MSS 5	2.70 a^{z***}	1.01 a	3.71 a$^{*\,**}$	2.016**	5.98 a
Arka	1.99 b	1.15 a	3.14 b***	0.791 b	6.15 a
Neera	1.75 b	1.89 b***	3.64 a$^{*\,**}$	1.592 c	6.75 b$^{*\,**}$
Control	1.62 c	1.01 a	2.63 c	1.247 c	8.65 c

^{z} Mean values in columns with similar alphabet combinations as superscript do not differ significantly at 1% level of significance by t-test. * Significantly different at *p=0.05, **p=0.01

maximum at lowest Fe level. Growth of the Fe-deficient plant decreased with decrease in Fe in the solution. The low availability of Fe to *Mentha spicata* plants affected the oil constituents. Maximum tissue concentration of Fe was observed in leaf tissue. Fe is reduced to ferrous form at the root surface if necessary and transported to the leaf through the xylem in a combined form. In the leaf it is used for chlorophyll formation as well as for the functioning of various Fe containing enzymes (Marsh *et al.*, 1963, Agarwal *et al.*, 1964). Synthesis of chlorophyll is more in MSS 5 and Neera than Arka. Fe uptake showed a negative correlation with Fe-stress tolerance. The most susceptible MSS 5 showed higher Fe uptake in root and less Fe translocation in the shoot than Arka and Neera. Therefore, tolerance and susceptibility are not dependent. Moreover, the results have suggested that the Fe deficiency affects the carvone content and herb yield and ultimately the economic losses of spearmint in Fe-efficient genotypes MSS 5 cultivar are lesser.

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