
C S I R O P U B L I S H I N G

AUSTRALIAN JOURNAL OF PLANT PHYSIOLOGY

Volume 26, 1999
© CSIRO Australia 1999

AJPP

An international journal of plant function

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Australian Journal of Plant Physiology

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Variation in transpiration efficiency and carbon isotope discrimination in cowpea

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Abstract. Genotypic variation in transpiration efficiency (TE) was investigated in a set of cowpea (*Vigna unguiculata* (L.) Walp.) genotypes grown as isolated plants in pots and under canopy conditions in the field. In the field, plants were grown in mini-lysimeters embedded in the ground around which a crop was grown, to simulate crop canopy condition. Two moisture regimes (100 and 60% of field capacity) were imposed from 30 to 60 days after sowing in both pot and field experiments. TE was determined by measuring transpiration and dry matter (DM) produced by the genotypes during the treatment period. Genotypes differed significantly in DM although the variation in the amount of water transpired (T) was relatively small. The TE ranged from 2.2 to 3.7 g kg⁻¹, representing a significant genotypic and environmental effect on the variation. There was a significant negative correlation ($r = -0.77$, $P < 0.01$) between TE adjusted for prevailing vapour pressure deficit and carbon isotope discrimination ratio (Δ) across all the experiments and treatments. A significantly positive correlation of TE measured in pot and field experiments suggested a low G \times E interaction. There was a negative correlation ($r = -0.62$, $P < 0.01$) between T and TE while the correlation of TE with net assimilation rate was non-significant, suggesting that the major cause for variation of TE in cowpea was in general associated with stomatal rather than mesophyll factors. Because of the positive relationship between T and DM, and negative relationship between TE and T, selection for high TE might therefore be associated with reduced T and hence lower DM. However, the present investigation showed a possibility of identifying specific genotypes with a combination of high TE and high net assimilation rate. The genotypes with high TE and high net assimilation rates were able to produce high DM under moisture deficit conditions.

Keywords: cowpea, transpiration efficiency, carbon isotope discrimination, net assimilation rate, drought, genotypic variation.

Introduction

Improvement in adaptation of cowpea (*Vigna unguiculata* (L.) Walp.) to drought-prone areas has been a long-term goal of crop improvement programs. However, progress in breeding for drought tolerance has been limited due to lack of selection criteria and simple and rapid tools to select for desirable traits from large populations under field conditions. Analysis of the crop performance using physiological models would not only enable identification of the traits contributing to the superior performance of drought tolerant genotypes under water deficit conditions, but also indicate scope for utilising the valuable traits in crop improvement programs.

The crop dry matter (DM) produced under water limited conditions is a product of the amount of water transpired (T) and the ratio of dry matter produced per unit T, termed as transpiration efficiency (TE) or water use efficiency. At leaf level, TE is strongly influenced by the ratio of CO₂ assimilation rate to transpiration rate. Transpiration however depends on the supply of water by the roots to meet the environmen-

tal demand, which is sensed by the plants through the vapour pressure difference between leaf and air. Thus, efficient roots and TE are important traits, which can contribute to the crop productivity under water-limited conditions.

A significant genotypic variation in TE has been observed in various crops e.g. grass species (Farquhar and Richards 1984; Frank *et al.* 1985), wheat (Condon and Richards 1993; Edhaie and Waines 1993), bean (White 1993) and cowpea (Hall *et al.* 1990, 1992). TE is estimated as the ratio of DM produced to the amount of water lost through transpiration over a period of time. The measurement of TE is cumbersome and labour-intensive because of the practical difficulties associated with measurement of transpiration and root dry matter.

A significant relationship between carbon isotope discrimination in leaf (Δ) and TE has been shown in many C₃ crops e.g. peanut (Wright *et al.* 1988, 1994), wheat (Condon *et al.* 1993; Edhaie and Waines 1993), C₃ grasses (Farquhar and Richards 1984; Frank *et al.* 1985) and cowpea (Ismail

and Hall 1992). These results suggested a possibility of using Δ as a rapid tool to assess TE in various C_3 crops. Farquhar *et al.* (1982) and Hubick *et al.* (1986) have shown that TE and Δ are related to each other through the ratio of CO_2 partial pressures in the mesophyll cell and the ambient air (P_i/P_a). The P_i/P_a (thus TE or Δ) can be influenced by a change in either or both stomatal (conductance type) and mesophyll (capacity type) factors. Therefore, Δ can be used as a surrogate measure of TE both in conductance types or capacity types of plants (Udaya Kumar *et al.* 1998a).

