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Analysis of growth, physiology, photosynthesis, essential monoterpene oil(s) yield and quality in *Ocimum sanctum* L genotypes

A. Misra*, S. Dwivedi, A. K. Srivastava, D. K. Tewari, A. Khan and R. Kumar

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

***Corresponding author**

Basil (*Ocimum sanctum L.)* is an important monoterpene essential oil(s) bearing crop with main essential oil constituents being eugenol and linalool. Genotypic and environmental factors plays an important role in growth and physiology of this plant. Genotypic variation have been observed among the 6 variants and indigenous one as a control. An efficient genotype OSP-6 of Ocimum sanctum found to be with higher photosynthetic pigments (1.81 mg g^{-1} f.wt.) and 6.94 mg g^{-1} (Co_2) m^{-2} $second^{-1}$ carbon assimilation rate. Maximum essential oil(s) was reported in 4KR (NM) genotype with 1.5 % oil formation where as 30.44% of total oil was found in OSP-6 genotype. The maximum peroxidase activity was obtained in OSP-6 genotype, with the maximum production of biomolecule eugenol. 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 photosynthates.

Key words: Chlorophyll, dry mass, leaf area, net photosynthetic rate, plant height, saccharides.

Basil (*Ocimum sanctlum 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) (Putievsk and Galambasi 1997). French basil oil is used in perfumery, cosmetics, confectionaries and in pharmaceutical industries. From the industrial side, Ocimum species with oil rich in camphor, citral, geraniol, linalool, linalool acetate, methylchavicol, eugenol, thymol, etc are the important one 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 saccarides formation and high value of photosynthetic efficiency as a performance indicator. The Crop with 7 successions was recently introduced in controlled condition in Lucknow, northern Indian plains. In this agro climatic condition the best efficient genotype of french basil has not been worked out, further so the detailed physiological studies has not been done so for. Therefore, conducted the studies on the physiologically active growth and its attributeson the biochemical changes and in the oil quality and quantity with the efficient genotypes of of Ocimum sanctum will be done during the present study.

Micronutrients, especially Fe and Zn are 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). Essential oil biosynthesis in basil is strongly influenced by Fe and Zn and the stresses caused by extrinsic and intrinsic factors affect the over all nutrition and growth. Zn further, involved in carbon assimilation, saccharide accumulation, free radical removal, antioxidant enzymes(Chakmak and Engels 1999), carbon utilization in terpene biosynthesis, and the overall growth of the 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 antioxidants enzymes peroxidase for free radical quenching in the basil have not been fully documented.

In the present paper we report on the role of micronutrients with extrinsic and intrinsic environmental factors and antioxidant peroxidase on and in the existing genotypes have not been fully studied as a stimulant of quenching of free-radicals through in - situ micronutrients levels in the physiology and biochemical changes. 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.

Plantlets (12.5–15.0 mm) with 3–4 leaves of seven *Ocimum sanctalum* genotypes KR (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 10000 cm^3 earthen pots filled with purified silica sand (Agarwala and Sharma 1961) for the development of roots. After 15 d, rooted cuttings were transferred to 2500 cm^3 pots. The salts used in nutrient solution of Hoagland and Arnon (1952) were purified for Zn (Hewitt 1952). The nutrient solution was used in the experiment except Fe which was supplied as Fe-EDTA. The low Fe supply was provided to screen out the better genotype in stress conditions. Three pots of each genotype were provided with Fe treatment of 2.8 ppm as Fe-EDTA and 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. 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*). 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 significantly in OSP-6 genotype compared with Sudha check (Table 1). Maximum fresh and dry biomass and leaf area were also observed in OSP-6. Plant height was minimum in 4KR (NM) and it was maximum in OSP-6. Chlorophyll formation was maximum in Sudha check. Oil production was maximum in OSP-6 (1.5%) that may be due to higher Carbon dioxide assimilation rate and saccharides formation due to Fe and Zn uptake. that may be due to the free radical quenching by higher peroxidae activity and its isozymes antioxidation processes. The maximum P_N was also found in OSP-6 that may be due to eliciting the genes of OSP-6 genotype by higher Zn concentration in this genotype which plays as antioxidant and secondly due to peroxidase isozymes during the general

Table 1. Effect of low Fe (2.8 *ppm*) culturing genotypes of *Ocimum sanctum L*. on growth attributes and other physiological parameters

