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Effect of heavy manuring of phosphorous and its toxicity on growth, photosynthesis and photosynthetic pigments in Zn-efficient genotype of spearmint MSS-5

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Abstract

Changes in growth attributes, photosynthesis (Pn), photosynthetic pigments with γ-Glu.cys peptidase peptide and Zn accumulation in a Zn-efficient genotype of spearmint MSS-5 were investigated. Effect of phosphorus toxicity on MSS-5 were significantly different than the other genotypes; Arka, Neera and control (the local strain), in terems of phenotypic changes in height and a decrease in chlorophyll contents and CO_2 exchange rate. Heavy P manuring lead to the tolerance of Zn accumulation in MSS-5 with γ-Glu.cys. peptidase peptide with high protein contents and Pn. Hence, the P toxicity induced a differential utilization of γ-Glu.cys.peptidase peptide for higher accumulation of Zn in MSS-5 spearmint with higher photosynthetic rate for increasing the height and essential monoterpene oil(s). The study also indicated that accumulation of toxic heavy metal-Zn with γ-Glu.cys.peptidase peptide made protein synthesis easier with antioxidants Zn cofactor enzymes.

Key words: Spearmint, *Mentha spicata*, Zn-efficient genotype MSS-5, protein, photosyntheis, photosynthetic pigments, Zn toxicity.

Introduction

The heavy and unmanaged fertilization of major nutrients *viz.*, N, P & K for the improvement of crop yields, leads to the deficiency of micronutrients. Heavy doses of P and its precipitation to phosphate salts makes Zn unavailable to the plant (Marschner, 1986). Zn shows the toxicity in medicinal and aromatic plants cultivation (Misra and Ramani, 1991). Furthermore, the acidic soil of hills bears the Fe toxicity in rose cultivation. One way or other, the toxicity of Zn and Fe especially in micronutrients usage affects the productivity and secondary plant products. The phytoremediation is a technique that plants uses to cleanup contaminated soil and water. No work has been reported so far on the cultivation of medicinal and aromatic plants on Fe efficient genotypes to cleanup the contaminated soil with heavy metal Zn.

Spearmint (*Mentha spicata* L.) is widely cultivated to obtain the essential monoterpene oil(s). Zn efficient genotype, MSS-5 has been taken up for the complete exploitation of monoterpene oil(s), with the aim of phytoremediation and to extract out Zn from Zn toxic fields. The aim to use this Zn efficient genotype MSS-5 was to know the physiology and growth behaviour in the wake of phytoremediation processes. In phytoremediation some plants tolerate high levels of toxic micronutrient metals by a variety of mechanisms such as reduced uptake, active efflux and intracellular and/or extracellular sequestration. The most predominant molecules in intracellular detoxification, widely prevalent in eukaryotes are thiol tripeptides, γ-Glu-Cys peptides or phytochelatins. These are synthesized by phytochelatin synthase which is activated by subleathal metal concentrations and play a crucial role in cytosolic metal detoxification (Steffens, 1990; Zenk, 1996). The physiological concentrations of these intracellular metal binding ligands have sometimes been used as a specific indicator of metal tolerance in plants (Grill *et al.*, 1988), and the role of heavy metals in decline and damage to forest ecosystem (Gawell *et al.*, 1996). Zinc is potentially toxic metal when transferred from plants via food chain to human. Agriculture system is also the principle sources of Zn, includes the heavy P fertilization for crops (Misra and Sharma, 1991).

Possibly Zn tolerant spearmint efficient genotypes MSS-5 cultivation in fields leads, for the removal of toxic heavy metal Zn, from heavily phosphorous fertilized soil, for the establishments of tolerant varieties capable of synthesizing more γ-Glu-cys peptides or phytochelatins. The essential monoterpene oil(s) of this efficient genotypes is commercially important for pharmaceutical and aromatic industries. The efficient genotype MSS-5 due to toxicity of heavy metals produces the Zn induced Fe deficiency symptoms. Therefore, a detailed study on the growth and physiology of an efficient genotype MSS-5 alongwith Arka and Neera was conducted to compare Zn accumulation and tolerance against the phosphorous toxicity and to show the Zn induce Fe deficiency in genotypes with primary plant products –photosynthetic pigments and Pn, and simultaneously the essential monoterpene oil(s)in spearmints, in heavly phosphorus fertilized crops.

Materials and methods

The experiment was conducted in controlled glasshouse condition from December to March at an ambient temperature of 27$\pm$3^{o}C and with 11 h day length. Uniform suckers of spearmint cultivars *viz.*, MSS-5, Arka, Neera and a local strain (control) were grown in 5,000 cm^3 plastic containers containing nutrient solution (Hoagland and Arnon, 1950). Each 2.8 Fe µg mL^{-1} treatment and mint strain were replicated 6 times and put in completely randomized block design with complete 5.6 Fe µg mL^{-1} strength, nutrient solution, were taken under the existing studies. The composition of nutrient solution was (as mg L^{-1}): 102 K, 100 Ca, 70 N-NO_3, 16 S, 12 Mg, 9 Cl, 5 P, 0.52 B, 0.33 Mn, 0.33 Mo, 0.10 Zn, 0.02 Cu and Fe was as Fe-EDTA (Ferric ethylinediamine tetraacetate). During the study, instead of 5 mg L^{-1}, 10 mg L^{-1} phosphorous was added in nutrient solution for toxicity in each treatments of 2.8 and 5.6 µg Fe mL^{-1}. Initial pH of the nutrient solution was 6.7 to 6.8, which was monitored and adjusted periodically Zn with 1.0 m M KOH or 2.0 m M H_2SO_4 to maintain a value of 7.2.

