Ratio of stable carbon and oxygen isotope discrimination ($\Delta^{13}C/\Delta^{18}O$) reflects variability in leaf intrinsic carboxylation efficiency in plants

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In this paper we discuss a time integrated approach based on stable isotope ratios of carbon and oxygen ($\Delta^{13}C/\Delta^{18}O$) to determine the variability in mesophyll carbon assimilatory capacity of plants. Identifying crop genotypes with high mesophyll capacity for carbon assimilation has specific advantage in crop improvement, since such genotypes besides sustaining productivity under water-limited conditions can also save substantial amounts of irrigation water. We believe that this approach would provide a strong impetus to plant breeding efforts with assured success to improve productivity.

Keywords: Carboxylation efficiency, cowpea, groundnut, stable isotope ratio, water use efficiency.

SUSTAINABLE crop productivity under water-limited conditions can be achieved only through breeding for relevant drought tolerance traits[1–3]. Water use efficiency (WUE), the amount of biomass produced per unit water used, is one such potential physiological trait that can be exploited in crop improvement[3]. As a stress-adaptive strategy, plants have naturally maximized WUE through a reduction in transpiration by partial stomatal closure. Though this reduction in stomatal conductance (g_s) can help to conserve water, it inadvertently decreases CO_2 entry into the leaf for photosynthesis. Therefore, selection of high WUE normally results in reduced total biomass[4]. However, this negative trade-off between WUE and biomass would be weaker or may not exist if the intrinsic carbon assimilatory capacity determines WUE[3,5,6]. Thus, selection for high WUE from such types would not be associated with reduced biomass and hence is most desirable for crop improvement.

Unlike g_s, determination of mesophyll carbon assimilatory capacity is rather difficult and only indirect approaches are being used for assessing variability in this trait. The response of photosynthesis to increase in CO_2 concentrations (dA/dCi) is often considered as a fairly good estimate of carboxylation efficiency[7]. Alternatively, we have shown that the ratio of intercellular CO_2 concentration (Ci) to g_s is a rapid estimate of mesophyll capacity[8,9]. Though rapid, gas-exchange measurements are instantaneous and hence not reliable, especially in highly changing environments. Furthermore, assessing gas-exchange parameters in a large number of genotypes will be extremely difficult. Therefore, for exploiting this trait in breeding programmes for crop improvement, more robust and time-averaged measurement techniques need to be developed.

Plants discriminate against heavy isotope of carbon (^{13}C) during photosynthesis caused primarily by stomatal diffusivity and carboxylation by RuBisCO[10–13]. As the same factors regulate carbon isotope discrimination ($\Delta^{13}C$) as well as Ci, a strong association between these parameters is normally noticed[14]. The incorporation of isotopic signatures is a continuous phenomenon that accurately integrates the diurnal as well as day-to-day variations of the growing conditions. Thus, $\Delta^{13}C$ is a good time averaged surrogate for Ci.

Similarly, oxygen isotope fractionation ($\delta^{18}O$) has been shown to occur during evaporation[15] as well as during transpiration[16–19]. We have provided experimental evidences indicating that $\delta^{18}O$ is a potential surrogate for stomatal conductance as well (H. Bindumadhava, Ph D thesis, unpublished)[3,4,20,21].

Here we demonstrate that the stable isotope ratios substituting for Ci and g_s can accurately reflect the mesophyll capacity on a time-integrated scale. This hypothesis was tested in separate experiments using selected contrasting genotypes of cowpea and groundnut. Five genotypes of cowpea (APC-123-V683, APC 4125, V585, APC-121-P132 and APC 40-GC 20) and four genotypes of groundnut (NCAC-17090, VRI-4, ICGS-11 and Sen Nghean) were selected based on differences in growth rates as well as WUE. Seed materials of cowpea genotypes were procured from the AICRP on Pulses, UAS, Bangalore and groundnut genotypes from ICRISAT Asia Centre, Hyderabad.

Seedlings of cowpea and groundnut were raised in carbonized rubber containers measuring 45 × 15 × 20 cm, filled with 20 kg rooting mixture (red sandy loam soil and farmyard manure in a ratio of 3 : 1 v/v). Two healthy plants were maintained in each container and were provided with optimum nutrient and water requirement.