Since selection for TE is more relevant for drought-prone environments, moisture stress has been the most extensively studied environmental variable. A substantial increase in TE under drought conditions has been shown in a number of species: peanut (Wright *et al.* 1988, 1994), wheat (Condon *et al.* 1993) and cowpea (Hall *et al.* 1992). An increase in TE under moisture stress has often been attributed to a reduction in stomatal conductance under stress. But the physiological factors contributing to the observed genetic variability have not been elucidated. Since TE and T are inter-related, the physiological factors contributing to the variation in TE require greater attention and analysis. Otherwise, selection for high TE alone may result in low biomass types (Udaya Kumar *et al.* 1998a). The extent to which such quantitative traits as TE are applied in crop improvement programs also depends on the comprehensive knowledge of various factors, such as the physiological basis of variation for TE, its relationship with other yield traits, genotype (G) \times environment (E) interaction and heritability.

Hall and his co-workers (Hall *et al.* 1992, 1993; Ismail and Hall 1993; Ismail *et al.* 1994) investigated genotypic variation in TE and its association with Δ , the G \times E interaction for TE in cowpea, extensively. They showed that genotypic ranking for Δ was remarkably consistent when the same genotypes were grown under different drought conditions, years and dates of sampling, but at the same location. However, the ranking varied for when the genotypes were grown in different locations suggesting that the G \times E is low in a particular zone but not across the zones (Hall *et al.* 1993).

The present study was aimed at investigating the genetic variability in TE for selected cowpea plants grown in isolated conditions in pots and under canopy conditions using mini-lysimeters in the field, and examining the physiological traits associated with the observed differences in TE.

Materials and methods

All experiments were conducted at the main research station, at the University of Agricultural Sciences, Bangalore (12°58'N, 77°35'E), on red sandy loam/alfisols (pH 6.5). During the 1993 rainy season, 1600 cowpea germplasm lines were grown in single rows of 5-m length, each under adequately irrigated condition, in a non-replicated trial. Based on canopy development and growth characteristics, 12 genotypes of similar maturity were selected (Table 1) for further investigations.

Three separate experiments, two with pot grown plants and one with plants in mini-lysimeters under canopy conditions, were conducted to

examine genotypic variability for TE and its relationship with leaf carbon isotope discrimination.

Experiment Ia and Ib: assessment of genotypic variability in TE in isolated plants

The two pot culture experiments were conducted during the rainy seasons of 1994 (Experiment Ia, August–October) and 1995 (Experiment Ib, July–September). The seasonal vapour pressure deficit (VPD) varied between Exp. Ia (1.23 kPa) and Exp. Ib (0.95 kPa).

The 12 genotypes (Table 1) were grown in carbonised rubber containers of 30 \times 15 \times 45 cm, filled with 18 kg of sandy loam soil with farm yard manure in a ratio of 4:1. Five seeds were sown in each pot and the seedlings were later thinned out to two seedlings per container. The plants were adequately irrigated to field capacity (FC) until 30 days after sowing (DAS), after which the two moisture regimes i.e. 100% FC (I_1) and 60% FC (I_2) were imposed until 60 DAS. Plants were irrigated using a feeder pipe (high density polythene tube of 40-cm length and 50-mm inner diameter with perforations on side wall, buried to 25-cm depth in the soil). Water applied through the feeder pipe was uniformly distributed in the subsoil layers through the perforations made to the sidewalls. The soil surface was mulched with plastic pieces to minimise surface evaporation. The pots were arranged randomly in an open area adjacent to a glasshouse. Three portable rainout shelters (ROS) (Chauhan *et al.* 1997), each of which covered an area of 7.2 \times 15 m, were positioned on railings which facilitated the movement of ROS as well as a pot weighing device (details provided below).

Moisture regimes were maintained by adding appropriate amounts of water as described by Udaya Kumar *et al.* (1998b). Briefly, the amount of water held at 100 or 60% FC of the soil was first measured. Then, the pots were weighed daily and the weight loss was replaced to either 100 or 60% FC by adding water through the feeder pipe.

The pots were weighed daily using an electronic load cell with an accuracy of ± 0.01 kg. The load cell was fitted to a mobile pot-weighing device that moved along the axis of a 'C' channel. A mobile platform was fitted to the horizontal beam of the gantry, which housed the load cell and display device. A lever and fulcrum with a 4:1 ratio was attached to the lower plate of the mobile platform. A battery-operated load cell balance was placed on the upper plate of the mobile platform. The pots containing plants were lifted clear off the ground by depressing the lever with a foot-operated pedal and weights were recorded.

The pots were weighed daily to measure the amount of water lost through evapo-transpiration (ET). Simultaneously, 'bare' pots (without plants) were also weighed, to quantify the evaporation (Es) component of ET. Transpiration (T) was calculated as ET–Es.

