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Research article

Nitrate reductase and peroxidase activity in growth and productivity of *Santalum album* L.

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Abstract: *Santalum album* is an important oil bearing species contains 4–6% oil rich in α-santalol and β-santalol. Accessing genetic variation within the population, 30 sandal trees of different age and girth were evaluated for their leaf nitrate reductase (NR) activity and peroxidase activity. A significant variation in leaf NR activity and peroxidase activity in selected trees was observed. NR activity ranges from 0.062 to 0.219 µmol NO_2^- g.fr.wt^{-1}.h^{-1} and peroxidase activity from 15.400 to 69.480 Δ_{470} $min^{-1}mg^{-1}$protein. However, correlation of girth of the trees (GBH) with NR and peroxidase activity was not significant thus, these three parameters are independently affecting the growth and oil yielding capacity. The maximum phenotypic and genotypic coefficient of variance was recorded for peroxidase activity (PCV 58.02, GCV 29.16) followed by NR activity (PCV 30.68, GCV 28.03). More PCV for both NR and peroxidase activity indicates that the variation is influenced by the environment.

Keywords: Genotypic variation - Nitrate reductase activity - Peroxidase activity - *Santalum album*.

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INTRODUCTION

Santalum album L. (Family- Santalaceae), a small evergreen tree, commonly known as East Indian Sandalwood is a partial root parasite and is widely distributed throughout India, Australia and Pacific Islands. Species is hemiparasitic, having photosynthetic capacity but water, mineral nutrients and organic substances are acquired via the host plant (Nagaveni & Srimathi 1985, Radomiljac *et al.* 1998, Radomiljac *et al.* 1999). This means that natural regeneration or artificial establishment is dependent on the presence of suitable host plant as well as suitable environmental conditions. In addition, sandalwood is vulnerable by various abiotic and biotic factors including illicit felling, poaching, change in land use and low natural regeneration (Srinivasan *et al.* 1992).

Indian sandalwood is highly valued for its fragrant heartwood containing oil which is used for centuries for religious and customary purposes and contributing to the economy of the country by its use in cosmetics, aromatherapy, scenting of soaps, perfumery and medicines. However, trees vary in their oil content depending upon the site conditions where they grow. The heartwood formation generally begins after 8 years of plantation and the best heartwood form in more than 20 years old trees having girth of above 50 cm (Sen-Sarma 1977). Though, the heartwood formation governed by genetic factors (Srimathi & Kulkarni 1980) but the age of the tree and colour of heartwood is indicative to the quality and content of sandal oil. Generally there is a decrease of about 45% in oil content from root to tip and about 20% from core to the periphery. The root portion roughly contains 3–6 %, stem 3–5 % and branches 1–3 % of the oil. Light coloured wood generally contain a higher percentage of oil than the dark coloured ones (Shankaranarayana 1985). Identification of superior genotypes with a higher quantity of heartwood was emphasized during the second All India Sandal Seminar held in 1981. Data on heartwood yield for different girth size trees was recorded by Rai & Kulkarni (1986). The majority of the oil is composed of sesquiterpene alcohols, dominated by α and β-santalol (Verghese *et al.* 1990, Jones *et al.*

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2006). Imbalance in commercial production and harvesting resulted in a sharp decline in the natural supplies of sandal (Brennan & Merlin 1991). International prices for sandalwood have therefore been consistently rising over the past few decades (Page *et al.* 2012). Sandalwood plant material has both genuine demand in India and abundant exporting potential (Arun Kumar *et al.* 2012). Present need is to expand its distribution range by raising plantations and also protection of existing natural populations.

