PHOTOSYNTHETICA 39 (3): 473-476, 2001

BRIEF COMMUNICATION

# Influence of Mn deficiency on growth, chlorophyll content, physiology, and essential monoterpene oil(s) in genotypes of spearmint (*Mentha spicata* L.)

P. SINGH, A. MISRA, and N.K. SRIVASTAVA

*Central Institute of Medicinal and Aromatic Plants, P.O. CIMAP, Kukrail Picnic Spot Road, Lucknow-226015, India*

## Abstract

Spearmint cultivars MSS-5, Arka, and Neera grown in nutrient culture in controlled conditions differed in plant height, number of tillers, internodal position, fresh mass, dry mass, leaf stem ratio, and chlorophyll contents. Initial transpiration rate, stomatal conductivity, and $CO_2$ exchange rate showed better increase in MSS-5 genotype. Mn stress decreased oil content whereas the content of oil constituent carvone increased in MSS-5 and Arka.

*Additional key words*: carvone; dry and fresh mass; leaf stem ratio; limonene; net photosynthetic rate; protein; stomatal conductance; tiller; transpiration rate.

Manganese is an essential microelement for plant growth and photosynthesis (Bertrand 1987, Fales and Ohki 1982, Ohki 1976, 1984, 1985), including medicinal and aromatic plants. Reduction in plant dry mass and net photosynthetic rate ($P_N$) of spinach was reported for Mn deficiency (Bottrill *et al.* 1970). The limitation of photosynthesis has major influence on growth (Fales and Ohki 1982). The requirement for Mn in photosystem 2 (PS2) $O_2$ evolution might corroborate this hypothesis (Packham and Barber 1984, Yamamoto and Nishimura 1983; but see Nedunchezhian *et al.* 2000). Nevertheless, high dose of Mn is toxic (Subrahmanyan and Rathore 2000). Also metabolic processes other than photosynthesis, biochemical reactions, and physiological systems require Mn. Deficiency of Mn causes chlorosis in plants. The low chlorophyll (Chl) content is either the result of few photosynthetically active chloroplasts or many disabled chloroplasts (Homann 1967). Mn deficiency severely disorganises normal arrangement of grana in spinach chloroplasts (Mercer *et al.* 1962). Information on the effect of Mn deficiency on transpiration rate (*E*) is sparse. However, Terry and Ulrich (1974) reported that Mn deficiency had no apparent effect on stomata opening. This would indicate that *E* would not likely be affected by Mn deficiency.

The isoprenoid biosynthetic pathway produces several important metabolites such as carotenoides, chlorophyll, gibberellic acid, and sterols that are essential for growth, photosynthesis, and plant development. This pathway also produces essential oil. Lower or higher concentrations of Mn affect several metabolic pathways (Knotz *et al.* 1977, Maudinas *et al.* 1977) and also the isoprenoid biosynthesis (Wilkinson and Ohki 1988). Thus optimum Mn requirment is essential for enzyme activities directly involved in the synthesis of chloroplast pigments. The present study explores the effect of Mn deficiency on growth, photosynthetic characters, and essential oil content and constituents in cultivars of spearmint.

Uniform sized plantlets of spearmint cvs. MSS-5, Arka, and Neera obtained from the farm nursery of the Central Institute of Medicinal and Aromatic Plants, Lucknow were planted in 10 000 $cm^3$ ceramic pots filled with silica sand. The sand had previously been cleaned by acid digestion in steam to purify and remove impurities of Mn (Agarwala and Sharma 1961). After 2 weeks, rooted suckers were transferred to 25 $cm^3$ pots containing nutrient solution. The salts used in solution culture were purified to remove impurities of Mn by dithizone (Hewitt 1966). Nutrient solution of Hoagland and Arnon (1950) was used in the experiment, except Fe that was supplied as Fe-EDTA. In the deficient medium Mn was omitted. Three pots each (six plants per pot) of deficient (–Mn) and control (+Mn) treatments were maintained in glasshouse at temperature of 30-35 °C and irradiance of 800-

---

*Received* 12 July 2001, *accepted* 13 September 2001.

Fax: 0522-342666, e-mail: cimap@satyam.net.in

*Acknowledgement*: The authors are grateful to the director CIMAP for providing necessary facilities and encouragement during the study.