Leaf area and oven dry weight of the plants were recorded at the beginning (30 DAS) as well as at the end of the experiment (60 DAS). Based on these observations, the change in dry matter during the treatment period (DM), leaf area duration (LAD, cm^2 days), net assimilation rate (NAR, $mg\ dm^{-2}\ day^{-1}$), mean transpiration rate (MTR, $mg\ dm^{-2}\ day^{-1}$), crop growth rate (CGR, $g\ m^{-2}\ day^{-1}$) and TE and K were computed as follows:

$$DM = \text{Total dry matter at 60 DAS} - \text{Total dry matter at 30 DAS},$$

$$LAD = \{(LA1 + LA2)/2\} \times d,$$

$$NAR = DM/LAD,$$

$$MTR = T/LAD,$$

$$CGR = DM/d,$$

$$TE = DM/T,$$

$$K = TE \times VPD\ (kPa),$$

where LA1 and LA2 were the leaf areas of plants at 30 and 60 DAS, respectively (Hunt 1982), 'd' was the duration of the experimental period in days (30 days), and K was the TE coefficient adjusted for the prevailing VPD.

Experiment II: assessment of TE under canopy conditions in the field

Experimental procedures used for the mini-lysimeter studies were described in detail by Udaya Kumar *et al.* (1998b). Mini-lysimeters of 90-cm length and 30-cm inner diameter were constructed using concrete and

fitted with strong MS loops to facilitate weighing. The mini-lysimeters were filled with soil excavated in 30-cm layers up to 0.9 m deep from the adjoining plots of the experimental site. Care was taken to ensure that the natural soil profile to a depth of 0.9 m was simulated. The mini-lysimeters were filled with soil at least three months before planting to allow settling of the soil core. The mini-lysimeters were placed in pits 0.9 m deep and 0.3 m in diameter, which had been dug in a 2.5 × 1.5-m matrix. In all, a total of 66 mini-lysimeters were installed at the experimental site with 22 located in each replication. In each plot, there were two mini-lysimeters spaced at a distance of 1.5 m. In each replication, two mini-lysimeters were left unplanted to monitor soil evaporation. A basal fertiliser containing 18 kg N/ha and 20 kg P₂O₅/ha was incorporated into the top soil in both the mini-lysimeters and surrounding soil.

The experimental area consisted of three replicate blocks of 7.2 × 15 m, excluding an area of similar size over which the three ROS were parked when not in use. The ROS was operated manually. The experiment was set up as a split plot design with two moisture regimes (100 and 60% FC, main plots) and five selected genotypes (Table 2, subplots).

Seeds were treated with Captan (fungicide) at a rate of 4 g/kg and *Rhizobium* strain (TAL 169) were hand dibbled, with 30 cm between rows and 10 cm between plants within each row. Each plot consisted of four rows of 6 m length, with two mini-lysimeters spaced at 1.5 m in the third row. Sowing was done simultaneously in the mini-lysimeters and the surrounding area. The crop was adequately irrigated until 30 DAS after which the moisture regimes were imposed.

The mini-lysimeters were weighed at 3-day intervals using an electronic load cell mounted on a mobile weighing device (Udaya Kumar *et al.* 1998b). The mini-lysimeter weighing device consisted of a truss made from mild steel T sections and frames 7.2 m long and 0.7 m tall. The truss also housed a platform rolling on six castor wheels on which a seat for the operator and a hydraulic jack were situated. A mild steel frame housed a load cell balance of 300 kg capacity and was attached to the hydraulic jack. The other end of the load cell was fitted with two chains and a hook to lift the mini-lysimeters about 30 cm clear of the ground. The platform with the seating arrangement could be moved across the horizontal beam to access and weigh mini-lysimeters.

The crop around the mini-lysimeters was watered using a drip irrigation system and the water input into each main treatment was measured and controlled by water meters (with an accuracy of ± 2%) fitted to control valves. The desired water regimes were maintained in the mini-lysimeters by applying the required quantity of water.

Growth observations including leaf area and total dry matter were recorded at 30 DAS and 60 DAS (at the end of the experiment) and physiological parameters such as NAR, CGR, LAD, MTR and TE were computed as outlined in Exps 1a and 1b. In both experiments, the third fully expanded leaf from the apex was sampled at 45 DAS. The leaf samples were oven-dried and ground into a fine powder for analysis of ¹³C isotopic discrimination (Δ) at the Department of Environmental Biology, Australian National University, Canberra, as described by Hubick *et al.* (1986).

