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BRIEF COMMUNICATION

Zn-acquisition and its role in growth, photosynthesis, photosynthetic pigments, and biochemical changes in essential monoterpene oil(s) of *Pelargonium graveolens*

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Abstract

Culturing geranium at different doses of Zn from 0–1.0 g m^{-3} (Zn_0 to $Zn_{1.000}$) revealed that Zn is an antioxidant promoter, apart from its micronutrient essentiality. $Zn_{0.250}$ was the critical concentration for maximum content (0.21 %) of total essential monoterpene oil(s). At $Zn_{0.005}$–$Zn_{0.250}$, net photosynthetic rate, and contents of chlorophyll and essential monoterpene oil(s) were affected. The maximum peroxidase activity was obtained at $Zn_{0.250}$, with the production of biomolecule geraniol. We found an oxido-reducible reaction of Zn in the formation of monoterpene essential oil(s) and possibly major constituents of geraniol.

Additional key words: chlorophyll; Cu; dry mass; Fe; leaf area; Mn; net photosynthetic rate; plant height; saccharides; Zn.

Geranium, *Pelargonium graveolens* L. Her. *ex* Ait. (synonymum *P. roseum* Willd.) of the family Geraniaceae is the only source of one of the most important essential monoterpene oil(s) called the oil of geranium. It is commonly known as rose geranium. It is distinctly different from the horticultural geraniums, which are basically ornamental and have no commercial usage in perfumery industries (Douglas 1969). *P. graveolens* widely grown cultivars Algerian/Funition, Kelkar/Egyption, and Bourbon/ Reunion (Rajeshwar Rao and Bhatacharya 1992) are commonly cultivated in India. Steam distillation of shoot biomass of geranium yields geraniol and citronellol rich monoterpene oil(s), extensively used for perfuming soaps and cosmetics to which they impart a pronounced and lasting rose odour. It is also largely used in flavouring tobacco products, tooth pastes, ointments, and other pharmaceutical preparations.

Zn is an essential micronutrient that acts either as a metal component of various enzymes or as a functional, structural, or regulatory cofactor, and is thus associated with saccharide metabolism, photosynthesis, and protein synthesis (Marschner 1986). Zn-deficiency reduces plant growth and inhibits photosynthesis in many plants including forest trees (Dell and Wilson 1985), fiber crops (Ohki 1976), rice (Ajay and Rathore 1995), and spinach (Randall and Bouma 1973). Zn retards the activity of carbon metabolism enzymes such as carbonic anhydrase (Ohki 1976, 1978), ribulose 1,5-bisphosphate carboxylase/oxygenase and fructose-1,6-bisphosphate (Marshner 1986). Zn, Se, and Cr are antioxidants scavenging free radicals. Zn stimulates the removal of free-radicals (Chakamak and Engels 1999).

Essential oil biosynthesis in geranium is strongly influenced by Zn-acquisition and the stresses caused by Zn on nutrition and growth. Zn is involved in carbon assimilation, saccharide accumulation, free radical removal, antioxidant enzymes, carbon utilization in terpene biosynthesis, and the overall growth of the plants. The requirement of Zn for Japanese mint and its limitations imposed on photosynthetic carbon metabolism and translocation in relation to essential oil accumulation in mint were shown by Misra and Sharma (1991), whereas antioxidant enzymes for free radical quenching in geranium have not been fully documented.

In the present paper we report on the role of Zn as a stimulant of quenching of free-radicals through Zn affected antioxidant enzyme activity. Simultaneously, photosynthetic efficiency in terms of net photosynthetic rate (P_N), content of Chl, leaf fresh and dry mass, leaf area, Zn content in plant shoot biomass, and oil yield were also determined.

