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Oxygen isotope enrichment ($\Delta^{18}O$) is a potential screening approach for higher leaf yield in tea (*Camillia sinesis*) accessions

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Natural variation in $\Delta^{18}O_{lb}$ in existing tea accessions is significantly high (18.36–25.31‰) and also showed a strong positive relationship with the harvested leaf yield ($r = 0.484$, $P < 0.05$, $n = 20$). Interestingly, the relative values of $\Delta^{18}O$ of the crosses (genetic cross) were higher than either of the individual parent. This trend was also reflected in the leaf yield. This study highlights the relevance of ^{18}O enrichment approach for screening tea accessions for both higher transpiration rate and leaf yields.

Keywords: Leaf biomass/yield, oxygen isotope enrichment, tea, transpiration rate.

AMONG several physiological traits, total transpiration (T) strongly determines the biomass production of plants[1,2].

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Total transpiration of the plant canopy is a function (f) of the total leaf area cover and transpiration rate per unit leaf area [$T = f$ (leaf area × transpiration rate)]. Transpiration rate at any given leaf area is influenced by the prevailing vapour pressure deficit (VPD) and stomatal conductance (g_s)[3]. Evolving a suitable technique for the determination of transpiration rate and/or g_s is hence important in identifying desirable genotype/s or accessions for crop improvement.

Transpiration can be measured through techniques like gas exchange, porometry and gravimetry but these methods have their own limitations. The instantaneous nature of gas exchange or porometry is tedious and destructive sampling nature of gravimetry limits the convenient use of these techniques while working with a large number of germplasm and segregating lines[4]. Hence, there is a need for a rapid and high throughput time integral surrogate of transpiration rate. One such approach is oxygen isotope enrichment. Water vapour molecules containing the lighter isotope of oxygen (^{16}O) diffuse relatively faster during evaporation compared to molecules with the heavier oxygen isotope (^{18}O)[5]. Hence, ^{18}O gets enriched in the water that is left behind. Further, transpiration being an evaporative process, several experiments have amply demonstrated strong positive relationships between ^{18}O enrichment and transpiration rate in a few crop species[4,6–10] (H. Bindhumadhava, unpublished) and in tree species like cashew[11] and coffee (P. Bhat, unpublished)[12].

In this study, the relevance of ^{18}O enrichment as a surrogate measure of transpiration rate as well as total leaf biomass (or leaf yield as the biological and economical yield is one and the same in the case of tea) of tea germplasm under natural growth conditions was well demonstrated in the tea gardens at Valparai, Tamil Nadu, India. Five-year-old bushes representing diverse tea accessions growing in natural field conditions were selected (fifty such bushes per germplasm as a replicate) and whole season leaf biomass was recorded. The tea plant density of 8000 per ha yields[12] around 3000 kg ha^{-1}. The leaf biomass was arrived in our experiment based on this calculation and presented as g/bush/season, which is an average of fifty such bushes. The third fully expanded foliage from the top of a healthy branch of fifty bushes of each accession was harvested and oven-dried at 80°C for 72 h for analysis of oxygen isotope. The relationship of leaf biomass per bush with oxygen isotope enrichment ($\Delta^{18}O$) was observed.

Prior to this study, we validated the relationship of transpiration rate with the rate of water uptake and $\Delta^{18}O$ in leaf biomass by randomly collecting leaves of local standard accession of tea maintained at different topography (slopes) starting from near the hill top to bottom flat basin.

For the determination of oxygen isotopic composition, the dried leaf powder (1.0 to 1.2 mg) was pyrolysed with glassy carbon catalyst in the complete absence of oxygen at 1400°C using a Temperature Conversion Elemental Analyzer (Thermo-Finnigan, Bremen, Germany) interfaced with IRMS. The analytical uncertainty for oxygen isotope measurement was less than 0.4‰. The ^{18}O enrichment ($\Delta^{18}O$) over the irrigation water was computed as follows. Oxygen isotope enrichment is represented in per mill (‰) notation (parts per thousand).

$$\Delta^{18}O_{bm}\ (‰) = \delta^{18}O_{bm} - \delta^{18}O_{ir},$$

where $\delta^{18}O_{bm}$ is the ^{18}O composition in relation to vSMOW (Vienna Standard Mean Ocean Water) in the biomass. $\delta^{18}O_{ir}$ of the irrigation water was determined by CO_2–H_2O equilibrating device (Gas Bench-II). Oxygen isotope measurement was made at the National Facility for Stable Isotope Studies, Department of Crop Physiology, UAS, Bangalore.

