

Stomatal responses of pearl millet (*Pennisetum americanum* [L.] Leeke) to leaf water status and environmental factors in the field

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Abstract. Factors affecting stomatal conductance (g_1) of pearl millet (*Pennisetum americanum* [L.] Leeke), cultivar BJ 104, were examined in the field in India during the dry season.

Diurnal changes in g_1 were evaluated for upper expanded leaves at flowering on two occasions using plants subjected to varying degrees of water stress. Except for the most severely stressed treatment, diurnal changes in g_1 closely matched changes in irradiance (I), the promotive effect of which largely overcame opposing influences on g_1 of increasing atmospheric vapour pressure deficit, and decreasing leaf water and turgor potentials (ψ , ψ_p).

Two main effects of water stress on g_1 were evident: (i) a decrease in the amplitude of the mid-day peak in g_1 , and (ii) a decrease in the time over which high g_1 was maintained, resulting in early (mid-day) closure and hysteresis in the relationship between g_1 and I .

Leaf conductance was greatest for upper leaves and decreased down the canopy. At equivalent depths in the canopy g_1 was higher in flowering than in photo-periodically-retarded plants of the same age. The magnitude of water stress-induced stomatal closure increased down the plant, and was more marked in retarded than in flowering plants.

Within individual stress treatments ψ of upper leaves decreased linearly as transpiration flux increased. It is concluded that stomatal behaviour of upper leaves of pearl millet at flowering largely operates to maximize assimilation rather than to minimize water loss.

Key-words: *Pennisetum americanum* (L.) Leeke; Gramineae; pearl millet; stomatal conductance; water stress.

Introduction

Pearl millet (*Pennisetum americanum* [L.] Leeke, syn. *P. typhoides* [Burm.f.] S. and H.) is commonly regarded as one of the most drought-resistant cereals in cultivation (Catherinet *et al.*, 1963; Cochemé &

Franquin, 1967; Arnon, 1972; Ferraris, 1973). It is grown extensively in semi-arid regions where insufficient and erratic rainfall constitutes a major limitation to crop growth. Despite its reputed ability to grow and produce grain under such conditions, the physiological basis for drought-resistance in pearl millet is little understood, and has been the subject of few investigations (Begg *et al.*, 1964; ICRISAT, 1977, 1978, 1980; Black & Squire, 1979; Gregory, 1979; Gregory & Squire, 1979; Squire, 1979).

Stomatal closure during water stress is a major factor mitigating the effects of drought. In a pot experiment with young pearl millet plants (Henson *et al.*, 1981a) stomatal conductance (g_1) was observed to decline rapidly with declining ψ . By contrast, in field experiments (Henson *et al.*, 1981; ICRISAT, 1980), stomatal closure in response to water stress was only partial despite ψ falling to values considerably lower than those required to induce almost complete stomatal closure in the pot-grown plants. The reasons for these differences between plants grown in pots and in the field, which have been observed frequently in several species (Turner, 1974a; Begg & Turner, 1976) are not fully understood, although the stage of plant development (Ackerson & Kreig, 1977; Morgan, 1977) and the degree of 'stress hardening' experienced by the plants (McKree, 1974; Brown, Jordan & Thomas, 1976) probably both contribute.

In order to characterize further the stomatal behaviour of pearl millet under conditions in the field, experiments were conducted at ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) Centre in India during the 1980 dry season (Jan–April). By obtaining information on stomatal responses to changing environmental factors under natural climatic conditions, it was hoped to obtain an insight into the strategy normally adopted by the plant for coping with water shortage.

Materials and methods

Plant culture

Seeds of pearl millet (*Pennisetum americanum* [L.]

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Leeke) cultivar BJ 104, were direct sown in late January or early February in rows 0.75 m apart on slightly raised ridges on either a medium (75–90 cm) or a deep (120 cm) alfisol soil at two sites on the ICRISAT farm, near Hyderabad, India (17° 30' north). Plants were subsequently thinned to about 10 cm apart within rows. All rows were initially irrigated by flooding between ridges to ensure establishment of the seedlings. The nature and frequency of subsequent irrigations varied according to the treatments imposed, as described below.

