Carbon isotope discrimination and specific leaf weight estimate transpiration efficiency indirectly in Stylosanthes under well-watered conditions

B.R. Thumma[1,2], B.P. Naidu[1], D.F. Cameron[1] and L.M. Bahnisch[2]

[1]CSIRO , Tropical Agriculture, St. Lucia, Qld 4067, Australia
[2]School of Land and Food, the University of Queensland, Gatton College, Qld 4343, Australia

Abstract

High transpiration efficiency (TE, dry matter produced per unit of water transpired) is an useful trait for plants adapted to dry regions. Conventional measurement of TE is tedious and not suitable for use in breeding programmes. We have evaluated two physiological and biochemical traits as indirect measures of TE in this study. Ten accessions from two species of Stylosanthes ?were grown in pots in controlled environment and water use of the plants was monitored for seven weeks. Significant variations were observed among the accessions for TE, carbon isotope discrimination (DELTA) and specific leaf weight (SLW, leaf weight per unit area). DELTA was significantly and negatively correlated with TE (r = -0.88), while, SLW was significantly and positively related with TE (r = 0.76). SLW and DELTA are currently being examined in a segregating population of Stylosanthes to investigate their suitability for a rapid screening technique to improve TE.

Key words: Transpiration efficiency, carbon isotope discrimination, specific leaf weight, drought resistance, Stylosanthes.

Cultivars from the genus Stylosanthes are the most important pasture legumes grown in the semi-arid tropics of northern Australia. Water is a major environmental constraint to plant growth in this region (7) and dry matter production is closely related to water use (9). ?Efficient use of water is therefore likely to be a key characteristic of pasture plants for the semi arid tropics. ?While water use efficiency or transpiration efficiency (dry matter produced per unit of water transpired-TE) has been extensively investigated as a trait associated with drought resistance of plants, no study has been undertaken to examine the genotypic variation for TE in Stylosanthes germplasm.

Measuring TE of field grown plants is labour intens-ive and time consuming. Farquhar et al. (4) showed that carbon isotope discrimination (DELTA) could be used to measure transpiration efficiency of plants indirectly. ?CO_2 in the atmosphere contains carbon mostly in the form of ^{12}C, but a fraction of CO_2 is also present in the stable isotope form of ^{13}C. During carboxylation, plants discriminate against ^{13}C present in ambient CO_2. Thus the dry matter of plants contains a lower proportion of ^{13}C compared to that of ambient CO_2. Farquhar and Richards (5) showed that ^{13}C discrimination (DELTA) is negatively related to TE in wheat. Thus, DELTA represents an integrated measure of TE of a plant over its life. The negative relationship between DELTA and TE has been established in many plant species (6).

Specific leaf weight (dry matter per unit leaf area-SLW) was shown to be positively correlated to TE in peanuts and in other species (10, 11). The reason for this relationship is not clear, but could be due to the associat-ion of thicker leaves with higher photosynthetic capacity (2).

The aims of the present study were:

? to determine the level of genotypic variation for TE in Stylosanthes accessions under well-watered conditions; and,

? to identify the relationship between TE and DELTA or SLW.

Materials and Methods

Seeds of ten accessions of Stylosanthes (nine accessions of S. scabra and one accession, CPI 41117A, of ?S. fruticosa; Table 1) were germinated in petri-dishes and three plants were transplanted to pots (58 x 15 cm) containing 11 kg of soil on 1 June 1996 in a controlled environment facility. Each genotype had three replicat-ions arranged in three randomised blocks. Three pots without plants were placed, one in each block, to estimate evaporation from the pots. The conditions of the growth room were 12 h photoperiod, photon flux intensity of 500 mmol/m2/s, vapour pressure deficit of 1.27 kPa, and a temperature regime of 30 /25^{o}C. Water use of the plants was recorded by weighing the pots manually. The pots were watered to field capacity on alternate days by adding water equal to the amount of water lost. Transpiration was estimated by deducting the evaporation, as measured from the unplanted pots, from the total water use of the plants. On 20 August 1996, shoots were cut and oven dried at 70^{o}C for 48 hours and the dry weights were recorded.