Results

Climate

The climatic conditions during the experimental period in both the years (1994 and 1995) were quite similar, except for vapour pressure deficit (VPD). The mean temperatures were 23.3 and 23.25°C during 1994 and 1995, respectively, with a range of 23.05–23.55°C during 1994 (August–September) and 23.2–23.3°C during 1995 (July–August). Similarly, the average incoming solar radiation was 16.62 MJ m⁻² day⁻¹ during 1994 and 15.17 MJ m⁻² day⁻¹ during 1995. However, the VPD values were 1.23 and 0.95 kPa, representing a substantial difference between 1994 and 1995, respectively. A

total rainfall of 210 mm was received during the 1994 experimental period while it was 276 mm during 1995. The daily sunshine hours ranged from 4.8 to 7.8 h in 1994 and from 4.7 to 5.9 h in 1995. The experimental plots (both in the field and pot culture experiments) were protected from rain by covering them with portable ROS during the rainfall events.

Genotypic variation in dry matter production and transpiration efficiency

There was a significant variation among genotypes for dry matter (DM including roots) production in both experiments (Tables 1 and 2). The water deficit treatment (I₂) resulted in a mean reduction of 22% in DM in Exp. 1b (Table 1) and 15% in Exp. II (Table 2). However, there was a large variation among genotypes with the reduction in DM under I₂ ranging from 13 to 34% in Exp. 1b and from 3 to 18% in Exp. II. The root dry matter (data not presented) ranged from 4 to 10 g per plant, representing a significant variation. However, data pooled over the two experiments showed that there was a significant positive relationship between root and DM ($r^2 = 0.37$, $P < 0.01$). Genotypes transpired comparable amounts of water in both experiments, although the T was significantly lower in I₂ than in I₁ (Tables 1 and 2). Despite the relatively small variation in T compared with DM, T accounted for 75% of variation in DM across the treatments ($r^2 = 0.75$, $P < 0.01$, Fig. 1).

Transpiration efficiency ranged from 2 to >3 g kg⁻¹ in I₁ and from 3 to 3.8 g kg⁻¹ in I₂ among genotypes, representing a significant variation ($P < 0.05$). TE was 25% greater in I₂ than in I₁ in both experiments. The stress induced increase in TE was strongly associated with the reduction in mean transpiration rate ($r = 0.73$, $P < 0.05$). In order to examine the relationship between TE and Δ across environments, the TE was adjusted for the prevailing VPD and expressed as K (see Materials and methods). A strong negative relationship was observed between Δ and K values across all the experiments ($r = -0.77$, $P < 0.001$, Fig. 2). A similar negative relationship between Δ and K for the mini-lysimeter experiment ($r = -0.66$, $P < 0.05$) suggests that Δ can be a surrogate estimate of TE even under the canopy conditions in the field.

The TE values were in general lower in Exp. II than in Exp. 1b. However, a significant positive correlation of TE between I₁ and I₂ ($r = 0.65$, $P < 0.05$) and between Exp. 1b and II ($r = 0.84$, $P < 0.01$) suggests low G × E interaction (Table 3). Further, there was also a significant positive correlation between the Exp. 1a and Exp. 1b for K ($r = 0.72$, $P < 0.01$, Fig. 3a) as well as for Δ ($r = 0.79$, $P < 0.01$, Fig. 3b) suggested a low G × E interaction for these parameters (Table 3).

It was also apparent from the results that the errors associated with TE measured with DM excluding root dry matter could be minor. This is evident from the significant positive relationship ($r = 0.96$, $P < 0.001$) between TE values measured with DM including root dry matter and with DM including shoot dry matter only (without roots) (Table 3).

Table 1. Total dry weight (TDM, with root), net assimilation rate (NAR), mean transpiration rate (MTR), transpiration (T), transpiration efficiency (TE) and carbon isotope discrimination in leaf (Δ , ‰), in 12 cowpea genotypes grown under two water treatments (I₁, 100% FC; I₂, 60% FC) in Exp. I