Experimental design, treatments, and sampling procedures

Diurnal studies. Plants were sampled from two sites on the ICRISAT farm, both 43 d after sowing (DAS). Site 1 plots on a deep alfisol soil included both fully furrow-irrigated plots and plots which received no further irrigation after stand establishment, resulting in a control and a 'medium'-stress treatment with minimum mid-day ψ at sampling of 0.66 and -1.17 MPa respectively. Plants were flowering when sampled. The penultimate leaf (i.e. the leaf below the flag leaf) was taken. There were three replicate plots per treatment and leaves were sampled from two randomly selected plants within each plot. Sampling commenced just prior to dawn (c. 6.00 Indian standard time) and continued at 60–90 min intervals until just after dusk (c. 19.00).

At site 2, on a medium depth alfisol, plants were sampled at three positions along a water supply gradient established using a line of overhead rotary sprinklers (Hanks *et al.*, 1976). Plant rows, orientated parallel to the sprinkler line, received amounts of irrigation water which depended on their distance from the line. Rows close to the line (position 1) received sufficient water to recharge the profile to field capacity. These plants were about 85–90 cm tall and in full flower at the time of sampling, having produced c. 31.5 visible panicles per m length of row. Plants in rows some 11 m from the sprinkler line (position 2) were shorter (63 cm) and produced fewer panicles (25.0 per m), while in rows at 14 m (position 3), plants were only 44 cm tall and had only 13.2 panicles per m. Plants in these three positions were estimated to have received 150, 62 and 30 mm irrigation water respectively, in addition to that given initially to ensure stand establishment. On the day of sampling (8 d after the last irrigation) plants in the three positions had minimum mid-day ψ of -1.74, -2.14 and -2.90 MPa respectively. Sampling procedure was as at site 1, except that where panicles had not yet emerged the youngest fully emerged leaf and not the penultimate leaf was selected.

Leaf position. Vertical profiles within the canopy in g_1 , ψ and ψ_p were investigated using plants grown either with full (furrow) irrigation or with irrigation withheld after the early vegetative stage. A photoperiodic treatment was superimposed on the irrigation treat-

ments, with half of each plot receiving the prevailing short days (SD; 11.5 to 13 h light per day), and half receiving 16 h photoperiods (LD) by extending the natural daylength with light from overhead incandescent lamps. Extension of the photoperiod retarded flower initiation by 18 d, and at the time of sampling (50 and 51 DAS) SD plants were in full flower while LD plants had no panicles visible (although stem extension had commenced).

Leaves were sampled from six randomly-selected plants along a single row, at four positions up the plant, between 11.00 and 13.00 h avoiding those portions of the SD sub-plots adjacent to the LD sub-plots which were subject to light spill-over. The flag leaf was sampled from flowering plants, and the uppermost fully-expanded leaf from non-flowering plants.

Measurements of stomatal conductance and leaf water status

Leaf conductance (g_1) was measured using an automatic diffusive resistance porometer (Delta-T Devices, Burwell, UK). The instrument was calibrated using a series of standard resistance plates just before and just after each set of test measurements. During measurements the instrument was shaded from direct sunlight. Measurements by the instrument of 'cup' and leaf temperature differed on average by less than 0.6 °C. Both leaf surfaces were monitored at a mid position along the leaf. Stomatal resistances ($s \text{ cm}^{-1}$) were calculated from the appropriate calibration curve and converted subsequently to conductances (cm s^{-1}). The conductance of each surface was summed to give a total g_1 for the leaf.

Following conductance measurements the leaf was enclosed in moist muslin wrapping, excised, and the leaf water potential (ψ) determined using a pressure chamber. A longitudinal half of each leaf was immediately frozen using solid CO_2 and subsequently used to determine solute potential (ψ_s) and, by derivation, turgor (pressure) potential (ψ_p), as described elsewhere (Henson *et al.*, 1982).

Environmental measurements

Standard meteorological data were obtained from a weather station on the ICRISAT farm, about 1000 m from the experimental sites. During diurnal sampling, measurements were also made within the crop area. Total irradiance (I) was measured at 30 min intervals using a pyranometer sensor located above the crop and linked to an integrator (Lambda Instrument Co., Nebraska, USA). Wet and dry bulb temperatures were recorded every 15 min using an Assman type psychrometer attached to a chart recorder (Cambridge Instruments Ltd., UK), these instruments being enclosed in a Stevenson screen.

