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BRIEF COMMUNICATION

Low iron stress nutrition for evaluation of Fe-efficient genotype physiology, photosynthesis, and essential monoterpene oil(s) yield of *Ocimum sanctum*

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Abstract

Genotypic variations were observed among 6 variants and an indigenous one as a control. An efficient genotype OCD(L) of *Ocimum sanctum* had the greatest content of chlorophylls [2.15 g kg^{-1}(FM)], Chl *a/b* ratio of 1.86, net photosynthetic rate of 0.72 mg(CO_2) m^{-2} s^{-1}, and 1.56 % oil formation. Methyl chavicol formed 50.31 % of total oil in OSP-6 genotype, which had also maximum peroxidase activity [ΔOD 2.4 mg^{-1}(protein)] and maximum production of eugenol (30.44 % of total oil). We found an oxido-reducible reaction of peroxidase and high bands of peroxidase isoenzymes in this OSP-6 genotype for the formation of monoterpene essential oil(s) and possibly the major constituents of eugenol through the high production of photosynthates.

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

Basil *(Ocimum sanctum* L.) of the family Labiateae is the only source of one of the most important essential monoterpene oil(s) called the oil of French basil or sweet basil. It is cultivated in many parts of the world for its essential monoterpene oil(s) (Douglas 1969, Putievsky and Galambqsi 1997). French basil oil is used in perfumery, cosmetics, confectionaries, and in pharmaceutical industries (Erickson 1976). From the industrial side, *Ocimum* species with oil rich in camphor, citral, geraniol, linalool, linalool acetate, methyl chavicol, eugenol, thymol, *etc*. are important and the efficient genotype of French basil with high value of methyl chavicol, linalool, and eugenol will be studied through physiologically active biochemical changes with end products of photosynthates—the saccharide formation and great photosynthetic efficiency as a performance indicator. The crops with 7 accessions were recently introduced in controled condition in Lucknow, northern Indian plains. In this climatic condition the best efficient genotype of French basil has not been found yet, so we studied physiologically active growth, biochemical changes, and oil quality and quantity in the efficient genotypes.

Micronutrients, especially Fe and Zn, act either as metal components of various enzymes or as functional, structural, or regulatory cofactors, and are 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 (Marschner 1986). Essential oil biosynthesis in basil is strongly influenced by Fe and Zn and the stresses caused by extrinsic and intrinsic factors affect the overall nutrition and growth. Zn is involved in carbon assimilation, saccharide accumulation, free radical removal, antioxidant enzymes, carbon utilization for terpene biosynthesis, and the growth of plants. The requirement of micronutrients 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 effects of antioxidant enzyme peroxidase in free radical quenching in the basil have not been fully documented.

We report the role of micronutrients with extrinsic and intrinsic environmental factors and antioxidant peroxidase enzyme effect on existing genotypes, as a stimulant for quenching of free-radicals (Chakmak and Engels 1999), and active oxygen species (AOS) such as

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Table 1. Growth attributes and other physiological parameters of genotypes of cultivated *Ocimum sanctum* on low Fe (2.8 g m^{-3}). Chl = chlorophyll, P_N = net photosynthetic rate. $^{*, **}$significant at 5 and 1 % levels, respectively.