Nitrate reductase is a key enzyme in the assimilation of exogenous nitrate and thus, responsible for entry of nitrogen in the biological system particularly in non-nitrogen fixing plants. Its activity gives a good estimate of the nitrogen status of the plant and is very often correlated with growth and yield (Srivastava 1980). In higher plants, cytosolic NAD(P)H-nitrate reductase (NR) is rapidly modulated by environmental conditions such as light, CO_2, or oxygen availability. Photosynthesis activates 60–80 % NR activity in leaves whereas after stomatal closure, leaf NR is inactivated down to 20 or 40% of its maximum activity (Kaiser *et al.* 1999). Peroxidases (donor: H_2O_2-oxidoreductase) are a single polypeptide chain, iron containing enzymes participating in many physiological processes *viz.* lignification, suberization, cross linking of cell wall proteins, stress response, defense against pathogens, salt tolerance and senescence has often been used as a marker enzyme. Peroxidases exists in several isozyme forms and the expression pattern of these enzymes is organ-specific and developmentally regulated (Ghamsari *et al.* 2007).

Indian sandalwood exhibits very high heritable value (h^2=0.92) for heartwood production (Ansari *et al.* 2008). However, information on genotypic variation for developing conservation strategies, genetic improvement and sustainable use of the species is inadequate. Considering the economic importance of the species, NR activity and peroxidase activity based assessment of genotypic variation may be used as a marker for the conservation of superior genotypes.

MATERIAL AND METHODS

Present work was conducted on 30 trees of different age and girth after randomization of trees on the basis of a random number for experimental sampling purpose (Gomez & Gomez 1984). Leaf samples were taken from the selected trees for the assay of nitrate reductase activity and peroxidase activity. Nitrate Reductase (EC: 1.6.6.1) activity was estimated following method of Jaworski (1971). 500 mg leaf pieces were incubated for 2 hrs in dark in a reaction mixture. Absorbance recorded at 540 nm using a UV-vis spectrophotometer (GBC, USA). NRA was expressed as µmol NO_2^- $h^{-1}.g^{-1}$ fresh leaf tissue and compared with a standard curve prepared from a known concentration of KNO_3.

Peroxidase activity (EC 1.11.1.7) was measured from acetone powder formed after homogenization of 100mg leaf samples in chilled acetone (Mahadevan & Sridhar 1986). Acetone powder was suspended in 1ml 0.02 M sodium phosphate buffer (pH 6.4) in an eppendorf tube and centrifuged for 10 minutes at 10000 rpm. The supernatant was used for estimation of peroxidase activity following the method of Rama Rao *et al.* (1982). Absorbance recorded at 470 nm for 3 min with an interval of 30 sec. The soluble protein content in the enzyme extract was estimated following the method of Lowry *et al.* (1951) and the absorbance was measured at 750 nm. A standard curve from known concentrations of bovine serum protein was prepared for quantifying protein content. The peroxidase activity was computed in accordance with protein content and expressed as Δ_{470} protein $min^{-1}.mg^{-1}$.

The data recorded for five replications each of nitrate reductase and peroxidase activity was subjected to analysis of variance (ANOVA) by the standard statistical procedure (Gomez & Gomez 1984). The significance of the data was ascertained by F-test and critical difference at p=0.05 computed for comparison of various treatments (selected trees) means.

RESULTS

Data of GBH, NR activity and peroxidase activity are depicted in figure 1A, B, C. Statistical analysis (Table 1) reveals a significant genotypic variation in NR and peroxidase activity among selected trees. Variation in NR activity ranges from 0.062 (Tree 17) to 0.219 (Tree 13) µmol NO_2^- $g.fr.wt.^{-1}.h^{-1}$. Peroxidase activity varied between 15.400 (Tree 30) to 69.480 Δ_{470} $min^{-1}.mg^{-1}$ protein (Tree 6) in selected trees. The maximum phenotypic and genotypic coefficient of variance was for peroxidase activity (PCV: 58.02, GCV: 29.16) followed by NR activity (PCV: 30.68, GCV: 28.03). PCV for peroxidase activity was about of the double magnitude of GCV. Simple linear regression analysis exhibits NR and peroxidase activity as an independent parameter for growth and productivity in sandal (Fig. 2A, B).