Transpiration efficiency (TE) was calculated as shoot dry matter produced per unit of water transpired. Specific leaf weight (SLW, dry weight/unit leaf area) was estimated on 20 August by harvesting the two youngest fully expanded leaves from each plant. Leaf areas were measured with a leaf area meter and the leaves were oven dried at 70^{o}C for 48 hours for dry weight measurement. The leaf samples previously used for specific leaf weight measurements were finely ground and sub-sampled to provide 10 mg of leaf material for measurement of carbon isotope composit-ion (5) and elemental nitrogen analysis. Specific leaf nitrogen (SLN) was calculated as leaf nitrogen per unit leaf area.

Results

Significant differences in transpiration and dry matter production were recorded among the different access-ions of Stylosanthes (Table 1). Consequently the access-ions differed significantly for TE, values ranging from 1.61 to 2.43 g/kg (Table 1). Similarly, DELTA values varied significantly from 22.5 to 24.7_ (Table 1).This variat-ion in DELTA of about 2.2_ is comparable to the variation in DELTA observed in a range of crop species (6).

Values of leaf area measured for SLW, ranged from 11 to 34 cm^{2} (Table 1). There was no ?relationship between SLW and leaf area (of the leaves used for measuring SLW) (Table 2). ?CPI 93116 which had the highest SLW, also maintained high leaf area; Seca which had the second highest SLW, had medium leaf area and Fitzroy with a high leaf area maintained low SLW.

A significant and negative relationship was found between DELTA and TE (r = -0.88; Table 2). Similarly, the relationship between SLW and TE was significant but positive (r = 0.76), indicating that high TE is associated with high SLW (thicker leaves). Specific leaf nitrogen (SLN) was significantly and positively correlated with SLW, TE ?and negatively with DELTA , indicating that TE and DELTA were influenced more by photosynthetic capacity than by stomatal conductance.

Table 1. Mean transpiration (T), shoot dry weight (S.dwt), transpiration efficiency (TE), carbon isotope discrimination (Δ), specific leaf weight (SLW) specific leaf nitrogen (SLN), and leaf area (of the leaves used for SLW- LA) of *Stylosanthes* accessions grown for seven weeks under well-watered conditions in a controlled environment facility.

Accession / Cultivar	T (kg/ plant)	S.dwt (g/plant)	TE (g/kg)	Δ (‰)	LA (cm^2)	SLW (g/m^2)	SLN (g/m^2)
[a]cv. Seca	2.54	6.88	2.31	22.5	21	62	2.59
cv. Fitzroy	3.17	5.69	1.80	24.2	30	46	1.75
[b]CPI 92477	3.44	6.37	1.85	23.4	23	54	2.05
[c]Q10042	2.94	6.46	2.20	23.7	17	56	1.98
CPI 55860	3.26	6.11	1.87	24.3	15	53	2.07
CPI 55872	2.77	5.40	1.95	23.8	34	48	1.98
CPI 93116	4.61	11.2	2.43	22.8	29	75	2.53
CPI 93055	2.21	3.65	1.65	24.5	15	48	1.53
CPI 36260	1.14	1.84	1.61	24.7	11	58	2.16
CPI 41117A	0.39	0.70	1.79	24.0	17	52	2.08
LSD (P=0.05)	0.5	0.3	0.3	0.4	3.7	7.1	0.3

[a] cultivar
[b] Commonwealth plant introduction number
[c] Queensland collection number

Table 2 Correlation coefficients between carbon isotope discrimination (Δ), Transpiration efficiency (TE), Specific leaf weight (SLW), Specific leaf nitrogen (SLN), Shoot dry weight (S.dwt), and Leaf area (LA).