Table 1. Influence of Mn deficiency on growth attributes, chlorophyll (Chl) and protein contents, net photosynthetic ($P_N$) and transpiration ($E$) rates, stomatal conductance ($g_s$), and oil contents in cultivars MSS-5, Arka, and Neera of *Mentha spicata*. $^*p$ = 5 %, $^{**}p$ = 1 %.

| | MSS-5 | | Arka | | Neera | | CD | |
|---|---|---|---|---|---|---|---|---|
| | +Mn | –Mn | +Mn | –Mn | +Mn | –Mn | 5 % | 1 % |
| Plant height [cm] | 65 | $51^{**}$ | 60 | $42^{**}$ | 47 | $41^{**}$ | 3.04 | 4.30 |
| Number of tillers | 16 | $11^{**}$ | 15 | $12^{**}$ | 12 | $8^{**}$ | 1.81 | 2.56 |
| Fresh mass per plant [g] | 52.50 | $42.51^{**}$ | 47.13 | $42.94^{**}$ | 40.46 | $15.72^{**}$ | 2.70 | 3.80 |
| Dry mass per plant [g] | 9.18 | $7.43^{**}$ | 8.24 | $7.51^{**}$ | 7.07 | $2.74^{**}$ | 0.47 | 0.67 |
| Leaf stem ratio | 0.70 | 0.85 | 0.74 | 1.41 | 0.95 | 1.28 | | |
| Chl ($a$+$b$) [g kg$^{-1}$(f.m.)] | 1.90 | $1.58^{*}$ | 1.72 | $1.36^{*}$ | 1.92 | 1.76 | 0.31 | 0.44 |
| Protein [g kg$^{-1}$(f.m.)] | 3.20 | $2.51^{**}$ | 2.82 | 2.72 | 2.52 | $1.79^{**}$ | 0.18 | 0.26 |
| $P_N$ [μg(CO$_2$) m$^{-2}$ s$^{-1}$] | 8.34 | $5.79^{**}$ | 8.08 | $6.12^{**}$ | 7.99 | $5.24^{**}$ | 1.36 | 1.93 |
| $E$ [μmol m$^{-2}$ s$^{-1}$] | 343.5 | $312.8^{**}$ | 335.3 | $304.6^{**}$ | 318.5 | $288.5^{**}$ | 0.02 | 0.03 |
| $g_s$ [nmol m$^{-2}$ s$^{-1}$] | 466.9 | $416.5^{**}$ | 424.5 | $414.7^{**}$ | 438.8 | $346.0^{**}$ | 0.03 | 0.04 |
| Oil [%] | 1.30 | $0.83^{**}$ | 1.14 | $0.70^{**}$ | 1.29 | $0.51^{**}$ | 0.30 | 0.42 |
| Carvone [%] | 67.90 | 66.98 | 66.42 | $72.23^{**}$ | 71.19 | $61.11^{**}$ | 2.97 | 4.21 |
| Limonene [%] | 12.24 | $4.86^{**}$ | 13.86 | $12.94^{**}$ | 2.52 | $6.74^{**}$ | 0.03 | 0.04 |

1 000 μmol m$^{-2}$ s$^{-1}$). The nutrient medium was changed weekly. Plant height, number of tillers, internode length, fresh biomass, and dry biomass were recorded. From these values leaf stem ratio (LSR) was calculated (Radford 1967). A known mass of third leaf tissue (fully expanded) was extracted with 80 % acetone and Chl absorbance was recorded on a *Spectronic 21D* spectrophotometer (*Milton Roy*, USA) and calculated according to Arnon (1949). $P_N$ of the 3rd leaf was measured in a closed system using a computerised portable photosynthesis system (*Li-6000*, *LiCOR*, Lincoln, USA) (Srivastava and Luthra 1991).

Protein content in fresh leaf tissue was determined by the method of Lowry (1951). Total oil content of leaves was estimated by hydro-distillation of 100 g fresh chopped leaves in an apparatus of Clevenger (1928). Carvone and limonene contents were determined by gas chromatography (*Perkin Elmer*, model *3920 B*). The column was TCD stainless steel packed with 10 % carbowax coated on *Chromosorb* (120 mesh) WNAW. Injector and detector temperatures were maintained at 250 °C. The flow of $H_2$ gas was 467 mm$^3$ s$^{-1}$ (Misra and Sharma 1991).

All the spearmint cultivars studied were of similar physiological stage and exhibited typical Mn deficiency symptoms such as marginal chlorosis and necrosis of leaves. The visual symptoms varied, with cv. MSS-5 being least susceptible and cv. Neera being most susceptible. This was further substantiated by growth parameters. There were significant differences in plant height, number of tillers, fresh mass, dry mass, and leaf stem ratio (Table 1). Mn deficiency significantly reduces growth in other plants (Burke *et al.* 1990, Behera and Behera 1993, Wissermeier and Horst 1991). This is because of Mn requirement in metabolic processes for plant growth and development, limitation of metabolic synthesis in secondary plant products, whose decreased activity ultimately results in a limitation of growth (Wilkinson 1982). The Chl content decreased significantly under Mn stress in all cultivars (Table 1). This decrease in Chl content under Mn deficiency is in accordance with the findings of Behera and Behera (1994), Mercer and Graham (1987), and Wilkinson and Ohki (1988). Mercer *et al.* (1982) reported that Mn deficiency severely disorganises the normal arrangement of the grana in spinach chloroplasts.