Treatment	Genotype	TDM (g pl ⁻¹)	NAR (mg dm ⁻² day ⁻¹)	MTR (mg dm ⁻² day ⁻¹)	T (kg)	TE (g kg ⁻¹)	Δ ‰
I ₁	APC 982	54.8	0.16	58.0	20.6	2.67	19.63
	APC 370	45.7	0.15	56.0	17.1	2.68	20.44
	APC 478	50.9	0.17	47.9	19.3	2.64	19.84
	APC 229	54.4	0.15	56.3	20.9	2.62	20.61
	APC 580	52.6	0.20	72.8	18.9	2.79	19.46
	APC 540	50.4	0.13	55.8	21.5	2.36	20.15
	APC 412	56.3	0.17	62.1	20.5	2.75	19.60
	APC 714	55.2	0.12	47.1	21.2	2.61	20.30
	APC 689	56.2	0.17	60.9	20.1	2.82	19.81
	APC1452	56.7	0.18	57.2	18.2	3.13	20.09
	APC 761	52.3	0.16	73.1	24.0	2.21	20.63
	APC 569	49.9	0.19	81.4	21.2	2.35	20.50
Mean		52.9	0.16	60.7	20.3	2.63	20.09
I ₂	APC 982	46.7	0.15	43.5	13.6	3.44	19.24
	APC 370	33.9	0.14	33.4	9.9	3.41	19.63
	APC 478	42.5	0.12	32.3	12.3	3.57	19.24
	APC 229	39.5	0.16	53.8	13.3	2.98	19.56
	APC 580	45.3	0.19	54.6	13.4	3.40	18.93
	APC 540	33.5	0.13	42.5	10.9	3.05	19.84
	APC 412	43.9	0.19	55.3	12.9	3.39	19.56
	APC 714	41.7	0.14	41.4	12.5	3.38	19.17
	APC 689	37.3	0.17	45.5	9.9	3.77	18.93
	APC1452	47.3	0.19	58.5	14.0	3.37	19.56
	APC 761	42.0	0.15	47.6	13.3	3.17	19.66
	APC 569	42.4	0.17	56.9	13.9	3.06	20.06
Mean		41.3	0.16	47.1	12.5	3.33	19.45
LSD ($P < 0.05$)	Genotypes	2.17	0.01	2.48	0.89	0.16	0.20
	Treatment	5.31	0.03	6.08	2.17	0.38	0.50
	G × T	7.51	0.04	8.60	3.07	0.54	0.70

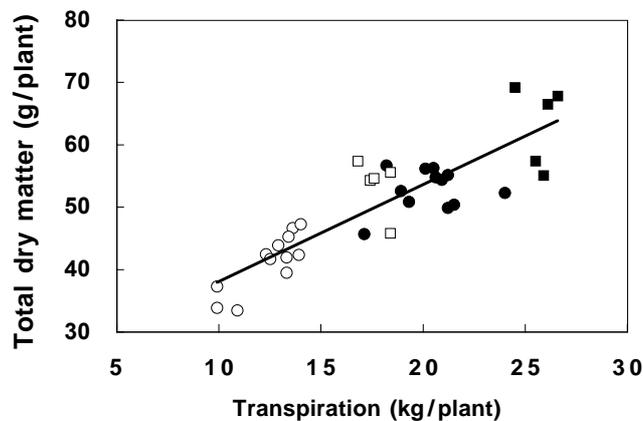


Fig. 1. Relationship between transpiration (kg plant⁻¹) and total dry matter (g plant⁻¹) in cowpea genotypes grown as isolated plants in pots (circles) and under canopy conditions in the field (squares), under irrigated (I₁, closed symbols) and water limited (I₂, open symbols). (DM = (1.55 × T) + 22.5; $r = 0.86$).

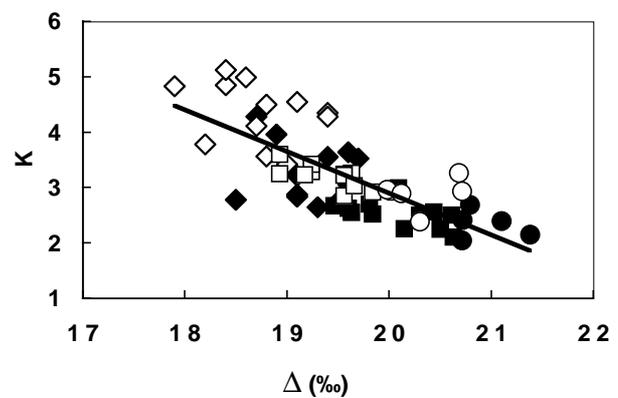


Fig. 2. Relationship between K and carbon isotope discrimination (Δ , ‰) in cowpea genotypes grown under irrigated (closed symbols) and water limited conditions (open symbols) in Exp. Ia (◆, ◇), Ib (■, □) and Exp. II (●, ○). Regression line drawn over all data points. ($K = (-0.75) \Delta + 17.98$; $r = -0.76$, $P < 0.01$).

