

Effect of water stress on carbon isotope discrimination and its relationship with transpiration efficiency and specific leaf area in *Cenchrus* species

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Abstract: Carbon isotope discrimination (CID) has been proposed in estimating transpiration efficiency (TE) in plants indirectly. To identify variations for TE and specific leaf area (SLA) and their association with CID, a glasshouse experiment was conducted using six prominent species of *Cenchrus*. A significant increase in TE (3.50 to 3.87 g kg⁻¹) and decrease in SLA (219.50 to 207.99 cm² g⁻¹) and CID (13.72 to 13.23‰) was observed from well watered to stress condition. Results indicated a direct relationship of SLA with CID ($r = 0.511^*$ and 0.544^*) and inverse relationship between TE and CID ($r = -0.229$ and -0.270). However, the relationship of TE with CID was insignificant. A positive and significant relationship was visualized between TE and dry matter production in both control ($r = 0.917^{**}$) and stress (0.718^{**}) treatments. Relationships of total dry matter with SLA and CID were monitored insignificant and negative in control and positive in stress treatment indicated difference in dry matter production under two treatments. It seems that, in *Cenchrus* species, CID was influenced more by the photosynthetic capacity than by stomatal conductance, as indicated by its positive relationship with SLA in both control ($r = 0.511^*$) and stress ($r = 0.544^*$) conditions and negative relationship with root dry matter production under control ($r = -0.921^{***}$) and stress ($r = -0.919^{***}$) condition. Results showed good correspondence between CID and SLA, indicating that lines having high TE and biomass production can be exploited for their genetic improvement for drought.

Key words: Carbon isotope discrimination, *Cenchrus* species, Specific leaf area, Transpiration efficiency
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Introduction

Among many abiotic factors, drought is the principal stress leading to significant crop loss in arid and semi-arid environments (Bray, 1997). Identification of the traits conferring drought tolerance and incorporating them into plant improvement programmes is a challenging task. Transpiration efficiency (TE) or water use efficiency (WUE) is one such trait, which is known to contribute to drought tolerance of plants (Ehleringer, 1993; Tilki and Dirik, 2007). The C₄ tropical grasses that are adapted to arid conditions have evolved an efficient water conservation mechanism associated with reduced stomatal conductance. A well developed CO₂ concentrating mechanism in the C₄ plants renders them to have significantly higher water use efficiency. Because of this important trait, the C₄ species like sugarcane, maize and many tropical grasses have significantly higher productivity compared to the C₃ species like *Stylosanthes* under arid and semi-arid tropical environments where water availability is the major constraints (Sheshshayee *et al.*, 2003).

Functional and comparative studies of plants in arid zones shows that water use efficiency is an adaptive trait in drier environments (Ehleringer and Cooper, 1988; Dudley, 1996; Thumma *et al.*, 2001) and increased TE is useful for plant survival in water limited environments (Ehleringer, 1993; Thumma *et al.*, 1998; Impa *et al.*, 2005). However, measuring TE at field level is difficult and measurements of instantaneous TE *i.e.*, CO₂ assimilation per unit of water transpiration (A/E) may not accurately represent water use efficiency over the whole season. Additionally, SLA have been shown to be negatively related to transpiration efficiency in

many plants including sunflower and *Stylosanthes* (Virgona *et al.*, 1990; Brown and Byrd, 1997; Thumma *et al.*, 1998). However, the underlying cause for this is not understood well. One of the reasons for this could be that plants with low SLA (*i.e.* thicker leaves) may have more mesophyll cells which might result in higher photosynthetic rate (Thumma *et al.*, 2001; Aniya and Herzog, 2004).

Stable carbon isotopes have been extensively applied to understand carbon and water relations in plants (Farquhar and Cernusak, 2005). Water use efficiency defined as the ratio of dry matter produced to total transpiration during specific growth period is one such trait extensively studied using stable isotopes. Plants discriminate against ¹³C(Δ¹³C) (carbon isotope discrimination, CID), during photosynthesis and the extent of this discrimination shows a strong inverse relationship with WUE or TE. Farquhar and Richards (1984) proposed that CID may be used to estimate TE indirectly as CID is a measure of stomatal conductance and assimilation rate integrated over time. CID is positively related to the ratio of intercellular CO₂ concentration to ambient CO₂ concentration (C_i/C_a), which is a balance between stomatal conductance and photosynthetic capacity (Farquhar *et al.*, 1989). Low C_i/C_a and hence low CID can result from either low stomatal conductance or high photosynthetic capacity. Consequently low C_i/C_a and low CID will result in higher TE. Shaheen and Hood-Nowotny (2005), reported that measurement of CID may prove to be a useful trait for selecting wheat cultivars with improved harvest index in salt affected areas.