There was highly significant decrease in $P_N$ in cv. Neera than in other cultivars. Initial $E$ decreased from 8-9 % in all genotypes over control, while stomatal conductance ($g_s$) also significantly decreased (Table 1). Reduction in photosynthetic parameters under Mn deficiency conditions is in agreement with findings in spinach (Bottrill *et al.* 1970), soybean (Ohki 1976), wheat (Ohki 1984, 1985), or sugar beet (Terry and Ulrich 1974).

Mn is utilised in many enzymic electron transport reactions (Packham and Barber 1984). Contradictory results were reported for sorghum (Pandey 1989), beans (Garg *et al.* 1986), and maize (Nenova and Stoyanov 1995). The leaf protein content showed decline at Mn deficiency in all genotypes with respect to control. Maximum decrease in protein content was in cv. Neera [1.79 g kg$^{-1}$(FW) over control], while minimum decrease was observed in cv. Arka (Table 1).

Oil content was reduced under Mn deficiency (Table 1). Maximum oil content decrease was observed in cv. Neera (60 % over control) and minimum in MSS-5 (36 % over control). Content of the oil constituent carvone was significantly greater in Arka, whereas limonene content increased significantly in cv. Neera but it did not increase in the other two cultivars.

Effects of nutrient stress on growth, metabolism, and oil content were reported in aromatic crops such as *M. arvensis* (Misra and Sharma 1991) and citronella (Misra and Srivastava 1994). Cv. Neera was most susceptiable to

Mn deficiency, whereas cultivar MSS-5 was the least susceptible and most efficient genotype. The significant positive association ($r = 0.947$, $p = 0.01$) between biomass yield and essential oil content in cv. MSS-5 indicated the association of primary metabolism with secondary metabolite accumulation. Similar results were found in *M. arvensis* under Fe-stress (Misra and Srivastava 1989, Srivastava and Luthra 1991). In conclusion, these studies highlight genotype differences in response to Mn deficiency and its effect on various physiological parameters that severely limit the terpene metabolism and accumulation.

## References

Agarwala, S.C., Sharma, C.P.: The standarisation of sand and water culture technique for the study of macro and micro (trace) element deficiencies under Indian condition. – Curr. Sci. **30**: 424-426, 1961.

Arnon, D.I.: Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. – Plant Physiol. **24**: 1-15, 1949.

Behera, B.C., Behera, P.K.: Manganese tolerance of *Amaranthus* species during seedling growth. – Ann. Plant Physiol. **7**: 153-159, 1993.

Behera, B.C., Behera, P.K.: Chlorophyll *a/b* and catalase activity in four species of *Amaranthus* in relation to their tolerance to manganese. – Indian J. Plant Physiol. **37**: 259-263, 1994.

Bertrand, G.: Sur intervention du manganese dans les oxidations provoquees par la laccase. – Compt. rend. Acad. Sci. Paris **124**: 1032-1035, 1355-1358, 1987.

Bottrill, D.E., Possingham, J.V., Kriedemann, P.E.: The effect of nutrient deficiency on photosynthesis and respiration in spinach. – Plant Soil **32**: 424-438, 1970.

Burke, D.G., Watkins, K., Scott, B.J.: Manganese toxicity effects on visible symptoms, yield, Mn levels and organic acid level in tolerant and sensitive wheat cultivars. – Crop Sci. **39**: 275-280, 1990.

Clevenger, J.F.: Apparatus for the determination of essential oil(s). – J. amer. pharm. Assoc. **17**: 345, 1928.

Fales, S.L., Ohki, K.: Manganese deficiency and toxicity in wheat: Influence on growth and forage quality of herbage. – Agron. J. **74**: 1070-1073, 1982.

Garg, O., Hemantranjan, A., Ramesh, C.: Effect of Fe and Zn fertilization on senescence in French bean (*Phaseolus vulgaris*). – J. Plant Nutr. **9**: 257-266, 1986.

Hewitt, E.J.: Sand & water culture method used in the study of plant nutrition. – Commun. Bur. Hort. Plant Crops Tech. Commun. **22**: 405-439, 1966.

Hoagland, D.R., Arnon, D.I.: The water culture method of growing plants without soil. – Calif. Agr. Exp. Stat. Circ. **347**: 1-32, 1950.