Table 2. Total dry weight (DM, including root), net assimilation rate (NAR), mean transpiration rate (MTR), transpiration (T), transpiration efficiency (TE), carbon isotope discrimination in leaf (Δ , ‰) in five cowpea genotypes grown under two watering regimes (I₁, 100% FC; I₂, 60% FC) in mini-lysimeters

Treatment	Genotype	DM (g pl ⁻¹)	NAR (mg dm ⁻² day ⁻¹)	MTR (mg dm ⁻² day ⁻¹)	T (kg)	TE (g kg ⁻¹)	δ ‰
I ₁	APC 982	69.2	0.29	102.9	24.5	2.82	20.79
	APC 370	67.8	0.26	102.3	26.6	2.51	21.10
	APC 478	57.4	0.23	103.2	25.5	2.25	21.38
	APC 229	55.1	0.27	127.4	25.9	2.14	20.71
	APC 580	66.5	0.28	108.1	26.1	2.53	20.72
Mean		63.2	0.27	108.8	25.7	2.45	20.94
I ₂	APC 982	54.3	0.29	95.8	17.4	3.11	19.98
	APC 370	57.4	0.36	104.9	16.8	3.42	20.20
	APC 478	55.6	0.27	90.1	18.4	3.03	20.12
	APC 229	45.8	0.29	121.6	18.4	2.50	20.30
	APC 580	54.6	0.35	111.9	17.6	3.08	20.71
Mean		53.5	0.31	104.8	17.7	3.03	20.26
LSD ($P < 0.05$)	Genotype	7.68	0.04	4.34	1.41	0.25	0.30
	Treatment	12.14	0.07	8.28	2.22	0.40	0.47
	G \times T	17.17	0.10	12.7	3.15	0.56	0.67

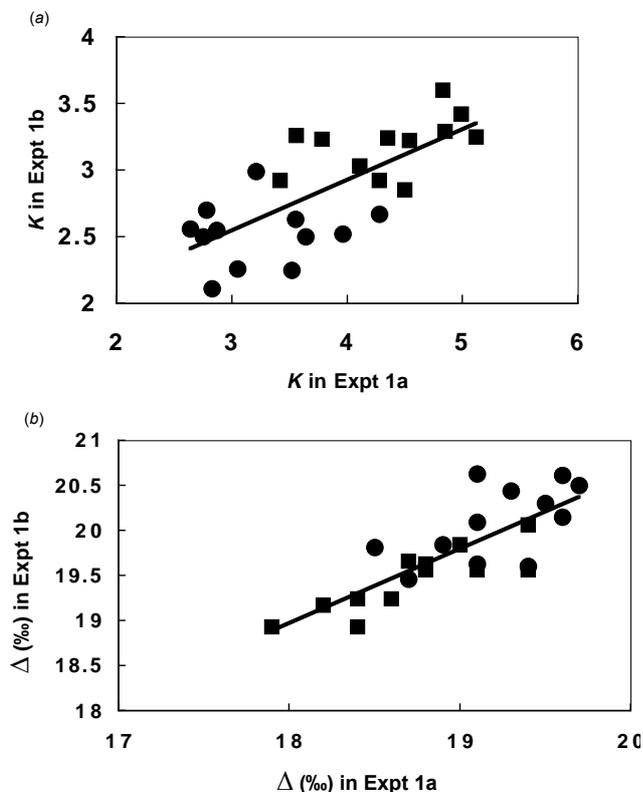


Fig. 3. Relationship of K (Fig. 3a, $r = 0.72$, $P < 0.01$) and carbon isotope discrimination (Δ , ‰) (Fig. 3b, $r = 0.79$, $P < 0.01$) between Exp. 1a and 1b for cowpea genotypes grown under irrigated (●) and water limited (■) conditions.

To examine the physiological basis for genotypic variation in TE, physiological parameters such as NAR and MTR were computed using variables such as DM, functional leaf area duration (LAD) and transpiration. The interrelationships between TE and other physiological traits (NAR and MTR) were examined separately for I₁ and I₂ treatments. A significant negative correlation ($r = -0.62$, $P < 0.01$) was observed between TE and T in both of the watering regimes and experiments, suggesting that increased TE is achieved by superior control of transpiration (Table 4). This finding was further substantiated by the negative relationship observed between transpiration rate and TE in both water regimes.

Net assimilation rate represents an integrated measure of photosynthetic capacity over a period of time. There was no relationship between TE and NAR or CGR under both water regimes, indicating that the genotypes that are intrinsically high in TE may not be high biomass producers (Table 4). However, in some genotypes high TE was associated with high DM and NAR. For example, the genotypes APC 580, APC 412 and APC 1452 showed high DM coupled with high TE, whereas the genotypes APC 370 and APC 478, despite a high TE, had a relatively low DM (Table 5). The genotypes with high CGR and high TE were classified as capacity types, since the high TE was brought about by higher NAR. On the other hand, the genotypes that had high TE primarily because of low MTR (APC 370 and APC 478) were classified as conductance types. The latter types were often associated with low CGR.