Our initial experiment with local standard tea accession maintained at different slopes (Figure 1) revealed that tea bushes grown at the flat basin showed maximum $\Delta^{18}O$ of 25.80‰ compared to those growing near the hilltop (24.77‰). $\Delta^{18}O$ of midway plants (intermediate to near hilltop and flat basin) was 25.15‰. At the lower slopes, more available water facilitates higher uptake by tea bushes. Water availability and uptake are directly related to transpiration rate[13]. Since transpiration rate is directly correlated with $\Delta^{18}O$ as evidenced earlier in a few crop species[7–10] (H. Bindhumadhava, unpublished) and in tree species like cashew[11] and coffee (P. Bhat, unpublished), the leaves of tea bushes in the region of lower slopes recorded higher $\Delta^{18}O$ compared to near the hilltop, due to more availability of water.

In tea accessions, difference in leaf biomass was prominent in the crosses, varying from 313 g/bush/season in the cross U9 × S3A1 to 189.4 in the cross CB43 × CR6017 (Table 1). Among the straight lines, U3 recorded maximum leaf biomass of 242.1 g/bush, compared to SA6, which recorded lowest yield of 69.6 g/bush (Table 1). The extent of variability of 65% in leaf yield among the crosses suggested the intrinsic genetic gain of the combination over

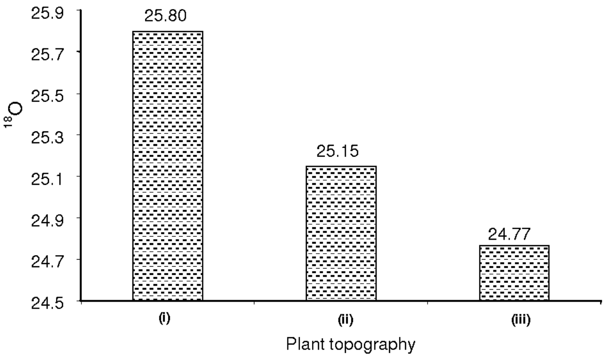

Figure 1. Variation in $\Delta^{18}O_{lb}$ of a standard tea accession maintained at different slopes. To examine the relationship of transpiration rate (natural variation because of availability of groundwater) with oxygen isotope enrichment, leaf biomass of a standard accession of tea was collected at different slopes from near the hilltop to flat basin (at three locations). (i) Flat basin; (ii) Middle slope (intermediate), and (iii) Near hill top) and analysed for ^{18}O.

individual parents. The magnitude of biomass accumulation is directly proportional to the amount of water transpired in the species wherein leaf area differences are not that large[2,4,6,13]. Hence it can be expected that ^{18}O enrichment is an indirect measure of biomass, as leaf biomass showed strong positive correlation with Δ^{18}O (Figure 2). Though Δ^{18}O reflects the variations in transpiration rate and not the total water transpired, it can still be considered for predicting the leaf yield in tea accessions and in a few other crop species[6,8–10] (H. Bindumadhava, unpublished).

