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Nitrate reductase: A biochemical marker for screening superior strain of lemongrass [*Cymbopogon flexuosus* (Steud) Wats]

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The aim of this study was to ascertain whether nitrate reductase (NR) and alkaline phosphatase activity (ALP) can be used as biochemical marker to screen lemon grass [*Cymbopogon flexuosus* (Steud) Wats] genotypes for higher herbage yield. In the study, 11 strains of essential oil bearing cash crop lemongrass were used. The results revealed that *in vivo* NR activity has significant positive correlation with herbage yield; however, no significant correlation was found for ALP. Thus, NR activity can be used as biochemical marker for screening lemon grass genotypes for growth and herbage yield.

Keywords: Alkaline phosphatase (ALP), biochemical marker, *Cymbopogon flexuosus*, herbage yield, nitrate reductase (NR)

Essential oils distilled from *Cymbopogon* species are of immense commercial value in pharmaceutical industries for the synthesis of vitamin A, β-ionone and methyl ionone. Two major constituents of the essential oil, geraniol and citral, due to their specific rose and lemon like aromas, are widely used as flavours, fragrances and cosmetics[1]. The oil of lemon grass [*C. flexuosus* (Steud) Wats] has shown promising anticancer activity and caused loss in tumour cell viability by activating the apoptotic process as identified through electron microscopy[2].

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Unlike major grain crops, in cash crops like lemongrass, the harvest product is fresh herbage which is dependent upon growth and vigour of the plant. Therefore, for crops like lemongrass, selection criteria should be aimed at assessing growth and vigour. But growth and vigour are highly influenced by soil condition, climate, cultivation practices, etc., apart from genotype. So it is necessary to develop stable and reliable criteria to sort out superior strains for better growth and vigour followed by higher herbage yield. Nitrogen application enhances fresh herbage yield and oil quality in lemongrass (*C. flexuosus*) in the semi-arid tropical conditions of South India[3]. However, essential oil yield has shown no direct co-relation with nitrate reductase (NR) activity. NR activity directly correlates with herbage yield. From more fresh herbage yield more oil will be produced but oil yield percentage (v/w) and quality show no difference.

NR is a key enzyme at the initial step in the pathway of nitrogen assimilation in plants and, hence, it is an important factor to be assessed for growth and vigour. NR activity has been proposed as a selection criterion in breeding programmes for screening cereal germplasms for higher productivity[4]. Similarly phosphatases are key enzymes in phosphorous metabolism during early stage of growth[5] and, hence, significantly influence the growth, vigour and plants metabolisms. Phosphatases are known to occur in all higher plants as well as microorganism and considered as essential enzymes for biochemical reactions.

In general, a biomarker or biological marker is a substance used as an indicator of a biological activity/state, which can be used to distinguish the genotypes. As NR and alkaline phosphatase (ALP) are as NR and alkaline phosphates (ALP) are correlated with growth and vigour of the plant, in the present investigation, we are trying to find out whether any correlation exists with the herb yield and NR or/and ALP activity. *In vivo* NR and ALP assay are relatively simple and large number of samples can be handled within a short time and, hence, chosen for the present study.

Ten varieties of lemongrass, *viz*., RLJ-TC-1 to -10, were maintained in the experimental field of Northeast Institute of Science and Technology, Jorhat,

India and used for the present study. Another high yielding and widely cultivated strain OD-19 was also taken as standard for comparison. Except OD-19, others are indigenous and originally collected from natural habitats of different states of North-eastern India, viz., Arunachal Pradesh, Assam, Meghalaya, Nagaland, and latter evaluated as distinct strain[6]. Field experiment was conducted in a randomized block design with three replications. The plot size was 7×5 m with 1 m gap between plots. Healthy uniform size slips were planted at a spacing of 60×70 cm. Farm yard manure was added at the time of land preparation. Harvest was made at 2 month interval and the data were taken for 3 yr and pooled.