Leaf temperatures were determined during diurnal sampling using an infra-red thermometer (Barnes Engineering Co., Stamford, Connecticut). Prior to

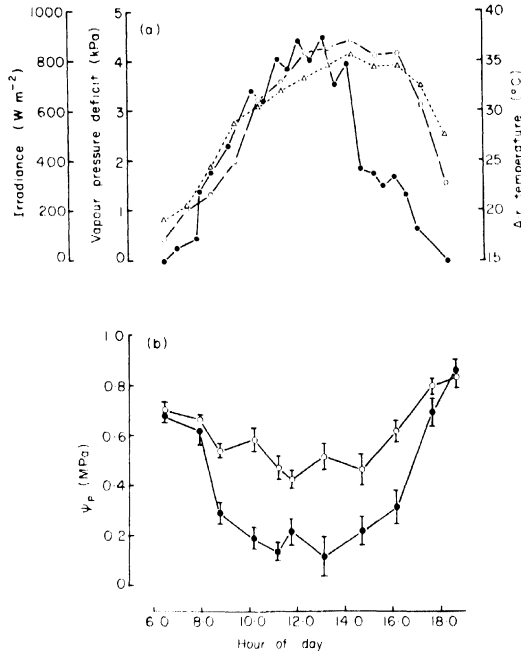


Figure 1. (a) Diurnal changes in irradiance (\bullet), air temperature (Δ) and vapour pressure deficit (\circ) on 19.3.80. (b) Diurnal changes in ψ_p of upper expanded leaves of irrigated (\circ) and non-irrigated (\bullet) pearl millet BJ 104 on 19.3.80 (site 1). Vertical bars indicate \pm s.e. ($n=6$).

sampling each plot, the thermometer calibration was checked against a standard calibrating device supplied by the manufacturers.

Results

Diurnal changes in conductance

Site 1, control versus moderate stress. On the day of measurement (19.3.80, 43 DAS) climatic conditions normal for the season prevailed, with high mid-day irradiances and high afternoon air temperatures and atmospheric vapour pressure deficits (Fig. 1a). Imbalance between water uptake and water loss led to a decline at mid-day in leaf ψ , ψ_s and ψ_p , with ψ_p dropping 0.3–0.4 MPa lower in non-irrigated than in irrigated plants (Fig. 1b). (For the diurnal changes in ψ and ψ_s , see Henson *et al.*, 1982.) Despite these diurnal reductions in bulk leaf potentials, adaxial g_1 increased in both treatments reaching a peak near to mid-day before declining (Fig. 2b). There was no such consistent change with time in abaxial g_1 , and within treatments the effect of sampling time was not significant. There was, however, a highly significant ($P < 0.001$) reduction in mean abaxial g_1 due to water stress (Fig. 2a). Though the mean adaxial g_1 of stressed

plants was also reduced, this reduction was only significant at $P < 0.05$.

Visual inspection of the data (Figs 1a, 2b) suggested that adaxial (and also total) g_1 closely followed changes in I . This impression was confirmed following correlation and multiple linear regression (MLR) analysis with all 'internal' (ψ , ψ_p , ψ_s , leaf temperature) and environmental (I , leaf-to-air vapour pressure deficit [lvpd], air temperature) parameters being included as independent variables in the analysis. For the adaxial surface 62.8% (control) and 58.6% (stressed) of the variance in g_1 could be accounted for by changes in I alone, while lvpd (the next most influential variable) accounted for only 16.1 and 7.3% of the variance in g_1 when I was omitted from the analysis. (However, when allowance was made for the influence of I , a negative effect of lvpd on g_1 was generally more evident, as shown by the change in the regression coefficients [Table 1; also see below].) Adaxial g_1 thus closely matched I , and continued to increase with increasing I with no evidence of saturation (Fig. 3b). The slopes of the regressions of adaxial g_1 on I for control and stressed leaves did not differ significantly.