MSS-5, Arka and Neera genotypes when subjected to Fe deficiency stress (2.8 mg Fe mL^{-1} treatment), resulted in root exudation, which decrease the pH of the nutrient medium and showed the chlorosis of younger leaves whereas older one remains green. The root exudation and their ability to absorb and utilize iron in the ferrous form vary, with the genotypes of the crop plants. The efficient genotype of spearmint MSS-5 only turned green after the Fe deficiency visualizes characters, where as the severe chlorotic Arka and Neera did not turn green. Chlorosis in terms of total chlorophyll were estimated for the cultivar MSS-5 genotype which behaves as a Fe efficient genotype with more root exudation which was measured with the method of Arnon (1949).

The cultivar MSS-5 genotype behaves as a Fe efficient genotype with more root exudation of phenolic compounds, especially the caffeic acids. The phenolic compounds, the caffeic acids were estimated in root exudation by the method of Singh *et al.* (2001). The plan tissue Fe and Zn contents were estimated with 1 N HCl extracts on atomic absorption Pye Unichem, 2900 (Misra, 1992). The lignin was estimated by the Kalson method of Browning (1967), 5 g samples were digested with 72% H_2SO_4, then diluted with acid; to hydrolize and solubilize the polysachharides. The insoluble residue was dried and weighed as lignin on % basis. This partially solubilized as acid soluble lignin from spearmint, were further quantified on UV absorbance at 410 nm.

HPLC analysis of γ-Glu-cys peptides or phytochelatins (PCs): For the separation of PCs, HPLC analysis was performed in crude extracts of plant tissue following the method of Grill *et al.* (1991). Frozen plant tissue (1 g FW) was homogenized in 0.5 ml 1 N NaOH containing 1 mg mL^{-1} sodium borohydride. Alter centrifugation at 13,000 g at 4°C, the supernatants were acidified by adding 3.6 N HCl and precipitated protein was removed by centrifugation. The protein was estimated by the method of Lowry *et al.* (1951). Separation of PC peptides was done on a reverse phase C-18 column (µ Bondapack, RP 4 µm) with a linear gradient 0.1% Trifluoro acetic acids at a flow rate 0.5 ml min^{-1} using the applied biosystem HPLC (model No. 783A) at 220 nm. Experiments were done and HPLC in triplicates, and repeated thrice.

Pn and essential monoterpene oil(s): Initially, Pn of the third leaf was measured in a closed system using a poratable computerized photosynthesis model Li-6000(Licor, Lincoln, USA), as described in Singh *et al.*(1999) and total essential monoterpene oil(s) were extracted by 100 g fresh chopped leaves in Clevenger's apparatus (Clevenger, 1928).

All measurements were taken in triplicate and the results are given as means±SE. The data were analyzed statistically by two way ANOVA followed by 't-test' for comparing the means following Armitage (1971). The correlation coefficient among the characters were also analyzed further the values are statistically analysed by paired t-test.

Results

The efficient genotype of spearmint cv MSS-5 exudes more root exudates in the nutrient medium (0.54 mg g^{-1}) followed by more acidic medium than the Arka and Neera Chlorosis of younger leaves were more pronounced in MSS-5 with more and more root exudates the older leaves became green, then the Arka and Neera cultivars (Table 1). Results showed that the most Fe efficient genotype is MSS-5, where the iron uptake is 1440 µg g^{-1} in roots tissues of spearmint with further more the recovery of chlorotic younger leaves. Converting more Fe for Fe uptake in an efficient genotype by the help of maximum production of 0.82 mg g^{-1} phenolic compounds and root exudates -Fe^{+++} reductants chelation to produce more Fe^{++} availability to the plant. Table 2 indicates more toxic Zn uptake in tissue concentrations in MSS-5 cultivars (94 µg Zn g^{-1}), with maximum production of protein 1.49 mg g^{-1} and phytochelatins 45.79 m mol g^{-1} FW. The role of Zn excess in spearmint is to behave as an antioxidant, as a scavenger to the excess free radicals removal during the

Table 1. Effects of different genotypes of spearmint in deficient Fe nutrition in younger leaves (at 2.8 µg Fe ml^{-1}) in phosphorous excess