	TE	SLW	SLN	S.dwt	LA
Δ	-0.88**	-0.66*	-0.76*	-0.63 n.s.	-0.44 n.s.
TE		0.76*	0.71*	0.77*	0.41 n.s.
SLW			0.83**	0.59 n.s.	0.01 n.s.
SLN				0.40 n.s.	0.10 n.s

*P < 0.05; **P < 0.01; n.s. not significant

Discussion

This study has revealed significant genotypic variat-ion for TE in Stylosanthes under well-watered condit-ions (Table 1). Moreover, DELTA was negatively and sign- ificantly correlated with TE (r = -0.88) as expected from the work of Farquhar and Richards (5). ? DELTA can be various- ly influenced both by stomatal conductance and photo-synthetic capacity (3). If DELTA is influenced more by photo-synthetic capacity negative relation develops between DELTA and biomass production and positive relationship develops between DELTA and biomass production if DELTA is influenced more by stomatal conductance. However, in Stylosanthes it appears that DELTA was influenced more by photosynthetic capacity than by stomatal conductance, as indicated by the negative relationships of DELTA with SLW (r = -0.66), and with yield (r = -0.63).

The positive relationship between SLW and TE implies that SLW may also be used to estimate TE indirectly. Such correlation has been found in other plant species also (10, 11). However, the relationship between SLW and TE is not as strong as between DELTA and TE. As the measurement of SLW is rapid and inexpensive, it could be used in the initial phase of breeding programme to screen large number of lines, whereas DELTA could be used for the precise analysis of advanced lines. SLW and DELTA are currently being used in a segregating population of Stylosanthes to study the inheritance and stability of these traits under different environmental conditions.

SLW and leaf area were independent of each other in the accessions studied as indicated by the different combination of these two traits shown by the current set of accessions. Similar results were reported for alfalfa (1). Therefore, it should be possible to select for the desired combination of these two traits.

Recently Hall et al. (8) have reported that Seca, CPI 92477, CPI 93116, Q10042 and CPI 55872, have shown strong environmental adaptation among a group of 13 S. scabra accessions studied in northern Australia over four years of below average rainfall. DELTA values for these accessions obtained in the present study were low (Table 1), indicating that TE may contribute to successful adaptation to the dry tropics.

Conclusion

This study has identified significant genotypic variation for TE in Stylosanthes accessions under well-watered conditions. ? DELTA or SLW may be used to estimate TE indirectly. However, stability of the relationship between DELTA and TE or SLW and TE under different environmental conditions and inheritance of these traits should be established before attempting to use them in breeding programs.

Acknowledgements

B.R.T. is grateful to the School of Land and Food, the University of Queensland, Gatton College, for financial assistance in the form of a Tutorial Fellowship.

References

1. Barnes D.K., Pearce, R.B., Carlson, G.E., Hart, R.H., and Hanson., C.H. 1969. Crop Sci. 9, 421-423.

2. Brown R.H., and Byrd G.T. 1996. ?Crop Sci. ?36, 475-480.

3. Condon, A.G., Richards, R.A., and Farquhar, G.D. 1987. Crop Sci. 27, 996-1001.

4. Farquhar, G.D., O'Leary, M.H., and Berry, J.A. 1982. Aust. J. of Plant Physiol. 9, 121-137.

5. Farquhar, G.D., and Richards, R.A. 1984. Aust. J. of Plant Physiol. 11, 539-552.

6. Farquhar, G.D., Ehleringer, J.R., and Hubick, K.T. 1989. Annu. Rev. ?of Plant Physiol. 40, 388-397.

7. Gillard, P., and Winter, W.H. 1984. In "The Biology and Agronomy of Stylosanthes" Edited by H.M. Stace and L.A. Edye. Academic press, Sydney. pp. 405-432.

8. Hall, T.J., Edye, L.A., Middleton, C.H., Messer, W.B., and Walker R.W. 1995. Trop. Grassl. 29, 169-176.

9. McCown, R.L, Gillard, P., and Edye, L.A. 1974. Aust. J. ?Exp Agric . Anim. Husb. 14, 328-333.

10. Virgona, J.M., Hubick, K.T., Rawson, H.M., Farquhar, G.D., and Downes, R.W.1990. Aust. J. of Plant Physiol. 17, 207-214.

11. Wright, G.C., Nageswara Rao, R.C., and Farquhar, G.D. 1994. Crop Sci. 34, 92-97.