	4KR(NM)	15KR(OM)	OB	OSP-6	OCD(L)	OC-10	Sudha Check	LSD at 5 %	LSD at1%
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 plant^{-1}]	211.8	248.6*	204.8	257.1**	289.5**	245.5**	126.2	11.1	16.3
Dry mass [g plant^{-1}]	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	1.86	1.81	2.41	2.63	-	-
P_N [µg(CO_2) m^{-2} s^{-1}]	0.16	0.21*	0.65**	0.69**	0.72**	0.74**	0.32	0.03	0.06
Saccharide [mg (CH_2O) m^{-2} s^{-1}]	0.104	0.139	0.470	0.526	0.578	0.478	0.386	-	-
Oil %	0.9	1.16	0.917	1.50	1.21**	1.13	1.05	0.02	0.04
Methyl chavicol	30.21	32.27**	42.29**	50.31**	39.25**	40.18**	37.17	4.01	8.02
Geraniol	2.09	4.09	2.10**	3.87**	1.07**	2.12**	3.10	0.03	0.05
Linalool	17.00	16.00**	17.00**	19.91**	17.00**	11.00**	21.00	2.04	6.07
Euginol	21.00	11.20**	29.10	30.44**	28.20**	19.90**	17.70	9.01	1.02
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

Chl = chlorophyll; P_N = net photosynthetic rate; oil amounts in % of total oil.

metabolism and extrinsic and entrinsic factors. Low Fe suply for screening and selection of efficient genotype with the maximum uptake of micronutrient especially Fe and Zn in OSP-6 variety expressed the gene expressions and further eliciting the photsynthates and photosynthesis, in the formation of essential monoterpene Oil(s) in the efficient genotype. The antioxidants play an important role in scvanging the free radicals produced due to glycolysis and lipid peroxidation .The peroxidase and its isozymes plays an important role in quenching the free radicals. Findings of present study support this concept as maximum peroxidase activity and its isozymes was observed with maximum Zn contents in OSP-6 genotype. Maximum Zn tissue concentrations with low Fe supply, High saccharides formation and photosynthesis has been reported in cotton (Ohki 1976), peppermint (Srivastava *et al*. 1997), soybean (Ohki 1978), and sweet mint (Misra *et al.* 2004). Genotype 4KR(NM) and other inefficient genotypes along with Sudha check genotype showed decrease in Chlorophyll contents and formation of photosynthesis pigments. Thus 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 in OSP-6 while a decrease in this enzyme activity and its isozymes minimum number of isozymes band n the inefficient genotypes. The Zn deficient and low tissue concentrations of Zn and Fe in the cultured plants of less efficient genotypes revealed lesser peroxidase activity with lesser peroxidase isoenzyme band profiles. In Japanese mint similar report was given for Mn nutrition (Misra 1996). The maximum monoterpene oil(s) was found in OSP-5 genotype. However, relative contents of citronellol, geraniol, linalool, and nerol varied in different other genotypes. As a result of seven treatments with different low Fe supply and culturing of plants, the contents of Fe, Mn, Zn, and Cu were smaller in shoots of French basil plants. Maximum Fe and Zn contents were observed in OSP-6 probably due to Fe and Zn efficiency resulting in maximum total essential monoterpene oil(s) production (1.5%) in this genotype under low Fe supply.

Statistical analysis showed a positive significant association between Zn content in leaf and P_N ($\gamma = 0.968 \leq p = 0.5$ %) and between P_N and content of saccharides ($\gamma = 0.809 \leq p = 0.05$ %). 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 = 779 ≤ p = 0.05 %), leaf dry mass (γ =782 ≤ 0.05%), leaf area and total monoterpene oil (s) (γ = 0.847 ≤ p=0.01). A positive significant correlation was also observed between saccharides and total oil (γ = 0.895 ≤ p=0.01%). A quadratic trend was observed for all these characters in OSP-6 then the inefficient genotypes which were comparable in low Fe than in plants having Zn tissue concentration.

We found that optimum tissue concentrations of Fe and Zn in OSP-6 along with higher saccharides formation secondary plant products and essential monoterpene oil(s) at low Fe suuply (2.28 ppm in nutrients solution) regulates the monoterpene productions. Utilization of metabolites from primary photosynthetic process in secondary metabolism advocates and further regulates the monoterpene total oil's 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 antioxidants stimulator Zn, in association with greater supply of photosynthates, which is important for screening and selection of an efficient genotype. Since essential oil biosynthesis occurs in these rapidly growing leaves, the initial growth period would require a still greater supply of photosynthates and energy in specific micronutrient i.e. Fe and Zn efficient genotype.

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