Though carbon isotope discrimination has been proposed as a selection criterion for improved transpiration efficiency in many



C_3 crops (Farquhar and Richards, 1984), such information is limited in C_4 tropical grasses. In semi-arid tropics, where different species of *Cenchrus* have their important role as pasture plants (Dabadghao and Shankamarayan, 1973), water is the paramount environmental constrains for their growth and persistence. The aim of the present study is to investigate the relationship between TE, dry matter production, CID and specific leaf area under control and stress conditions in *Cenchrus* species as well as to evaluate the suitability of specific leaf area (SLA) as a surrogate for CID and TE. Being apomictic in nature the selection of species/ lines of *Cenchrus* on the basis of resultant relationship will lead to screen lines having better drought tolerance behavior. The study will also lead to establish the nature of relationships among TE, SLA and CID first time in a C_4 tropical grass.

Materials and Methods

Seeds of six species of *Cenchrus* viz., *C. ciliaris* L. IG-69-3108 (India) *C. setigerus* Vahl. EC-397331 (Uganda), *C. pennisetiformis* Hochst and Steud. ex Steud. EC-397528 (Kenya), *C. echinatus* L. EC-397342 (Australia), *C. myosuroides* Kunth EC-397345 (Paraguay) and *C. glaucus* C. R. Mudaliar and Sundararaj EC-397614 (USA) were germinated and a nursery bed was prepared in the month of June at Indian Grassland and Fodder Research Institute, Jhansi. After 15 days of germination, three seedlings of each species were transferred to pots (20 cm x 15 cm) containing 2 kg locally available red laterite soil having 11-15% water holding capacity with farm yard manure (FYM) (3:1) in each pots. After two weeks of establishment each pot was thinned to one plant. Each species had three replications arranged in three randomized blocks. In total each species was represented nine times.

The relationship between transpiration efficiency and isotope discrimination was established by following the experiment of Virgona *et al.* (1990) for sunflower, Johnson and Tiezen (1994) for alfalfa and Thumma *et al.* (1998) for *Stylosanthes scabra*. After growing the plants for 30 days, two treatments were imposed. In controlled pots, water was given at field capacity (300 ml) and in stress treatment pots water was given in half of the field capacity. Both controlled and stressed plant were watered every 2nd and 3rd day after starting the experiment and on each watering day pots were weighed manually using electronic balance and the amount of water loss were calculated. After 60 days of water treatment, plants were harvested (3 pots in each treatment for each species) by carefully removing plants from the pots and separated them into root and shoots. Both shoots and roots were oven dried at 80°C for 48 hr. Transpiration was calculated by deducting evaporation (estimated from the unplanted pots) from the total water used by the plants of each pot. Transpiration efficiency (TE) was calculated as total biomass produced per unit of water transpired (Thumma *et al.*, 1998). At the time of final harvest (60 days of stress treatment) leaf area of six youngest fully trifoliate leaves was measured using leaf area meter. They were oven-dried at 80°C for 48 hr to calculate the SLA (leaf area divided by weight of the leaves).

The stable carbon isotope composition ($\delta^{13}C$) of dry leaf samples was determined by ratio mass spectrometry following Condon *et al.* (1987). Carbon isotope composition was measured at National Facility for Stable Isotope Studies in Biological Sciences, Department of Crop Physiology, University of Agriculture Science (UAS), GKVK campus Bangalore, 560065, India.

Osmolality in leaves in both control and stressed plants at the time of final harvest was measured using Vapor Pressure Osmometer model 5500 (Wescor, USA). Water potential (ψ) was measured in the same leaf using HR 33T Due Point Hygrometer employing C-52 sample chamber (Wescor, USA). The 0.1 M phosphate buffer (pH 7.0) was used to ground the collected leaves at the time of final harvest and supernatant was used to measure the soluble protein using Lowry's method (Lowry *et al.*, 1951). Statistical analyses like level of significance and correlation was performed by following the standard statistical methods (Gomez and Gomez, 1984). Significance was calculated on the basis of LSD as mentioned in the Table. Standard student 't' test was also carried out to know the significant difference between stress and control values.