The abaxial surface was much less responsive to I than the adaxial surface and was unaffected by I with water stress (Fig. 3a). This led to total g_1 of stressed

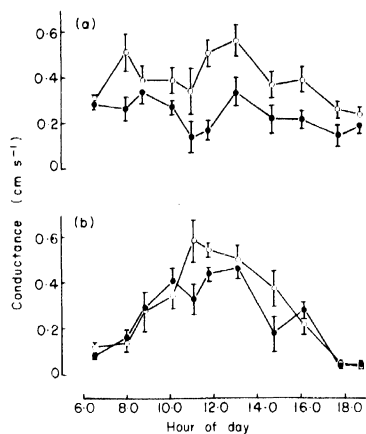


Figure 2. Diurnal changes in abaxial (a), and adaxial (b) conductance of upper expanded leaves of irrigated (○) and non-irrigated (●) pearl millet BJ 104 on 19/3/80 (site 1). Vertical bars indicate ± s.e. ($n = 6$).

plants being significantly ($P < 0.05$) less responsive to I than g_1 of irrigated plants.

Although within treatments, increasing I largely negated effects on g_1 of increasing water stress, stomata were still responsive to leaf water status. Thus, when I was high and essentially constant g_1 tended to be greatest in those leaves having the highest ψ_p (Fig. 4).

Site 2; 'severe' stress. Plants were again sampled 43 DAS, but on the day of sampling (7.3.80) maximum irradiance was slightly lower than for 19.3.80 and declined earlier in the afternoon due to cloud cover (Fig. 5a). Severe water stress was evident, however, as indicated by low ψ and ψ_p at mid-day (Fig. 5b; see also Materials and methods). Changes in adaxial and abaxial g_1 are shown in Fig. 6a, b. For the plants at positions 1 and 2 in the water supply gradient changes in g_1 closely followed I , but for plants at position 3 receiving less water, after an initial rise, g_1 declined sharply just prior to mid-day with falling ψ and ψ_p , and remained low for the remainder of the photoperiod. Maximum total conductances were lower than for control plants sampled on 19.3, but were not appreciably different from the moderately stressed treatment sampled on that date.

The two leaf surfaces responded similarly over the day (Fig. 6), with adaxial g_1 being generally higher than abaxial g_1 as with stressed plants at site 1. Also, as at site 1, water stress had a more significant effect in reducing mean abaxial g_1 ($P < 0.001$) than in reducing mean adaxial g_1 ($P < 0.01$).

The effects of water stress were evident in plots of g_1 v. I (Fig. 7a, b) which revealed (i), the 'saturation' of abaxial g_1 at an irradiance of $< 250 \text{ W m}^{-2}$ and (ii),

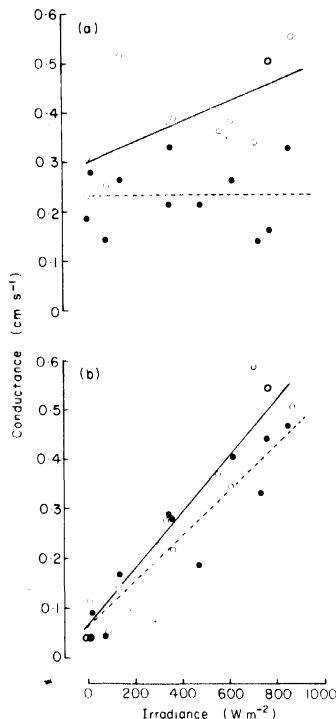


Figure 3. Relationships between abaxial (a), and adaxial (b) conductance (g_1), and irradiance (I), for upper expanded leaves of irrigated (○) and non-irrigated (●) pearl millet BJ 104 on 19/3/80. Each point is a mean of six. Lines are fitted linear regressions of g_1 on I for irrigated (—) and non-irrigated (---) plants. For (a) $r = 0.37$, significant at $P < 0.05$ (irrigated) and < 0.01 , not significant (non-irrigated), for (b) $r = 0.96$ (irrigated) and 0.93 (non-irrigated), both significant at $P < 0.001$.

hysteresis in the g_1 v. I relationship, with stomatal closure on both surfaces occurring at irradiances which sustained higher g_1 values during the opening phase before ψ_p declined to zero. Both these latter effects were present essentially in all three treatments and were in marked contrast to responses observed at site 1.