Genotype	Chlorophyll (mg g^{-1})		Phenolic compounds (mg g^{-1})	Caffeic acid (mg g^{-1})	Fe uptake (µg g^{-1})	Lignin (g g^{-1})	P value
	Deficient	Recovered					
Arka	2.01	2.07	0.65	0.41	1109	1.1	0.01
Neera	1.99	2.01	0.68	0.47	1163	1.2	NS
MSS-5	1.85	3.11	0.82	0.54	1448	1.4	0.01
Control	1.89	3.01	0.71	0.51	1437	1.1	0.01

Table 2. Effect of different genotype of spearmint in deficient Fe nutrition in younger leaves (at 2.8 µg Fe ml^{-1}) in phosphorous excess

Genotype	Height (cm)	Zn accumulation (mg g^{-1})	Pn (mg (CO_2) $m^{-2}s^{-1}$)	Protein (mg g^{-1})	Oil (%)	γ-Glu Cys peptide (m mol g^{-1} FW)	P value
Arka	31	70	124±7*	1.01	0.42	6.48±0.3	0.01
Neera	25	82	239±4	1.24	0.49	33.49±1.5	NS
MSS-5	42	94	249±2**	1.49	0.56	45.79±2.1	0.01
Control	32	90	241±3*	1.37	0.47	41.62±2.1	0.01

*, ** mean values significant at P=0.05 and P=0.01, respectively (Paired t test); NS - nonsignificant

Table 3. Correlation coefficients between different characters *viz.*, phenolic compounds, caffeic- acids, Fe uptake, Zn accumulation in *M. spicata*

Characters	Phenolic compound	Caffeic acid	Zn accumulation	Fe ptake	Lignin	Protein
γ-Glu.-Cys.-peptide	0.719**	0.712**	0.871**	0.641*	0.179	0.971**
Phenolic compound		0.699*	0.811**	0.821**	0.827**	0.642**
Caffeic acid			0.749**	0.714**	0.912**	0.617
Zn-accumulation				-0.497	0.679*	0.747**
Fe uptake					0.579	0.621*
Lignin						0.639*

*, ** values are significant P=0.05 or P=0.01, respectively

metabolism of essential monoterpene oil(s).

Table 3 indicated the γ-Glu Cys. peptidase peptide for significantly associated with toxic Zn-accumulation (r=0.871, $P \leq 0.01$). Zn accumulation and lignin (r=679, $P \leq 0.01$) and Zn accumulation with protein (r=747, $P \leq 0.01$), respectively.

Discussion

Results indicated the most efficient and inefficient genotype of all the existing cultivars *viz.*, Arka, Neera and MSS-5. The most Fe-efficient genotype is MSS-5. The root exudation of phenolic compounds (10.87 mg g^{-1}) and caffeic acid (0.54 mg g^{-1}), is more in MSS-5 cultivar where as Arka and Neera had lesser amount. Greening in the form of chlorophyll formation (3.11 mg g^{-1}) and Fe uptake (1448 µg g^{-1}) was found in MSS-5 genotype, whereas it was very less in Arka and Neera. Other workers also reported the same root exudation, and greening of the chlorosis of the genotypes, in different crops (Marschner, 1996; Brown and Jolly, 1986; Kannan, 1982).

Fe efficient genotype MSS-5 showed the Zn (94 µg Zn g^{-1}) and γ-Glu-cys peptide phytochelatin (45.79 n mol g F.wt.) then lesser concentrations of Zn and phtochelatins in Arka and Neera (Table 2). The heavy P fertilization produced Zn induced Fe-deficiency (Table 1). This Zn induced Fe deficiency was reported in *Mentha arvensis* (Misra and Ramani, 1991). Furthermore, the Zn toxicity facilitates the proteins metabolism due to the antioxidants usage of Zn (Table 2). Phytochelatins and Zn accumulation were more pronounced in spearmint MSS-5.

Plants tolerate high levels of toxic micronutrients – Zn, by a variety of mechanism, such as reduced uptake of Fe, active efflux and intracellular on extracllular sequestration of Zn. Predominant molecules in intracellular detoxification are γ-Glu-cys peptides or the phtochelatins. These phytochelatins are activated by sublethal metal concentrations and play a crucial role in cytosolic metal detoxification (Steffens, 1990; Zenk, 1996). These intracellular metal binding ligands are being used as a specific indicators of metal tolerance (Grill *et al.*, 1988). Zinc is potentially toxic metal when transferred from plants via a food chain to human and then to agriculture systems and vice-versa. Sometimes as an antioxidant dosages, in facilitating the protein synthesis in plants and also make them the Zn tolerant spearmint crops which thus leads to the phytoremediation process for excess Zn removal from the contaminated water and soil.

Moreover, the correlation coefficient in Table 3 indicated the Zn accumulation with phytochelatins (r=0.871, $P \leq 0.01$) and Zn accumulation with lignin (r=679, $P \leq 0.05$). The above significant association showed that the phytoremediation processes remove the toxic Zn from the heavily P fertilized fields by the Fe-efficient MSS-5 genotype. The lignin association with Zn accumulation further support for increased insect resistance in spearmint genotype MSS-5.

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