Results and Discussion

Significant decrease in water potential (-1.45 to -1.83 MPa) and increase in osmolality (695.78 to 835.99 mmol kg⁻¹) from control to stress treatment indicated that plant experienced a reasonable level of water deficit (Chandra *et al.*, 2004). Results also showed significant differences between the two watering treatments for the amount of water transpired (T), shoot dry matter (SDM), root dry matter (RDM), total dry matter (TDM) and shoot transpiration efficiency (shoot dry matter produced per unit of water transpired; STE) (Table 1). Mean SLA in stressed plants was significantly lower (6.7%) than those of control. This could be due to the reduction in leaf area expansion during stress treatment. The carbon isotope discrimination decreased from mean value of 13.72 in control to 13.23 in stress treatment. Farquhar and Richards (1984) and Shaheen and Hood-Nowotny (2005), have reported decrease in CID in response to water deficit and increased salinity. Root to shoot ratios increased under stress treatment but level of total soluble protein contents did not change, however, at species level a significant increase in protein content was observed in *C. pennisetiformis* in stress over control.

An insignificant negative relationship was observed between TE and CID in control ($r = -0.229$) and stress ($r = -0.270$) treatments (Fig. 1a,b). However, TE was positively related to RDM, SDM and TDM in both control and stress treatments and these relationships were significant to highly significant (Table 2). Carbon isotope discrimination (CID) was negatively related to RDM and TDM in control treatment whereas in stress treatment only RDM was negatively related with CID (Table 2). Though in stress condition the relation was positive between CID, SDM and TDM the correlation was insignificant. In both control and stress conditions root dry matter was significantly associated with CID (Table 2). Carbon isotope discrimination has been shown to be negatively related to TE in

Table - 1: Mean transpiration [T, kg], shoot dry matter [SDM, g], root dry matter [RDM, g], total dry matter [TDM, g], root to shoot ratio [R/S, g g⁻¹], shoot transpiration efficiency [STE, g kg⁻¹], total [root+shoot] transpiration efficiency [TE, g kg⁻¹], carbon isotope discrimination [CID, ‰], specific leaf area [SLA, cm² g⁻¹], plant height [cm], water potential [ψ , MPa], total soluble protein [mg g⁻¹ (f.m.)] and osmolality [mmol kg⁻¹] of six *Cenchrus* species grown in glass house