Plant tips (12.5–15.0 cm) with 3–4 leaves of *P. graveolens* of Bourbon genotype were obtained from the farm nursery of the CIMAP, Lucknow, India. Uniform cuttings

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were initially planted in 10 000 cm^3 earthen pots filled with purified silica sand (Agarwala and Sharma1961) for the development of roots. After 15 d, rooted cuttings were transferred to 2 500 cm^3 pots. The salts used in nutrient solution of Hoagland and Arnon (1959) were purified for Zn (Hewitt 1952). The nutrient solution was used in the experiment except Fe which was supplied as Fe-EDTA. Three pots each of Zn treatments ranging from 0.0 to 1.0 g(Zn) m^{-3} were maintained in controlled glasshouse condition at ambient temperature (30±5 °C) and irradiance (800–1 000 µmol m^{-2} s^{-1}). The nutrient solution in each treatment was added at alternate days. With onset of deficiency and toxicity (after 20 d), growth and detailed physiological and biochemical data characteristics were determined. P_N was measured using a computerized portable photosynthesis system *Li-COR 6000* (*LiCOR*, USA) (Misra and Srivastava 1991). Chl amount in 80 % acetone extracts from 3rd leaf was determined spectrophotometrically on *Pye Unicam PU8610* according to Arnon (1949). Leaf fresh and shoot dry mass and area (area meter *Li-3000*) were also recorded. For tissue element analysis 1 g dried leaf samples were digested with 1 M HCl at 60 °C for 24 h. Aliquot samples of the clear digest were diluted with water (10 cm^3) and analyzed for Zn by atomic absorption spectrophotometer (*Pye Unicam SP 2800*) (Misra and Sharma 1991). Antioxidant-reactive peroxidase enzyme activity was estimated as described in Sharon *et al*. (1960). 2 g of freshly chopped leaves at 3rd position were homogenized with 5 cm^3 of 0.1 M phosphate buffer (pH 6.8). Each treatment was replicated 3 times and assayed by SDS-PAGE electrophoresis.

Geranium oil was estimated by steam distillation of 100 g freshly plucked leaves in an apparatus of Clevenger (1928). Geraniol, citronellol, and other associated oil contents were determined by gas liquid chromatography (*Perkin-Elmer* model *3920 B*). The stainless steel column was packed with 10 % carbowax (20 mesh) on *Chromosorb WNAW*. Injector and detector temperature were maintained at 200 °C. The flow of H_2 was 0.47 cm s^{-1}; data processing for area % was done on a *Hewlett-Packard* integrator model *HP-33*.

The fresh and dry biomasses increased with increase in the supply of Zn (Table 1). Maximum fresh and dry biomass and leaf area were observed at $Zn_{0.250}$. Plant height was maximum at $Zn_{0.500}$. $Zn_{1.000}$ was toxic to all growth parameters. The Chl content increased up to $Zn_{0.250}$ and then decreased. The maximum P_N was found at $Zn_{0.250}$; at this Zn supply also the saccharide content was the highest. Zn deficiency and Zn toxicity inhibited P_N in cotton (Ohki 1976), peppermint (Srivastava *et al*. 1997), soybean (Ohki 1978), and sweet mint (Misra *et al*. 2004). A decrease in Chl content represents a decline in photochemical capacity of leaf at deficient Zn supply (Ohki 1976).

Maxima of peroxidase activity were observed at $Zn_{0.250}$. The Zn deficient and toxic cultured plants revealed lesser peroxidase activity with lesser peroxidase isoenzyme band profiles. In Japanese mint similar report was given for Mn nutrition (Misra 1996). The maximum of monoterpene oil(s) was found at $Zn_{0.250}$. However, relative contents of citronellol, geraniol, linalool, and nerol varied at different Zn treatments. As a result of different Zn supply the contents of Fe, Mn, Zn, and Cu were smaller in shoots of geranium. Their maximum contents

Table 1. Effect of Zn acquisition on parameters of geranium. Chl = chlorophyll; P_N = net photosynthetic rate; oil amounts in % of total oil. Saccharides means rate of (CH_2O) formation.