As with site 1 data correlation and MLR analysis confirmed the dominant effect of I on g_1 , with I accounting for 59 and 61% of the variance in total g_1 at positions 1 and 2 respectively, though only 16% of the variance for severely stressed plants at position 3. MLR analysis showed that lvpd was the factor with the most influence on g_1 after I . However, lvpd had little significant effect on g_1 alone (accounting for less than 8% of the variance in total g_1 when considered independently of other variables) and regression coefficients were in general non-significant. When the effect of I was allowed for by MLR (Table 1), both the

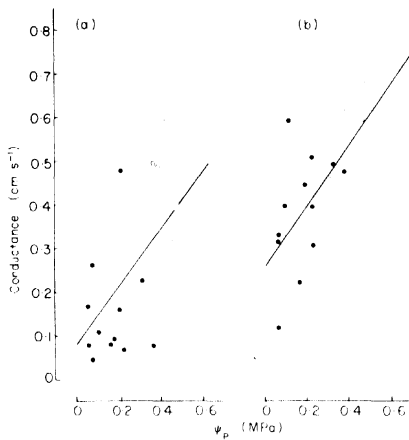


Figure 4. Relationships between abaxial (a) and adaxial (b) conductance (g_1), and bulk leaf ψ_p , for upper expanded leaves of pearl millet BJ 104 at constant irradiance (754 W m^{-2}) on 19/3/80. Points represent individual leaves from irrigated (○) and non-irrigated (●) plots. Lines are fitted linear regressions of g_1 on ψ_p . For (a) $r = -0.51$ ($P < 0.05$) and for (b) $r = 0.65$ ($P < 0.01$).

magnitude and significance of the regression coefficients (β) for g_1 on lvpd were increased, revealing a significant influence of lvpd on g_1 . This effect did not vary appreciably between surfaces, neither was there any general dependence of β on the degree of water stress. This contrasts with the observations of Black & Squire (1979) and Squire (1979) that only stomata of well-watered millet plants responded to changes in vpd.

Effects of leaf position and developmental stage

In this experiment no consistent differences were observed in the response to water stress by the two leaf surfaces and hence only total conductances are considered.

Total g_1 of leaves at different levels within the canopy are shown in Fig. 8a, b. In general, upper leaves had higher conductances than lower ones. However, there were significant differences in g_1 down the canopy only in flowering plants (the differences for leaf position being significant at $P < 0.05$ for control plants and $P < 0.001$ for stressed ones).

The effect of water stress on g_1 depended both on leaf position within the canopy and stage of plant development as influenced by photoperiod (Fig. 8c). The effect of water stress on g_1 increased with increasing depth in the canopy; conductance of the upper leaf layer being least affected by stress. At equivalent depths within the canopy, stress reduced the conductance of retarded plants more than that of flowering plants. Indeed, for flowering plants there was no effect of stress on g_1 of the topmost leaf layer. It was not possible, however, from these data, to determine whether stomata of

retarded plants were inherently more sensitive to stress than stomata of flowering plants. Although a greater degree of stomatal closure was evident in the former group and both groups had similar mean leaf ψ ($c. -1.5 \text{ MPa}$; Henson *et al.*, 1982), leaf ψ_p was about 0.14 MPa lower in retarded than in flowering plants.