Attribute	4KR(NM)	15KR(OM)	OB	OSP-6	OCD(L)	OC-10	Sudha	LSD	
								at 5 %	at 1 %
Plant height [cm]	35.0	57.0	61.7*	68.5**	60.4**	61.1**	49.0	2.5	4.1
No. of branches	8	11*	14**	19**	16*	11*	7	1.1	3.2
Fresh mass [g per plant, shoot+root]	211.8	248.6*	204.8	257.1**	289.5**	245.5**	126.2	11.1	16.3
Dry mass [g per plant]	11.11	16.63*	17.81*	16.37**	17.36**	17.46**	17.85	2.10	3.30
Leaf area [cm^2]	6.2	10.1*	21.2**	37.1**	42.3**	27.2**	10.2	3.5	6.2
Chl *a* [g kg^{-1}(FM)]	0.68	0.89*	0.90**	1.15**	1.38**	1.31**	0.72	0.11	0.15
Chl *b* [g kg^{-1}(FM)]	0.70	0.59	0.51*	0.79**	0.77**	0.28	0.38	0.08	0.12
Chl *a/b*	1.35	1.47	1.34	1.81	1.86	2.41	2.63	–	–
P_N [$\mu g(CO_2)\ m^{-2}\ s^{-1}$]	0.16	0.21*	0.65**	0.69**	0.74**	0.72**	0.32	0.03	0.06
Saccharides [$mg(CH_2O)\ m^{-2}\ s^{-1}$]	0.104	0.139	0.470	0.526	0.578	0.478	0.386	–	–
Total oil [%]	0.9	1.16	9.17*	1.50	1.56**	1.13	1.05	0.02	0.04
Methyl chavicol [% of total oil]	30.21	32.27**	42.29**	50.31**	39.25**	40.18**	37.17	4.01	8.02
Geraniol [% of total oil]	2.09	4.09	2.10**	3.87**	1.07**	2.12**	3.10	0.03	0.05
Linalool [% of total oil]	17.00	16.00**	17.00**	19.91**	17.00**	11.00**	21.00	2.04	6.07
Eugenol [% of total oil]	21.00	11.20**	29.10	30.44**	28.20**	19.90**	17.70	9.01	11.02
Fe [mg kg^{-1}]	88	118	148**	269**	527**	469**	347	21	42
Mn [mg kg^{-1}]	22	39**	44**	47**	92**	66**	48	9	11
Zn [mg kg^{-1}]	11	21*	37**	41**	61**	44**	39	7	9
Cu [mg kg^{-1}]	9	6	18**	15	13**	7	2	3	5

hydrogen peroxide, singlet oxygen, and hydroxyl radical, and *in-situ* micronutrient contents on the physiological and biochemical changes during antioxidant activities and autooxidation of lipids (Singh 1989, Aruoma 1996). Formation of these excess ROS and free-radicals are generated by stresses, which are harmfull to the plant cells (Richards *et al.* 1998). On the other hand, the production of AOS and free-radicals in plants is a developmentally regulated process important under non-stress conditions, elemental or environmental. These free-radicals and AOS are involved in many physiological responses such as stomatal closure, programmed cell death, peroxisome biogenesis, cell wall cross-linking, lignin synthesis, and signal transduction (Dat *et al.* 2000, Neil *et al.* 2002). Hydrogen peroxide production—AOS in intracellular sources such as mitrochondria, chloroplasts and peroxisomes—affect the Calvin cycle during general metabolism in intracellular cells. Simultaneously, photosynthetic efficiency in terms of net photosynthetic rate (P_N), contents of chlorophyll (Chl) and xanthophylls, leaf fresh and dry masses, leaf area, Zn content in plant shoot biomass, and oil yield affected by the stresses were determined to evaluate the Fe efficient genotype among the existing genotypes.

Plantlets of 12.5–15.0 mm height with 3–4 leaves of *O. sanctum* genotypes 4KR(NM), 15KR(OM), OSP-6, OCD(L), OB, OC-10, and Sudha-Check were obtained from the farm nursery of the CIMAP, Lucknow, India. Uniform plantlets were initially planted in 10 000 cm^3 earthen pots filled with purified silica sand (Agrawala and Sharma 1961) 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 (1952) were purified for Zn and Cu (Hewitt 1952). The nutrient solution was used in the experiment except Fe which was supplied as Fe-EDTA. Three pots each of Fe treatments (2.8 g m^{-3}) as Fe-EDTA for each genotype were maintained in controlled glasshouse at ambient temperature (30±5 °C), irradiance (800–1 000 μmol m^{-2} s^{-1}), and 11-h photoperiod. The low Fe concentration should screen the better genotype in stress conditions. The nutrient solution in each treatment was added on alternate days. With onset of deficiency and toxicity (after 20 d), growth and physiological and biochemical 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 the 3rd leaf was determined spectrophotometrically on *Pye Unicam PU8610*, UK), according to Arnon (1949). Leaf fresh and shoot dry mass and area (area meter *Li-3000, USA*) 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 Fe, Mn, Zn, and Cu by atomic absorption spectrophotometer (*Pye Unicam SP 2800,* UK) (Misra and Sharma 1991). Antioxidant-reactive peroxidase enzyme activity was estimated as described in Sharon *et al.* (1966). 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.

French basil oil was estimated by steam distillation of 100 g freshly plucked leaves in an apparatus of Clevenger (1928). Geraniol, methyl chavicol, linalool, euginol, and other associated oil contents were determined by gas liquid chromatography (*Perkin-Elmer* model *3920 B*, UK). The stainless steel column was packed with 10 % carbowax (20 mesh) on *Chromosorb WNAW*. Injector and detector temperatures 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* (UK).