Table 1. Analysis of variance (ANOVA) for NR Activity and Peroxidase activity.

Parameters	Source	df	Sum of squares	Mean square	F value	Standard Error	CD (P=0.05)
NR Activity	Replication	04	0.0550	0.013743	11.6479	0.021724	0.043123
	Genotype	29	0.21	0.007132	6.0445		
	Error	116	0.14	0.001179			
	Total	149	0.40				
Peroxidase Activity	Replication	04	18361.55	4590.387	6.8195	16.408763	32.571396
	Genotype	29	26119.55	900.6740	1.3380		
	Error	116	78081.78	673.1188			
	Total	149	122562.88				

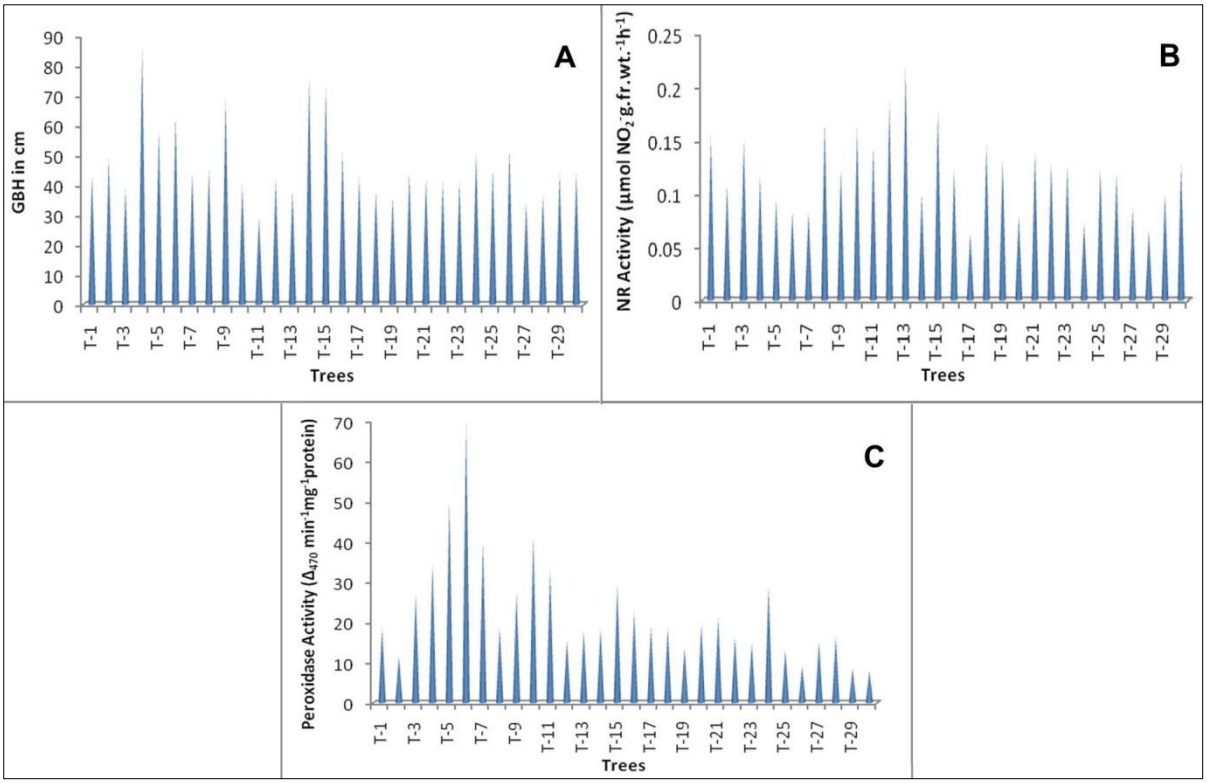

Figure 1. A, GBH; **B,** Nitrate reductase activity; **C,** Peroxidase activity of selected trees.