Table 1. Variations in mean leaf biomass (LBM, g bush^{-1} season^{-1}) and $\Delta^{18}O_{lb}$ (per mil) in a few selected tea accessions

Cross name	LBM	Δ^{18}O
U2 × BJ2	190 ± 10.1	18.36 ± 0.09
U2 × MB380	296 ± 9.0	19.36 ± 0.1
U10 × TV20	310 ± 5.3	24.32 ± 0.06
CR6017 × SA6	264 ± 6.1	25.31 ± 0.08
U9 × S3A1	245 ± 5.4	24.33 ± 0.12
U10 × ATK	241 ± 2.9	19.46 ± 0.11
U27 × TV20	175 ± 1.3	18.16 ± 0.07
U10B × TV20	320 ± 5.6	19.51 ± 0.11
U10 × MB380	212 ± 8.9	19.09 ± 0.09
CB43 × ATK	271 ± 7.6	23.33 ± 0.08
CB43 × CR6017	189 ± 4.4	19.96 ± 0.10
TR12025 × BJ2	247 ± 6.3	19.04 ± 0.08
MB380 × SA6	253 ± 6.1	19.27 ± 0.06
U8 × RD46	242 ± 8.0	18.49 ± 0.07
U14 × TR12025	280 ± 7.0	22.84 ± 0.06
U3	242 ± 6.1	18.74 ± 0.12
U8 × RD46	276 ± 6.0	23.42 ± 0.11
U9 × S3A1	313 ± 8.1	22.25 ± 0.10
ATK	195 ± 4.3	19.11 ± 0.09
SA6-self	69 ± 6.6	18.94 ± 0.09
F-test	**	**
CD at $P = 0.05$	45.82	0.982

Values are ± standard deviation. CD represents critical difference between any two treatment means at a probability level of 0.05.

Table 2. Variation in mean leaf biomass (g bush^{-1} season^{-1}) and $\Delta^{18}O_{lb}$ (per mil) of two contrasting groups of tea accessions

Accession (cross-combination)	Mean LBM	Δ^{18}O
Group-1 (low Δ^{18}O and low LBM)		
U2 × BJ2	190.0	18.36
U27 × TV20	175.3	18.16
U10 × MB380	212.9	19.09
CB43 × CR6017	189.4	19.96
SA6-self	69.6	18.94
Mean	167.43	18.91
Group-2 (high Δ^{18}O and high LBM)		
U10 × TV20	310.3	24.32
CR6017 × SA6	264.1	25.31
CB43 × ATK	271.6	23.33
U8 × RD46	276.9	23.42
U14 × TR12025	280.0	22.83
U9 × S3A1	313.1	22.25
Mean	286.00	23.57

The accession/s capable of extracting more water from deeper soil profiles with better functional root system are expected to produce more biomass, as water is the major constraint for productivity[1]. Such accessions have an advantage as high stomatal-induced transpiration facilitates more carbon availability for photosynthesis[13,14]. An accession that shows high transpiration rate would also show high photosynthetic rate[15,16] and thus high leaf productivity. Determination of differences in Δ^{18}O among accessions at a given water availability and agronomic location, seems to reflect the variations in leaf production potential. Since, in the present study, the tea accessions used are maintained at similar topography (slope) and also had conveniently similar maintenance canopy, Δ^{18}O could still reflect variations in leaf biomass.

We also observed that the leaf biomass and $\Delta^{18}O_{bm}$ of cross-combinations were apparently high compared to individual parents (e.g. ATK or SA6 alone; Figure 3 *a* and *b*). This suggests that in cross-combination, genetic recombination might result in some physiological alterations in transpiration as well as photosynthetic efficiency-driven biomass production, which would result in higher ^{18}O enrichment. The genetic effect of crosses on possible physiological and biochemical alterations needs to be examined and experiments have been designed to confirm this aspect.