Table 3. Correlation between TE of cowpea genotypes measured under various treatments and experiments*, **, significance at $P < 0.05$ and $P < 0.01$, respectively

Parameters	r
Stress vs control	0.65* ($n = 17$)
Mini-lysimeter vs pot culture	0.84** ($n = 10$)
Total biomass vs shoot biomass	0.96** ($n = 34$)

Table 4. Correlation of TE with other physiological parameters in cowpea genotypes*, **, significance at $P < 0.05$ and $P < 0.01$, respectively

Parameters	r in control ($n = 17$)	r in stress ($n = 17$)
T	-0.62**	-0.62**
MTR	-0.47*	-0.59*
NAR	-0.16	-0.39
CGR	0.30	-0.09

Table 5. Transpiration efficiency and total dry matter of selected capacity (high NAR) and conductance (low NAR) genotypes in cowpea‘Capacity types’ represent the mean values of genotypes, APC 580, APC 412 and APC 1452; ‘conductance types’ represent mean values of genotypes, APC 370 and APC 478. Data are means (\pm SE) over genotypes

	DM (g pl^{-1})	NAR ($\text{mg dm}^{-2} \text{ day}^{-1}$)	MTR ($\text{mg dm}^{-2} \text{ day}^{-1}$)	TE (g kg^{-1})
Control				
Capacity types	55.23 (± 1.86)	0.184 (± 0.014)	64.03 (± 6.49)	2.89 (± 0.17)
Conductance types	48.33 (± 2.61)	0.138 (± 0.012)	51.97 (± 4.03)	2.66 (± 0.02)
Stress				
Capacity types	45.48 (± 1.40)	0.191 (± 0.005)	56.12 (± 1.68)	3.39 (± 0.01)
Conductance types	38.22 (± 4.31)	0.115 (± 0.001)	32.87 (± 0.56)	3.49 (± 0.08)

Discussion

Transpiration efficiency is an important physiological trait that influences adaptation of crop plants to water limited situations. Several studies have shown genetic variability for this trait in a number of crop species e.g. cotton (Lu *et al.* 1996), spruce (Sun *et al.* 1996), and peanut (Nageswara Rao *et al.* 1993; Wright *et al.* 1988, 1993, 1994). Although variability in TE has been shown in many crops, rapid progress in assessing this trait was made possible only after the advent of carbon isotope discrimination in leaves, which has been used as a rapid and dependable surrogate method to assess TE in crops (Farquhar *et al.* 1989).

In the present study, genetic variability for TE and the relationship between TE and were examined in selected cowpea genotypes in isolated plants grown in pots and also under canopy conditions in the field. It was apparent that genotypic variation for TE in cowpea was significant with the variation amongst genotypes ranging in the order of 30–40% (Table 1). These results are in agreement with the findings of Hall *et al.* (1990, 1993) and Ismail and Hall (1992) for isolated plants. The present study demonstrated that the genotypic variation is also repeatable under field conditions (Table 2), suggesting a possibility of exploiting TE in cowpea crop improvement programs. Masle and Passioura (1987) have highlighted the importance of accounting for root biomass in determining TE. Although significant genetic variability in root biomass was observed in the present study, a strong positive association ($r = 0.96$, $P < 0.001$) between TE estimated with and TE estimated without root dry matter suggests that errors associated with measurement of TE based on shoot biomass only would be very small (Table 3).

A significant negative relationship between K and Δ (Fig. 2) suggests that Δ could be used as a rapid screening tool to assess genotypes for TE in cowpea, as observed in the earlier studies (Hall *et al.* 1990). Further, the present study also demonstrated a significant relationship between Δ and TE in the field grown plants under canopy conditions using mini-lysimeters ($r = -0.66$, $P < 0.05$). Similar relationships between TE and have been shown in several other crops (Farquhar *et al.* 1982; Farquhar and Richards 1984; Virgona *et al.* 1990; Johnson *et al.* 1995; Sun *et al.* 1996).