Discussion

Irradiance

The diurnal course of conductance in pearl millet appeared to be governed largely by changes in I , which modified stomatal response to water stress in a manner qualitatively similar to that observed for other crop species (Turner, 1974a; Biscoe, Cohen & Wallace, 1976). This finding is consistent with results of earlier studies (ICRISAT, 1980), and with those of Squire (1979), who found that canopy conductance of pearl millet plants growing in drying soil was mainly related to I , while being unaffected by changes in vpd. In the present study inclusion of environmental factors additional to I as independent variables in MLR analyses led to only small additional improvements in fit. In its effect on conductance, therefore, I largely overrode opposing influences of concomitant changes in lvpd and leaf water status. Because of this it is likely that ψ and ψ_p were to a large extent functions of g_1 rather than the converse, with low ψ occurring due to high evaporation rates, resulting in turn from high g_1 combined with high lvpd. Thus, when ψ was related to transpiration flux as estimated from the product of lvpd and g_1 (Kaufmann & Hall, 1974), significant negative correlations were obtained (Fig. 9). Although the estimates of transpiration were necessarily approximations (the contribution of boundary layer resistance in determining transpiration flux not having been assessed), the data support the view that ψ was a function of transpiration rate. Stomatal behaviour was thus most probably directed towards maximizing CO_2 assimilation rates rather than minimizing water loss. As assimilation was not measured no conclusions can be drawn regarding 'optimal' stomatal functioning. However, one might speculate whether water use efficiency in pearl millet, unlike in C_3 species, might not actually be favoured by maximizing g_1 (as concluded by Cowan (1977) to be the case for the C_4 desert species *Tidestromia oblongifolia*). For this to be so, assimilation would have to increase with I at least up to full sunlight and not be unduly limited by high temperatures or water stress. The first two conditions appear to be satisfied for millet both on the basis of laboratory studies (McPherson & Slatyer, 1973), and, in part, by the present field results (Fig. 3).

The effect of I on g_1 is generally accounted for by its effect, via photosynthesis, on intra-cellular CO_2 concentration (C_i), with low C_i favouring stomatal opening (Raschke, 1975). For this explanation to hold in the present case it would be necessary for rates of photosynthesis sufficiently adequate to reduce C_i (and hence

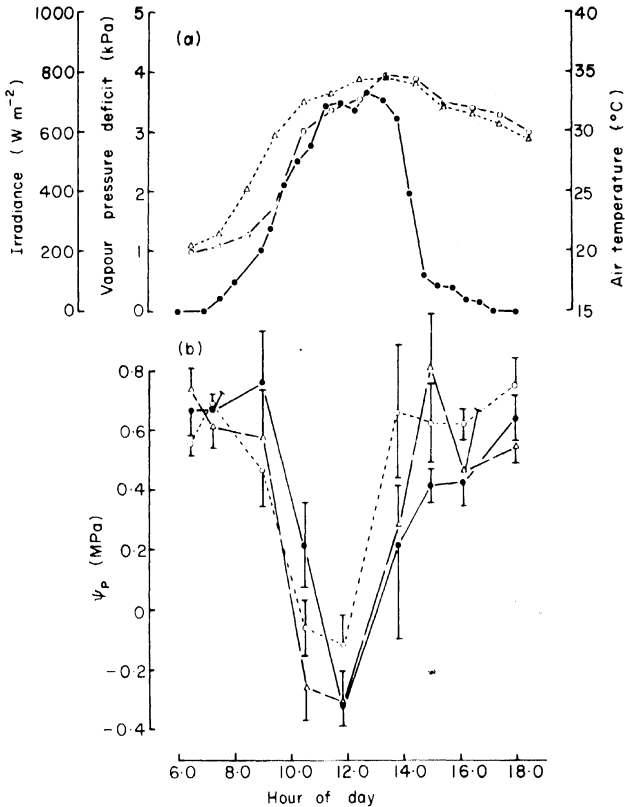


Figure 5. (a) Diurnal changes in irradiance (●), air temperature (△) and vapour pressure deficit (○) on 7/3/80. (b) Diurnal changes on 7/3/80 (site 2) in ψ_p of upper expanded leaves of pearl millet BJ 104 grown at three positions in a water supply gradient (○, position 1; △, position 2; ●, position 3; see text for further details). Vertical bars indicate \pm s.e. ($n = 6$).

increase g_1), to be attained despite severe internal water stress. The requisite robustness of photosynthetic activity in pearl millet to water stress has, however, yet to be demonstrated.

One benefit to accrue from high g_1 is lowering of leaf temperature below that of the air, so lessening the chance of irreversible tissue damage at high temperatures. In the present studies (data not presented) leaf temperatures were as much as 5°C below air temperatures at maximum conductance.