In tea improvement programmes, constant emphasis has been given to identify efficient accession from the available germplasm pool for higher leaf yields. Once such types are identified, they can be subjected to improving leaf quality parameters for further betterment. Hence in the present study, the emphasis was to identify efficient tea accession/s. The accessions were classified based on Δ^{18}O and LDM traits using standardized normal distribution (*Z*-plot). *Z*-distribution reveals two contrasting groups (Figure 4). Group-1 comprised of five accessions (having low Δ^{18}O and low LBM) and group-2 comprised of six accessions (having high Δ^{18}O coupled with high LBM). The average values of these groups for Δ^{18}O and LBM were compared. Significant variations in these two

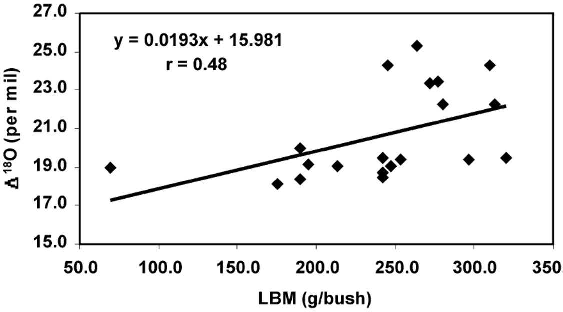

Figure 2. Relationship between leaf biomass (LBM, bush^{-1} season^{-1}) and $\Delta^{18}O_{lb}$ (‰) in 20 tea accessions ($r = 0.48$ is significant at $P < 0.05$).

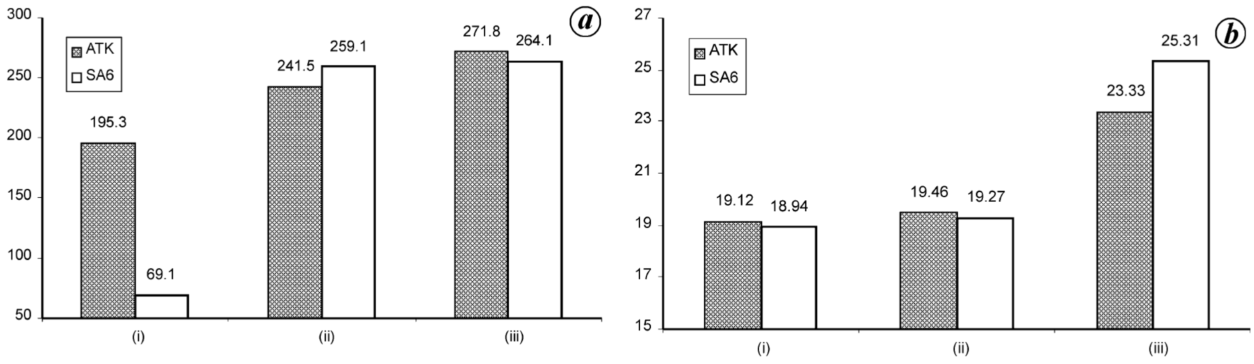

Figure 3. *a*, Leaf biomass (g bush^{-1}) of accessions ATK and SA6 alone (i), ATK cross with U10 and SA6 with MR380 (ii) and ATK with CB43 and SA6 with CR6017 (iii). ***b***, $\Delta^{18}O$ in leaf biomass (‰) of accessions ATK and SA6 alone (i), ATK cross with U10 and SA6 with MR380 (ii), and ATK with CB43 and SA6 with CR6017 (iii). Oxygen isotope composition in leaf biomass of U10, CB43, MR380 and CR6017 (as straight accession) was not analysed due to non-availability of these accessions during the experimental season.

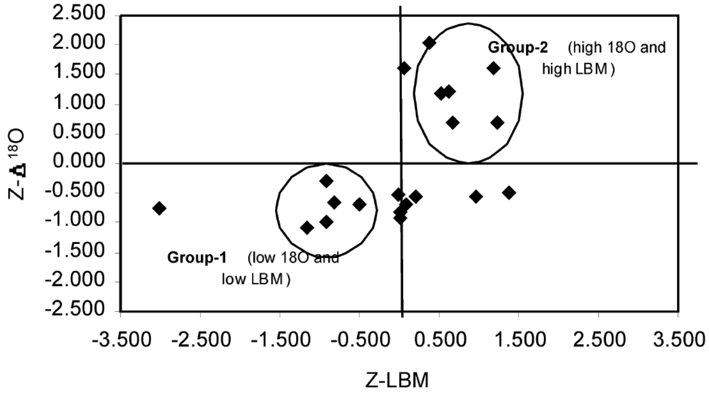

Figure 4. Grouping of tea accessions between leaf biomass and $\Delta^{18}O$ based on standardized normal distribution. Based on the distribution of points, two groups were made. Accessions belonging to group-1 (comprising of five lines having low $\Delta^{18}O$ and low LBM) and group-2 (comprising of six lines having high $\Delta^{18}O$ and high LBM) are encircled. LBM (g bush^{-1} season^{-1}) and $\Delta^{18}O$ (per mil) values of these groups are provided in Table 2.