	Zn_0	$Zn_{0.005}$	$Zn_{0.050}$	$Zn_{0.100}$	$Zn_{0.250}$	$Zn_{0.500}$	$Zn_{1.000}$	LSD at 5 %	LSD at 1 %
Plant height [cm]	57.0	58.0	61.0*	62.5**	63.4**	64.1**	59.0	2.5	4.1
No. of branches	9	10*	13**	18**	10*	10*	8	1.1	3.2
Fresh mass [g plant^{-1}]	218.8	238.6*	224.8	252.1**	282.5**	215.5**	196.2	11.1	16.3
Dry mass [g plant^{-1}]	14.11	16.33*	16.81*	17.37**	19.36**	18.46**	15.85	2.10	3.30
Leaf area [cm^2]	8.2	12.1*	25.2**	39.1**	40.3**	37.2**	11.2	3.5	6.2
Chl *a* [g kg^{-1}(FM)]	0.68	0.79*	0.94**	1.35**	1.48**	1.01**	0.82*	0.11	0.15
Chl *b* [g kg^{-1}(FM)]	0.50	0.56	0.61*	0.69**	0.79**	0.40	0.29	0.08	0.12
Chl *a/b*	1.36	1.41	1.54	1.96	1.87	2.53	2.83	-	-
P_N [µg(CO_2) m^{-2} s^{-1}]	0.15	0.19*	0.75**	0.76**	0.82**	0.71**	0.42**	0.03	0.06
Saccharides [µg m^{-2} s^{-1}]	0.102	0.129	0.510	0.516	0.558	0.483	0.286	-	-
Oil %	0.15	0.16	0.17*	0.19	0.21**	0.16	0.15	0.02	0.04
Citronellol	0.21	0.27**	0.29**	0.32**	0.25**	0.18**	0.17**	0.01	0.02
Geraniol	0.09	0.09	0.10**	0.11**	0.07**	0.12**	0.10**	0.01	0.01
Linalool	8.00	10.00**	9.00**	6.00**	7.00**	8.00**	7.00**	0.04	0.07
Nerol	1.00	1.20**	1.10	1.40**	1.20**	0.90**	0.70**	0.01	0.02
Fe [mg kg^{-1}]	98	112	142**	249**	537**	419**	312**	21	42
Mn [mg kg^{-1}]	26	37**	41**	57**	98**	62**	53**	9	11
Zn [mg kg^{-1}]	12	19*	34**	45**	64**	41**	36**	7	9
Cu [mg kg^{-1}]	7	9	11**	11	12**	7	5	3	5

were observed at $Zn_{0.250}$.

Statistical analysis showed a positive significant association between Zn content in leaf and P_N ($\gamma = 0.924 \leq p$=0.5 %) and between P_N and content of saccharides ($\gamma = 0.879 \leq p$=0.05 %). However, Zn content in leaf was negatively correlated with Chl *a/b* ratio. P_N showed a positive significant association with leaf fresh mass (γ = 791 $\leq p$=0.05 %), leaf dry mass ($\gamma = 692 \leq p$=0.05 %), leaf area and total monoterpene oil(s) ($\gamma = 0.721 \leq p$=0.01). A positive significant correlation was also observed between saccharides and total oil ($\gamma = 0.695 \leq p$=0.01%). A quadratic trend was observed for all these characters which were comparable in +Zn than in plants grown at Zn deficit or much higher Zn supply.

We found that optimum supply of Zn is $Zn_{0.250}$. Utilization of metabolites from primary photosynthetic process in secondary metabolism regulates monoterpene production (Gershenzon and Croteau 1991). Thus a close relation between photosynthesis, photorespiration, and terpenoid synthesis exists in essential monoterpene oil(s) bearing plants (Maffei and Codignola 1990). Moreover, the actively growing leaves require a larger supply of an antioxidant stimulator Zn, in association with greater supply of photosynthates. Since essential oil biosynthesis occurs in these rapidly growing leaves, the initial growth period would require a still greater supply of photosynthates and energy.

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