The fresh biomass was significantly greater in all tested genotypes in comparison with the checked Sudha genotype (Table 1). Maximum fresh and dry biomasses and leaf area were observed in OCD(L). Plant height was minimum in the 4KR(NM) genotype and maximum in OSP-6. Chl content was maximum in OCD(L) having the maximum total essential monterpene oil(s) production of 1.56 % (Table 1), which was due to high photosynthesis and large formation of saccharides (Table 1). This was probably connected with free radical quenching by high peroxidase activity and isozymes in antioxidation processes. The maximum P_N found in OCD(L) was due to eliciting the genes of OCD(L) genotype by high Zn content which functions as antioxidant during the general metabolism. Low Fe supply is important for screening and selection of efficient genotypes represented by maximum uptake of micro-nutrients, especially Fe and Zn. In OCD(L) the gene expression for enhanced biomass and dry matter production was triggered by high Fe and Zn contents in tissues. Great photosynthate formation and photosynthesis lead to the formation of essential monoterpene oil(s) in the efficient genotype with the minimum ½ strength Fe supply in Hoagland's solution. The antioxidants were important in scavanging the free radicals produced during glycolysis. The peroxidase and its isozymes are important in quenching free radicals. Our findings support the connection of maximum peroxidase activity and its isozymes with maximum Zn contents in OCD(L). Maximum tissue contents of Zn at low Fe supply and the high saccharide formation and photosynthesis were reported in cotton (Ohki 1976), peppermint (Srivastava *et al.* 1997), soybean (Ohki 1978), sweet mint (Misra *et al.* 2003), and geranium (Misra *et al.* 2005). 4KR(NM) and other inefficient genotypes with the checked Sudha genotype showed the decrease in contents of Chl. Thus a decrease in Chl content represents a decline in photochemical capacity of leaf at deficient Zn supply (Ohki 1976).

Maxima of peroxidase activity [ΔOD 2.4 mg^{-1} (protein)] were observed in OSP-6, but the decrease in activity of this enzyme activity and its isozymes with minimum number of isozyme bands was found in inefficient genotypes. The Zn deficiency and low tissue contents of Zn and Fe in less efficient genotypes revealed lesser peroxidase activity with lesser peroxidase isoenzyme band profiles. In Japanese mint a similar report was given for Mn nutrition (Misra 1996). The maximum content of monoterpene oil(s) was found in OCD(L). However, relative contents of methyl chavicol, geraniol, linalool, and euginol varied in different inefficient genotypes (Table 1). As a result of low Fe supply, the contents of Fe, Mn, Zn, and Cu were small in shoots of French basil plants. The maximum contents of Fe and Zn observed in OCD(L) were further due to Fe and Zn efficiency in the maximum total essential monoterpene oil(s) production (1.5 %).

Statistical analysis showed a positive significant association between Zn content in leaf and P_N ($\gamma = 0.968 \leq p = 0.05$ %) and between P_N and content of saccharides ($\gamma = 0.809 \leq p = 0.05$ %). However, Zn content in leaves was negatively correlated with Chl *a/b* ratio. P_N showed a positive significant association with leaf fresh mass ($\gamma = 0.779 \leq p = 0.05$ %), leaf dry mass ($\gamma = 0.782 \leq 0.05$ %), and leaf area and total monoterpene oil(s) ($\gamma = 0.847 \leq p = 0.01$). A positive significant correlation was also observed between saccharides and total oil ($\gamma = 0.895 \leq p = 0.01$ %). A quadratic trend was observed for all characters which were comparable at low Fe.

We found optimum tissue contents of Fe and Zn in the efficient genotype OCD(L) with higher formation of saccharides and secondary plant products. The essential monoterpene oil(s) at low Fe supply (2.8 g m^{-3}) in nutrient solution regulated the monoterpene production. Utilization of metabolites from primary photosynthetic process in secondary metabolism advocates and further regulates the monoterpene total oil 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 the antioxidant stimulator Zn (Misra *et al.* 2003), in association with greater supply of photosynthates. Since essential oil biosynthesis occurs in the rapidly growing leaves, the initial growth period would require a large supply of photosynthates and energy in the Fe efficient genotype.

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