| Species | T | SDM | RDM | TDM | R/S | STE | TE | CID | SLA | Plant height | ψ | Protein content | Osmolality |
|---------------------------|--------|--------|---------|----------|------|---------|---------|-------|-----------|--------------|---------|-----------------|------------|
| Control treatment | | | | | | | | | | | | | |
| <i>C. ciliaris</i> | 6.67 | 13.94 | 9.66 | 23.60 | 0.69 | 2.09 | 3.54 | 13.49 | 210.34 | 90.66 | -1.26 | 46.83 | 672.66 |
| <i>C. setigerus</i> | 5.66 | 17.69 | 2.29 | 19.98 | 0.13 | 3.12 | 3.53 | 14.13 | 225.51 | 88.33 | -1.57 | 24.99 | 795.00 |
| <i>C. pennisetiformis</i> | 6.93 | 14.25 | 6.45 | 20.70 | 0.45 | 2.05 | 2.99 | 13.72 | 244.13 | 67.33 | -1.73 | 28.63 | 811.00 |
| <i>C. echinatus</i> | 5.94 | 14.74 | 0.84 | 15.58 | 0.06 | 2.48 | 2.62 | 14.50 | 246.42 | 56.00 | -1.09 | 41.86 | 506.00 |
| <i>C. myosuroides</i> | 6.09 | 17.34 | 9.74 | 27.08 | 0.56 | 1.59 | 4.44 | 12.97 | 162.50 | 90.00 | -1.36 | 42.94 | 704.00 |
| <i>C. glaucus</i> | 5.89 | 13.73 | 9.39 | 23.12 | 0.68 | 2.33 | 3.93 | 13.56 | 228.14 | 74.00 | -1.68 | 36.75 | 686.00 |
| L.S.D. (p<0.05) | 0.99 | 1.57 | 4.88 | 5.12 | 0.41 | 0.68 | 0.84 | 0.79 | 37.49 | 18.37 | -0.27 | 10.28 | 132.80 |
| Mean | 6.19 | 15.28 | 6.39 | 21.67 | 0.43 | 2.27 | 3.50 | 13.72 | 219.50 | 77.72 | -1.45 | 37.0 | 695.77 |
| Stress treatment | | | | | | | | | | | | | |
| <i>C. ciliaris</i> | 2.77* | 6.12* | 3.8* | 9.92** | 0.62 | 2.21 | 3.58 | 12.67 | 209.34 | 78.33 | -1.90** | 42.07* | 680.66 |
| <i>C. setigerus</i> | 2.62* | 8.30** | 1.71* | 10.02*** | 0.21 | 3.16 | 3.82*** | 13.90 | 225.01 | 70.0* | -1.69 | 28.0* | 820.33 |
| <i>C. pennisetiformis</i> | 3.00** | 10.07* | 2.25* | 12.32*** | 0.27 | 3.35*** | 4.10*** | 13.52 | 229.14* | 59.33 | -1.91* | 32.83** | 1010.0* |
| <i>C. echinatus</i> | 2.87* | 7.11** | 0.40** | 7.47*** | 0.06 | 2.47 | 3.82** | 13.91 | 216.65* | 52.0 | -2.00* | 41.23 | 734.0*** |
| <i>C. myosuroides</i> | 3.09* | 10.37* | 3.9*** | 14.27** | 0.38 | 3.35** | 4.62 | 12.27 | 147.83 | 83.33* | -1.62 | 42.21 | 953.00** |
| <i>C. glaucus</i> | 2.22* | 7.02** | 2.46*** | 9.49** | 0.35 | 2.60 | 3.28* | 13.16 | 220.00*** | 58.0* | -1.84* | 36.33 | 818.00** |
| L.S.D. (p<0.05) | 0.52 | 2.20 | 1.66 | 2.89 | 0.28 | 0.53 | 0.61 | 0.99 | 36.70 | 15.04 | -0.17 | 7.06 | 152.87 |
| Mean | 2.75 | 6.99 | 2.40 | 10.58 | 0.31 | 2.85 | 3.87 | 13.23 | 207.99 | 66.83 | -1.83 | 37.11 | 835.99 |

* = The difference is statistically significant at p < 0.05, ** = p < 0.01 and *** = p < 0.001



Table - 2: Linear correlation among total transpiration efficiency [TE], carbon isotope discrimination [CID], root dry matter [RDM], shoot dry matter [SDM] and total dry matter [TDM] for six species of *Cenchrus* grown in a glass house

| Parameters | RDM | | SDM | | TDM | |
|------------|-----------|-----------|---------|--------|----------|----------|
| | Control | Stress | Control | Stress | Control | Stress |
| TE | 0.188 | 0.349 | 0.459* | 0.139 | 0.917*** | 0.718*** |
| CID | -0.921*** | -0.919*** | 0.286 | 0.126 | -0.368 | 0.237 |

* = the difference is statistically significant at $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$