On the 35th day after sowing, gas-exchange parameters were recorded and CO_2 response curves were developed on the third fully expanded leaf from the apex. A portable photosynthesis system (CIRAS-1, PP Systems, UK) was used to measure gas-exchange parameters. The built-in CO_2 dosing system of CIRAS-1 was employed to generate various CO_2 concentrations ranging from 50 to 1000 ppm. Carbon assimilation rates recorded at each CO_2 concentration were plotted against their corresponding Ci and a second degree polynomial function was fitted. The slope of the initial linear region of the CO_2 response curves (dA/dCi) is often considered as a reflection of the potential carboxylation efficiency. The initial slope of the A–Ci curves was computed by differentiating the polynomial equation with respect to Ci (at CO_2 compensation point).

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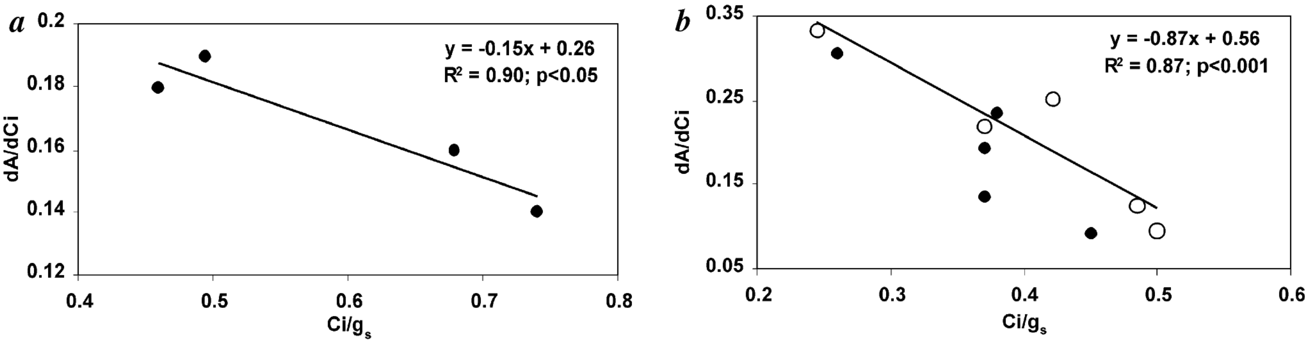

Figure 1. Relationship between Ci/g_s and dA/dCi in genotypes of (*a*) groundnut and (*b*) cowpea. Gas-exchange parameters were measured using a portable photosynthesis system. A polynomial function for CO_2 response curve was differentiated w.r.t. Ci to compute dA/dCi. All gas-exchange parameters were measured on the third fully expanded leaf from the apex. Gas-exchange parameters were recorded in two separate experiments for cowpea genotypes (open and closed symbols).

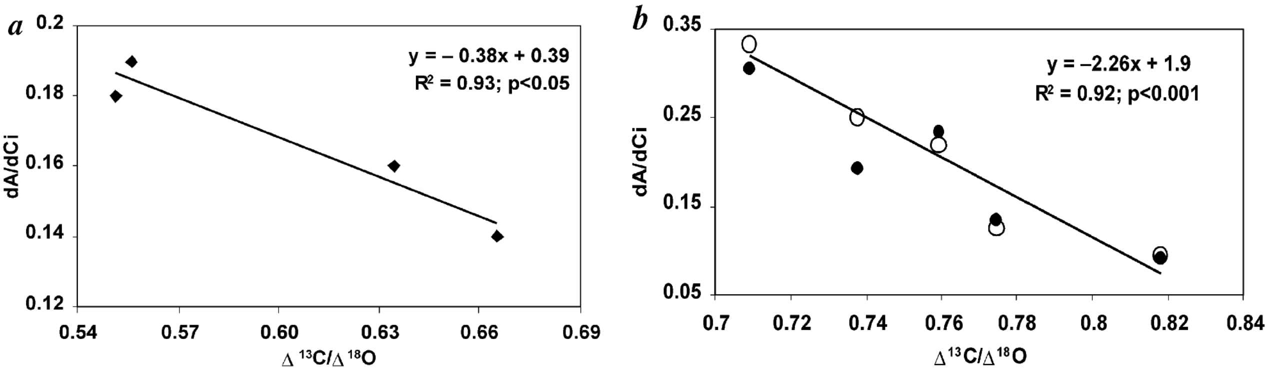

Figure 2. Relationship between dA/dCi and with $\Delta^{13}C/\Delta^{18}O$ in genotypes of (*a*) groundnut and (*b*) cowpea. Carbon and oxygen isotope ratios were determined using a Delta-plus Isotope Ratio Mass Spectrometer interfaced with an elemental analyzer via a continuous flow device. Gas-exchange parameters and stable isotope discrimination were recorded in two separate experiments for cowpea genotypes (open and closed symbols).