For a trait to be successfully exploited in breeding programs, in addition to significant genetic variability, a low $G \times E$ interaction is preferred. To study the $G \times E$ interaction in cowpea, the relative rankings of the genotypes for K in plants grown in pots during two different years were compared. Despite the variation in VPD, there was a significant relationship of K and Δ between the two experiments (Fig. 3a and b) indicating a low $G \times E$ interaction for these parameters. Though Exp. Ib and II were conducted in the same season, the growing conditions were different. For example Exp. Ib was conducted with isolated plants in pots which had a lower soil volume than those in Exp. II (mini-lysimeters). In spite of differences in growing conditions and, consequently, the microclimate, there was a strong positive relationship of TE between these two experiments ($r = 0.84$). The low $G \times E$ interaction was further substantiated by a significant positive relationship between the TE measured under two water regimes, although there was a consistent increase in TE under the water deficit treatment. Hall *et al.* (1993) also demonstrated a low $G \times E$ interaction for TE across different treatments within a location. However, the extent of differences in the environmental conditions experienced by the crops in our experiments might not be large enough to provide contrasting environments to assess $G \times E$ interaction for TE. As suggested by Hall *et al.* (1993) there is a need for

further research to examine the $G \times E$ interactions for TE under a wide range of environmental conditions. A low $G \times E$ interaction for TE has been reported in other legume crops such as peanut (Wright *et al.* 1993; Nageswara Rao and Wright 1994) and soybean (White 1998).

Unlike in the experiments of Ismail *et al.* (1994), where pot size differences influenced the changes in TE under stress, such an effect of the soil volume was not seen in our experiments. From this context, pots maintained in an open area can be conveniently used for an initial screening of genetic variability in TE among cowpea genotypes. The strong positive relationship of TE between Exp. Ib and II indicates that an initial assessment of genotypes for TE could be attempted using Δ as a selection tool.

To exploit the genetic variability in TE for crop improvement, it is important to understand the underlying physiological mechanism(s) contributing to the genotypic variability. It is well known that the stomatal conductance (g_s) or mesophyll efficiency (g_m) substantially influence the intercellular CO_2 partial pressures (P_i) and hence contribute to the variation in TE (Johnson and Tieszen 1993; Lu *et al.* 1996; Sun *et al.* 1996; Virgona and Farquhar 1996). In order to investigate the basis of variation for TE in cowpea, the relative contribution of each of g_s and g_m were examined separately under irrigated and water deficit conditions. The relevance of these physiological parameters in bringing about the genetic variability in TE can be best quantified only when the inter-relationships of these two traits are assessed separately in stress and non-stress conditions. Although direct measurements of stomatal conductance were not done in the present study, the rate of transpiration computed per unit leaf area per unit time (expressed as MTR) can represent an integrated measure of g_s over a period of time. Similarly, net assimilation rate (expressed as NAR) can represent an integrated measure of photosynthetic rate. Thus, NAR and MTR reflect the carbon gain and mean g_s integrated over the experimental period and hence are more reliable than spot measurements of conductance and photosynthesis (Udaya Kumar *et al.* 1998b).

A significant inverse relationship between MTR and TE and lack of correlation between TE and NAR in both of the watering regimes (Table 4) suggests that the genetic variability in TE among cowpea genotypes is predominantly manifested by stomatal rather than mesophyll factors. Such a strong stomatal control of TE has also been reported in a number of other species, including grasses (Johnson and Tieszen 1993) and cotton (Lu *et al.* 1996). The results of the present study suggest that cowpea could be classified as a conductance type, in contrast to other crops such as peanut (Wright *et al.* 1993, 1994; Udaya Kumar *et al.* 1998a), sunflower (Virgona *et al.* 1990) and spruce (Sun *et al.* 1996) in which photosynthetic capacity is the major cause for variability in TE.

Genotypic variability of TE in cowpea can be successfully exploited only if the variability in TE is associated with

mesophyll efficiency but not stomatal factors. Since the variation of TE in cowpea seem to be generally associated with stomatal factors, selection for TE alone is likely to result in low DM types. However, it was interesting to note the lack of relationship between TE and T in some genotypes. In these specific cases, variation in TE is most likely to be associated with mesophyll capacity. In fact, high DM (coupled with high TE) in these genotypes supports this observation. Hence these could be grouped as 'capacity types'. These results suggest that it is possible to identify genotypes with high TE without any reduction in T. For example, the genotypes APC 1452, APC 580 and APC 412 can be identified as high TE types associated with high DM (Table 5). These genotypes also had relatively high NAR, suggesting high mesophyll efficiency contributing to the high TE. Therefore, selection for TE will have potential for application in the improvement of cowpea if genotypes with a combination of high TE and high photosynthetic capacity are identified. The two-step selection process might involve first, selecting for TE using Δ , followed by screening for high NAR types within the subset. Further studies are necessary to develop cost-effective tools to select for these two traits in a large-scale screening. However, the present study suggests scope for selection of such cowpea genotypes with a combination of TE and DM traits.

Acknowledgments

This research was supported by the ACIAR project PN 9216. We also thank Professor G. D. Farquhar for his encouragement and analytical support during this project and Dr M. S. Sheshshayee for the suggestions during the preparation of the manuscript.

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Manuscript received 13 July 1998, accepted 18 June 1999