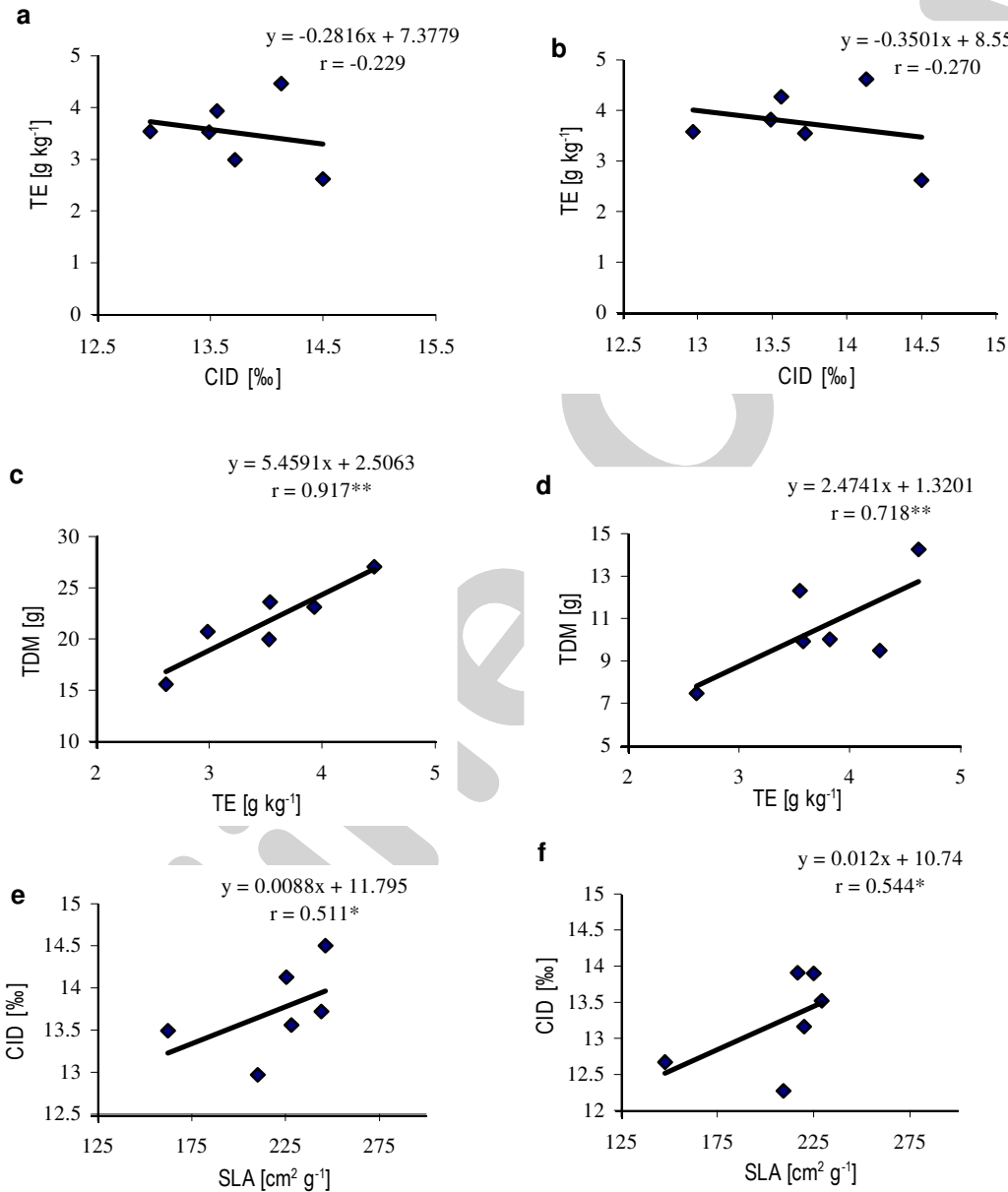


Fig. 1: Relationships of transpiration efficiency with carbon isotope discrimination (a,b) and total dry matter production (c,d), and of specific leaf area with carbon isotope discrimination (e,f) in control and stress conditions in six species of *Cenchrus* grown in glass house. The correlation coefficients in figures a and b was not significant whereas for c, d and e, f was significant at $p < 0.05^*$ and $p < 0.001^{**}$ respectively. Control = a, c and e; stress = b, d and f



many crops species like wheat (Farquhar and Richards, 1984), peanut (Hubick *et al.*, 1986; Wright *et al.*, 1988), barley (Hubick and Farquhar, 1989), *Stylosanthes* (Thumma *et al.*, 1998, 2001) and alfalfa (Johnson and Tieszen, 1994). Though present work indicated such relationship in *Cenchrus* species, the decrease in CID from control to stress was not significant as reported in other crops species (Thumma *et al.*, 1998; Condon *et al.*, 1990; Virgona *et al.*, 1990; Farquhar and Cernusak, 2005) indicating slow response of water stress on tropical grasses like *Cenchrus* which grow under extreme environmental conditions and thus they are highly adapted to such conditions.