After recording the gas-exchange parameters, the leaf was harvested and dried in a hot-air oven at 80°C for 48–72 h. The dried leaves were ground to a fine powder using a ball mill. Around 1 mg of dried leaf samples was combusted in the Flash Elemental Analyzer (NA 1112, Carlo Erba, Italy) interfaced to an Isotope Ratio Mass Spectrometer (IRMS; Delta-Plus, Thermo-Finnigan, Bremen, Germany) via a continuous flow device (Conflo-III). The carbon isotopic composition of plant samples ($\delta^{13}C_p$) was determined with an analytical precision of less than 0.1‰. Carbon isotope discrimination (Ä^{13}C) was computed as follows[12], assuming the isotopic composition of atmospheric air ($\delta^{13}C_a$) to be –8‰.

$$\Delta^{13}C\ (‰) = [\delta^{13}C_a - \delta^{13}C_p]/[1 + \delta^{13}C_p/1000].$$

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) 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;

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

where $\delta^{18}O_{bm}$ is the ^{18}O composition in relation to V_{SMOW} in the biomass. $\delta^{18}O_{ir}$ of the irrigation water was determined by CO_2–H_2O equilibrating device (Gas Bench-III). All stable isotope measurements were made at the National Facility for Stable Isotope Studies, Department of Crop Physiology, UAS, Bangalore.

Differences in mesophyll capacity were examined in the selected contrasting genotypes of cowpea and groundnut in two separate experiments. Intrinsic carboxylation efficiency (dA/dCi) varied between 0.095 and 0.332 μmol $CO_2\ m^{-2}\ s^{-1}\ ppm^{-1}$ (with standard errors less than 0.04) among cowpea genotypes. The dA/dCi values were determined again in a separate experiment and were found to closely correlate with those of the first experiment (data not shown). For groundnut, the dA/dCi ranged between 0.142 and 0.188 μmol $CO_2.m^{-2}\ s^{-1}\ ppm^{-1}$ (standard errors were less than 0.01), representing a significant variation. The Ci/g_s ratio, another estimate of photosynthetic capacity, also varied significantly among both cowpea and groundnut genotypes. The ratio of Ci/g_s was demonstrated as a rapid estimate of carbon assimilatory capacity[8]. A significant inverse relationship between dA/dCi and Ci/g_s observed both in cowpea and groundnut (Figure 1), is in confirmation with our earlier findings (H. Bindumadhava, Ph D thesis, unpublished)[8,9]. A reduction in Ci levels can be expected either when g_s is low or when efficiency of carbon assimilation is high. At a given g_s therefore, variation in *Ci* is mainly a function of carbon assimilatory capacity. The most prominent factor that determines the differences in carbon assimilatory capacity is the primary carboxylation enzyme, RuBisCO[9,22–24].

Carbon and oxygen isotope signatures of plant organic matter integrate the diurnal as well as seasonal variations in *Ci* and g_s respectively (H. Bindumadhava, Ph D thesis, unpublished)[3,12,20,25]. Therefore, the ratio of $\Delta^{13}C$ to $\Delta^{18}O$ would represent a time-averaged estimate of Ci/g_s and hence photosynthetic capacity. Accordingly, the ratio of two stable isotope discriminations ($\Delta^{13}C/\Delta^{18}O$) showed significant correlation with d*A*/d*Ci* in both cowpea and groundnut (Figure 2).

From the agricultural point of view, it is important to identify crop genotypes where WUE is predominantly regulated by intrinsic photosynthetic capacity. Such genotypes, besides sustaining productivity under water limited conditions can also save substantial amounts of irrigation water. Being rapid and accurate, stable isotope ratios ($\Delta^{13}C/\Delta^{18}O$) provide a powerful option in identifying the desirable genotypes with superior photosynthetic capacity. Such genotypes can be used in crop improvement programmes.

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