The variation in g_1 with leaf position within the canopy is most readily interpreted in terms of a vertical gradient in irradiance. Light penetration into a millet canopy was found to decrease linearly with depth (within rows) approaching extinction at about 75 cm below the top (Begg, 1965). Reductions in g_1 down the canopy were previously observed for pearl millet by Squire (1979), and for other crop species by Begg & Turner (1970), Turner & Begg (1973), Turner (1974b),

Jones (1977) and Sivakumar *et al.* (1979). In the present study an interaction was evident between leaf position (and therefore irradiance level) and water stress. Conductances of upper leaves exposed to full sunlight were least affected by stress, while stomatal closure occurred in response to stress more readily with increasing depth within the canopy (Fig. 8c). This response was not a result of increased stress at increasing depth, for ψ_p was similar at all leaf levels while ψ actually increased down the canopy (Henson *et al.*, 1982). Conductance was therefore inversely proportional to ψ . Although leaf age effects might also contribute, it is suggested that I was mainly responsible for the observed g_1 profile, overriding the effects of water stress as observed on a diurnal basis, again probably via its effects on C_i . Such differential stress sensitivity between leaf layers would be expected to be of considerable advantage to the plant, as high g_1 in leaves most exposed to light would serve to ensure

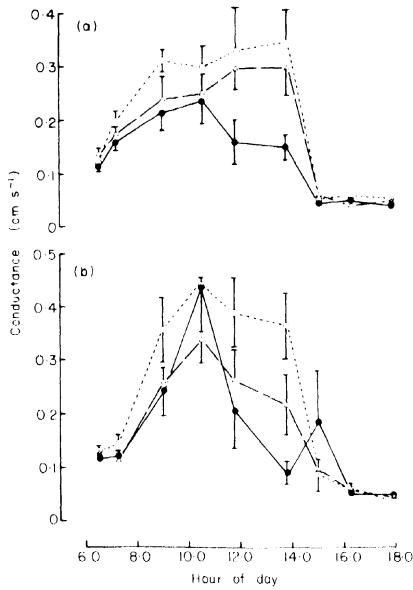


Figure 6. Diurnal changes on 7/3/80 (site 2) in abaxial (a) and adaxial (b) conductance of upper expanded leaves of pearl millet BJ 104 grown at three positions in a water supply gradient. For explanation of symbols see Fig. 5b. Vertical bars indicate \pm s.e. ($n = 6$).

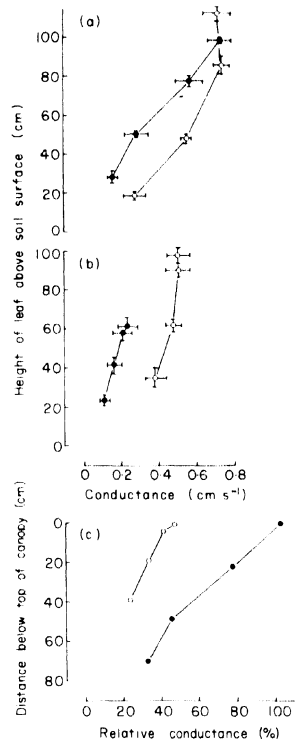


Figure 8. Effect of leaf position and water stress on total conductance (g_t) of pearl millet BJ 104. (a) Flowering plants, changes in g_t with height for irrigated (○) and non-irrigated (●) plants. Bars indicate \pm s.e.; $n = 6$. (b) As in (a) but for photoperiodically retarded plants. (c) Total g_t of non-irrigated plants as a percentage of total g_t of irrigated plants at equivalent distances from the top of the canopy (relative g_t) for flowering (●) and retarded (○) plants.

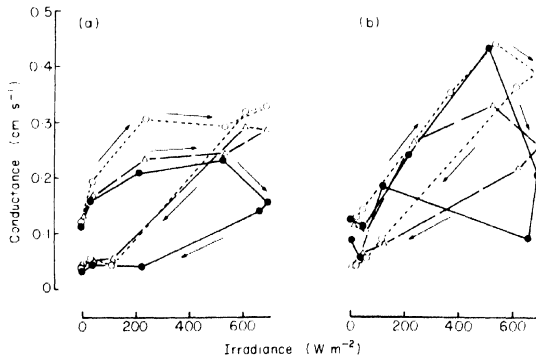


Figure 7. Relationships, on 7/3/80 (site 1) between abaxial (a) and adaxial (b) conductance and irradiance for upper expanded leaves of pearl millet BJ 104 grown at three positions in a water supply gradient. For explanation of symbols see Fig. 5b. Arrows indicate direction of change in time.