Cenchrus myosuroides maintained highest TE (lowest CID) under both well-watered and water-stressed treatments but the difference in two treatments was insignificant indicating limitation due to threshold values as in most of the C_4 species TE ranges from 4 to 6 g kg⁻¹. However, this emphasizes that selection of prominent species of *Cenchrus* based on CID can be done under well watered conditions to avoid confusion with the effects of water stress as suggested by Condon and Richards (1992). Apart from *C. myosuroides*, *C. glaucus*, *C. setigerus* and *C. echinatus* maintained low CID. Except *C. glaucus*, rest of five species showed increase in TE from control to stress. The highest TE and lowest CID in *C. myosuroides* as well as lesser values of CID in *C. glaucus* and *C. setigerus* along with higher TE suggested that TE could be one of the significant factors contributing to drought resistance in *Cenchrus*. High TE should help the plants to persist longer under competitive and water limited conditions (Ehleringer, 1993; Sheshshayee *et al.*, 2005). In major C_3 crops species like wheat, tomato, peanut, cotton, barley, sunflower and stylo genetic variation in TE have been negatively correlated with CID (Farquhar *et al.*, 1982, 1988; Thumma *et al.*, 2001). The relationship as observed in case of C_4 tropical grass indicated similar change in CID but at the cost of increase in TE which may be due to the increase in the proportion of carbon gained in form of total dry matter which is subsequently not lost in respiration under stress treatment. Contrary to this, in C_3 crops TE was relatively unaffected by the stress treatment, although CID values decreased under the stress treatment.

Among six species, *C. myosuroides* showed maximum biomass accumulation expressed as total dry matter (TDM) production also indicated highest TE in both treatments (Table 1) and correlation was positive and highly significant (Fig. 1c, d). Even when TDM was subdivided into root and shoot, both of these components showed positive relationships with TE in control and stress conditions. In the present study, CID was negatively related to root biomass ($r = -0.921$ and -0.919 , Table 2) under both control and stress treatments. Similar results have been reported in peanut (Wright *et al.*, 1988) and *Stylosanthes* (Thumma *et al.*, 1998, 2001) in semi arid tropics. This implies that in *Cenchrus* selection based on CID will improve TE along with root biomass, which is very crucial for the survival of the plants in semi arid tropics in which *Cenchrus* grow.

Specific leaf area decreased significantly from control to stress in *C. pennisetiformis*, *C. echinatus* and *C. glaucus*. Lowest specific leaf area was observed in *C. myosuroides* (Table 1). A positive and significant relationship was found between specific leaf area and CID in both control ($r = 0.511$; Fig. 1e) and stress conditions ($r = 0.544$; Fig. 1f). This shows that SLA can be used as a surrogate for CID as measurement of SLA is relatively easier, faster and cheaper than measuring CID (Rao and Wright, 1994). However, SLA as well as relative humidity, leaf temperature and diffusion resistance can be affected by many environmental factors such as irradiance and temperature (Brown and Byrd, 1997). Therefore, SLA could perhaps be used as a surrogate of CID in screening large numbers of accessions/ segregating lines in the early phase of breeding programmes, whereas CID may be more suitable for precise analysis of the advanced lines (Richards, 1996; Thumma *et al.*, 2001).

In several C_3 plant species like alfalfa SLA was negatively correlated with biomass production (Nelson, 1988; Wright *et al.*, 1994). Although, the reason for this is not established, the negative relationship may be due to the fact that plants with low SLA (thicker leaves) will have more mesophyll cells per unit area or larger mesophyll cells leading to higher rates of CO₂ assimilation (Thumma *et al.*, 1998). Indeed, a negative relationship has been observed between SLA and leaf photosynthesis per unit area in many plant species (Pearce *et al.*, 1969; Nelson, 1988; Sheshshayee *et al.*, 2003; Aniya and Herzog, 2004). Being C_4 crop, *Cenchrus* species used in the present study did not show strong negative relationship between SLA and biomass production because C_4 crop as such possessed high mesophyll cells per unit area in comparison to C_3 crops (Sheshshayee *et al.*, 2003). The direct and strong relationship between TE and total dry matter (Table 2) as monitored in the present study indicated better photosynthetic capacity of the crop especially when SLA was not visualized significantly correlated with biomass production. Similar to other C_3 crop species transpiration has been observed positively associated with biomass production in *Cenchrus* (Table 1). High transpiration not only leads to high photosynthetic rate, but also keeps the leaf surface cool especially under hot conditions (Lu *et al.*, 1994). Though *Cenchrus* is an apomictic crop low DNA polymorphism is normally expected, the variation in CID observed among species suggested to study the possible variations in CID among available 400 exotic lines of *Cenchrus* in the institute (Mishra *et al.*, 2002). Moreover, stability of relationship between CID and TE or SLA under different environmental conditions and inheritance of these traits should be established before attempting to use them in large scale breeding programmes.

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