For enzyme assay leaf sample were taken from the 2nd leaf at the time of first harvest. Previous experiments[7] show that NR activities of lemongrass leaf gradually decline with the age. Hence, in the present experiment, 2nd leaf (full grown) was selected to assay the maximum activity of the enzymes. *In vivo* NR activity was estimated as per the method outlined by Lodha[8] and the product (nitrite) values were expressed as µmol g^{-1} fresh wt (fw) h^{-1}. *In vivo* ALP activity (in terms of p-nitrophenol released) was also measured from 2nd leaf at the time of harvest as per the method outlined by Sadasivam and Manikam[9]. Three replications were made for each sample. The co-efficient of correlation between herbage yield and enzyme activity were worked out as per the method outlined by Panse and Sukhatme[10].

Herbage yield exhibited wide variability as evident from the range of variation among the strains (Table 1). Highest yield of 80.81 t/ha was recorded for RLJ-TC-8, while lowest yield was only 30.44 t/ha for RLJ-TC-6. OD-19, which was used as standard, had a productivity of 56.76 t/ha. Highest NR activities (enzyme product) were 2.43 and 2.46 µmol g^{-1} fw h^{-1} for RLJ-TC-8 and RLJ-TC-3, which recorded highest and second highest herbage yield, respectively. Statistical analysis revealed a strong positive correlation between herbage yield and NR activity (r=+0.713), which was found to be significant. However, in case of ALP the observation was not similar. Although RLJ-TC-3 recorded highest ALP activity (23.52 µmol g^{-1} fw h^{-1}), RLJ-TC-8 had a much lower value of 16.80 µmol g^{-1} fw h^{-1} (Table 1). Analysis showed that there existed a positive correlation between herbage yield and ALP activity, however statistically it was not significant. A number of workers have shown inherent differences in NR activity at both inter-specific and intra-specific level. It has been reported that 15 diverse species of dicot weeds belonging to 6 families had pronounced inter-specific differences and particularly in fast growing weeds like *Euphorbia hirta* and *E. geneculata*, NR activity was highest[11]. In the same way, 19 cultivars of Jute had significant intra-specific variation and positive correlation between NR activity and yield[12]. Similar pronounced intra-specific variation was also observed in fodder legume berseem (*Trifolium alexandrinum*), particularly in induced tetraploids where herb yield was high[13].

Table 1—Nitrate reductase and alkaline phosphatase activity in 11 strains of lemongrass and correlation with herbage yield

Accessions	NR (µmol g^{-1} fw h^{-1})	Herb yield (t/ha/yr)	ALP (µmol g^{-1}fw h^{-1})
RLJ-TC-1	2.43 ± 0.08	56.48	20.44 ± 0.15
RLJ-TC-2	2.30 ± 0.10	58.75	17.64 ± 0.19
RLJ-TC-3	2.46 ± 0.07	70.56	23.52 ± 0.18
RLJ-TC-4	1.98 ± 0.06	50.79	21.56 ± 0.20
RLJ-TC-5	2.08 ± 0.04	54.91	20.44 ± 0.09
RLJ-TC-6	2.30 ± 0.03	30.44	21.28 ± 0.21
RLJ-TC-7	1.62 ± 0.01	62.31	18.20 ± 0.18
RLJ-TC-8	2.43 ± 0.11	80.81	16.80 ± 0.11
RLJ-TC-9	1.32 ± 0.03	60.88	10.64 ± 0.08
RLJ-TC-10	2.36 ± 0.10	57.33	21.00 ± 0.22
OD-19	2.13 ± 0.04	56.76	20.16 ± 0.14

For nitrate reductase (NR) activity, r = (+) 0.713 > P 0.05 = 0.602; alkaline phosphates (ALP) activity, r = (+) 0.496 < P 0.05 CD (P=0.05) for NR-0.237; for ALP-0.865; for herbage yield-0.961

In the present study, all the strains of lemongrass were grown in identical condition and, hence, observed differences in NR activities are due to genotypic differences. Based on the present observation, it is clear that, in case of crop like lemongrass, *in vivo* NR activity can be considered as biochemical marker for herbage yield and, hence, it can be used as a selection criterion for screening out the superior genotypes for herbage yield.

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