Homann, P.H.: Studies on the manganese of the chloroplast. – Plant Physiol. **42**: 997-1007, 1967.

Knotz, J., Coolbaugh, R.C., West, C.A.: Regulation of the biosynthesis of ent-kaurene from mevalonate in the endosperm of immature marah macrocarpus seeds by adenylate energy change. – Plant Physiol. **60**: 81-85, 1977.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.: Protein measurement with the Folin phenol reagent. – J. biol. Chem. **193**: 265-275, 1951.

Marcer, N.E., Graham, R.D.: Genotypic variation for Mn efficiency in wheat. – J. Plant Nutr. **10**: 2049-2055, 1987.

Maudinas, B., Bucholtz, M.L., Papastephanou, C., Katiyar, S.S., Briedis, A.V., Porter, J.W.: The partial purification and properties of a phytoene synthesizing enzyme system. – Arch. Biochem. Biophys. **180**: 354-362, 1977.

Mercer, F.V., Nittim, M., Possingham, J.V.: The effect of manganese deficiency on the structure of spinach chloroplasts. – J. Cell Biol. **15**: 379-381, 1962.

Misra, A., Sharma, S.: Critical concentration of iron in relation to essential oil yield and quality parameters of japanese mint. – Soil Sci. Plant Nutr. **37**: 185-190, 1991.

Misra, A., Srivastava, N.K.: Iron nutrition related to growth and physiology of japanese mint (*Mentha arvensis*). – In: Proc. Int. Congress of Plant Physiology. Pp. 1115-1160. New Art Press, New Delhi 1989.

Misra, A., Srivastava, N.K.: Influence of Fe nutrition on chlorophyll content, photosynthesis and essential monoterpene oil(s) in java citronella (*C. winterianus* Jowitt). – Photosynthetica **30**: 425-434, 1994.

Nedunchezhian, N., Muthuchelian, K., Bertamini, M.: Photosystem 2 efficiency and thylakoid protein pattern in DCMU-treated wheat seedlings during senescence. – Photosynthetica **38**: 807-814, 2000.

Nenova, V., Stoyanov, I.: Physiological and biochemical changes in young maize plants under Fe deficiency. Catalase, peroxidase and nitrate reductase activity in leaves. – J. Plant Nutr. **18**: 2081-2091, 1995.

Ohki, K.: Manganese deficiency and toxicity levels for "Bragg" soybeans. – Agron. J. **68**: 861-864, 1976.

Ohki, K.: Manganese deficiency and toxicity effects on growth, development and nutrient composition in wheat. – Agron. J. **76**: 213-218, 1984.

Ohki, K.: Manganese deficiency and toxicity effects on photosynthesis, chlorophyll, and transpiration in wheat. – Crop Sci. **25**: 187-191, 1985.

Packham, N.G., Barber, J.: Stimulation by manganese and other divalent cations on the electron donation reactions of photosystem II. – Biochim. biophys. Acta **764**: 17-23, 1984.

Pandey, D.P.: Iron chlorosis and nitrate reductase activity in response to Fe stress and additional nitrate in sorghum. – J. Plant Nutr. **12**: 375-386, 1989.

Radford, P.J.: Growth analysis formulae. Their use and abuse. – Crop Sci. **7**: 171-175, 1967.

Srivastava, N.K., Luthra, R.: Interspecific variation in mints for photosynthetic efficiency, and $^{14}C$ primary metabolic pool in relation to essential oil accumulation. – J. Plant Physiol. **138**: 650-654, 1991.

Subrahmanyam, D., Rathore, V.S.: Influence of manganese toxicity on photosynthesis in ricebean (*Vigna umbellata*) seedlings. – Photosynthetica **38**: 449-453, 2000.

Terry, N., Ulrich, A.: Photosynthetic and respiratory $CO_2$ exchange of sugar beet leaves as influenced by manganese deficiency. – Crop Sci. **14**: 502-504, 1974.

Wilkinson, R.E.: Mefluidide inhibition of sorghum growth and gibberellin precursor biosynthesis. – J. Plant Growth Regul. **1**: 85-94, 1982.

Wilkinson, R.E., Ohki, K.: Influence of manganese deficiency and toxicity on isoprenoid synthesis. – Plant Physiol. **87**: 841-846, 1988.

Wissermeier, A.H., Horst, W.J.: Modified methods for screening cowpea cultivars for Mn leaf tissue tolerance. – Crop Sci. **31**: 435-439, 1991.

Yamamoto, Y., Nishimura, M.: Association of two manganese atoms with the reaction center of photosystem II in a highly active $O_2$ evolving photosystem II preparation. – Biochim. biophys. Acta **724**: 294-297, 1983.