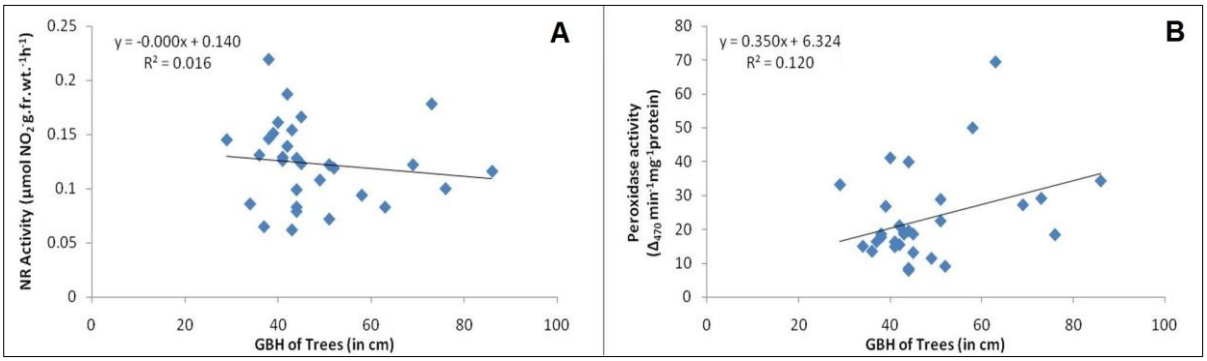

Figure 2. Linear regression between: **A,** Girth at breast height (GBH) and NR activity; **B,** GBH and Peroxidase activity.

DISCUSSION AND CONCLUSION

Roots of *Santalum album* depend upon other plants especially leguminous plants for nutrition and establishment corresponds with its slow growth and late heartwood production. A high Km value (0.255 mM KNO_3) of nitrate reductase enzyme (Tiwari 2008 unpublished) is an indication of low NR activity in *Santalum album* which is related with its hemiparasitic nature. Ananthapadmanabha *et al.* (1988) categorized host plants of sandal in three groups by different physiological activities. Significant NR activity of sandal plants growing

with different host species was observed by Nagaveni & Vijayalakshmi (2003). Poorly grown sandal plants showed low NR activity and hence directly proportional to the growth parameters of the plants. Among the various metabolic processes, nitrate reductase activity was found directly correlated with the biomass production (Pokhariyal *et al.* 1993).

The phenotypic variance was greater in magnitude compared with their corresponding genotypic variance for both the parameters *i.e.* NR activity and peroxidase activity. These two parameters show a moderate level of coefficient of variance. Further, fluctuation observed in peroxidase activity in different genotypes is much more influenced by the environment than the genotype. But in the case of nitrate reductase, the fluctuation is almost equally attributable to both environment and genotype. Parthasarthi *et al.* (1986) observed a negative correlation between the specific peroxidase isozyme activity and oil percent in the mature sandal plants. Mor *et al.* (2008) found peroxidase activity in the interaction between the host (*Arabidopsis thaliana*) and the parasite (*Orobanche aegyptica*) and proposed that peroxidases could have a role in generating extracellular reactive oxygen species (ROS) for a loosening of the cell wall of the host in order to facilitate penetration. Alternatively, the ROS could act in facilitating the root elongation of the parasite.

Correlation analysis reveals non-significant correlation of NR activity and peroxidase activity with the GBH of trees which is advocating the independent role of these three parameters in the growth and yield. It may be because of their genes are located at a distance beyond the linkage, *i.e.* > 1 c Morgan on different loci on the same chromosome or on different chromosomes. However, while working with 37 accessions of sandal, Arun Kumar *et al.* (2011) recorded a weak significant positive correlation between tree diameter and heartwood proportion. Since, the economic importance of sandalwood depends on the quantity and quality of heartwood and its oil-bearing potential, NR activity and peroxidase activity may be used as an independent biochemical marker for selection of high oil yielding trees of sandal for tree improvement. However, seasonal and diurnal changes also affecting these enzyme activities and thus growth and productivity.

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