traits were observed. The extent of variation in LBM and $\Delta^{18}O$ was 62 and 21% respectively, suggesting selection made from $\Delta^{18}O$ resulted in higher leaf yields (Table 2).

In the present investigation, we highlight the use of ^{18}O enrichment as a rapid and yet accurate surrogate for screening tea accessions for both higher transpiration rate and leaf yield. This is perhaps the first report on tea, demonstrating the possibility of using oxygen isotope enrichment for screening the variability in leaf yields among the accessions.

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Thickness estimation of Deccan Flood Basalt of the Koyna Area, Maharashtra (India) from inversion of aeromagnetic and gravity data and implications for recurring seismic activity

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Thickness estimation of volcanic suite and delineation of underlying Achaean basement topography using geophysical methods have always been a challenging-problem confronting the geoscientific community. In most cases, their estimations are unsatisfactory due to lack of quality dataset or inverse geological situation, where high susceptibility/velocity rocks at the surface are underlain by low susceptibility/velocity rocks. In order to circumvent the above situation, an inversion scheme has been attempted to model aeromagnetic and gravity datasets acquired over the seismically active Koyna region situated over the Deccan Traps of western Maharashtra. Inversion of aeromagnetic data results into a Deccan basalt thickness of about 1500 m below the Koyna region. Further, inversion of gravity data indicates that the entire column of lava below this region is made up of non-massive vesicular type of basalts having a low density of 2.58 g/cm^3 and a porosity of about 17%. Presence of vesicles, faults and fractures within the porous basaltic column appears to facilitate the diffusion of fluid in the surrounding medium and in the basement, thus causing the reactivation of faults which may be responsible for recurring seismic activity in this region.

Keywords: Aeromagnetic, gravity, inversion, induced seismicity, Koyna.

THE Koyna region of Maharashtra (Figure 1) assumed great importance globally among geoscientists after the occurrence of an earthquake with $M \sim 6.5$ on 11 December 1967. This region, considered to be a part of hitherto believed aseismic Indian peninsular shield, suddenly gained prominence after this earthquake and resulted in the accumulation of vast quantity of geophysical and geological data to (i) understand the nature and physical characteristics of the Pre-Deccan Trap topography which existed before extrusion of Deccan volcanism, and (ii) delineate the subsurface structural and tectonic configurations which may hold clues to the occurrence of the devastating earthquake. Recent analysis of geophysical datasets such as gravity, magnetic, deep electrical resistivity, magnetotellurics, seismics, etc.[1–7] has thrown significant light on the seismotectonics of Koyna Seismic Zone (KSZ). However, there does not seem to be any consensus on the cause of recurring seismic activity so far in this region.

Recently, Pandey and Chadha[8], based on pore fluid pressure study, concluded that the diffusion process within the volcanic lavas and to some extent within the basement has been quite prevalent, which facilitates reactivation of pre-existing faults causing earthquakes. A detailed magnetotelluric (MT) sounding study over this seismic zone[7] found a low apparent resistivity of 40 to 150 ohm-m, which compares with the resistivity of non-massive basalts. In contrast, the underlying basement is found to have high resistivity range of 5000–20,000 ohm-m. Basaltic thickness in this region was estimated to be 1.5 km. Thickness estimation of such rock types and delineation of basement topography from the potential field data have always been difficult due to (i) high velocity and highly randomly magnetized suite of basaltic rocks underlain by low velocity, low magnetic susceptibility granitic-gneissic

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