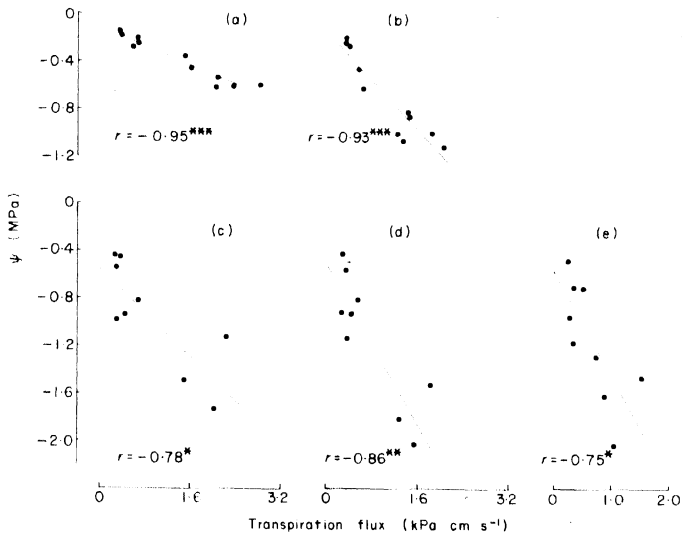


Figure 9. Relationships between leaf ψ and estimated transpiration flux ($lvpd \times g_1$) over the course of a day for pearl millet grown with varying soil moisture regimes. (a) and (b), irrigated and droughted plants respectively, site 1. (c), (d), (e) positions 1, 2 and 3 respectively, site 2. Points are means of six and represent different sampling times as shown in Figs 1b and 5b. Lines are fitted linear regressions. Correlation coefficients (r) are given; *, **, *** indicate significance at $P = 0.05, 0.01$ and 0.001 respectively. $1 \text{ kPa cm s}^{-1} = 7.16 \mu\text{g H}_2\text{O cm}^{-2} \text{ s}^{-1}$ at 30 C .

Table 1. Influence of irradiance (I) and leaf-to-air vapour pressure deficit ($lvpd$) on diurnal changes in conductance (g_1) of abaxial and adaxial leaf surfaces of pearl millet, cv. BJ 104. Data are regression coefficients, β , (where $y = \alpha + \beta x$) for the regression of g_1 on I and $lvpd$, either computed independently or in combination using multiple linear regression analysis. Levels of significance of β from zero are shown as: n.s., non-significant; *, **, ***, significant at $P = 0.05, 0.01$ and 0.001 respectively. The units of β are, for g_1 v. I , $\text{mm s}^{-1}/\text{W m}^{-2}$; for g_1 v. $lvpd$, $\text{cm s}^{-1} \text{ kPa}^{-1}$.

Site	Treatment†	Leaf surface	β , independent regression		β , multiple regression	
			g_1 v. I	g_1 v. $lvpd$	g_1 v. I	g_1 v. $lvpd$
1	Control	abaxial	0.201 **	0.011 ^{ns}	0.307 ***	-0.065 *
		adaxial	0.579 ***	0.117 ***‡	0.648 ***	-0.042 ^{ns}
	Stressed	abaxial	<0.001 ^{ns}	-0.048 **	0.131 *	-0.074 ***
		adaxial	0.443 ***	0.058 †‡	0.526 ***	-0.047 *
2	Position 1	abaxial	0.377 ***	-0.031 ^{ns}	0.424 ***	-0.056 ***
		adaxial	0.489 ***	-0.027 ^{ns}	0.539 ***	-0.059 ***
	Position 2	abaxial	0.317 ***	-0.019 ^{ns}	0.358 ***	-0.043 ***
		adaxial	0.306 ***	-0.029 ^{ns}	0.356 ***	-0.052 ***
	Position 3	abaxial	0.139 ***	-0.033 ***	0.214 ***	-0.053 ***
		adaxial	0.202 *	-0.029 ^{ns}	0.280 **	-0.055 **

† For further details see text.

‡ Note positive association.

maximum assimilation, while stress-induced stomatal closure in leaves less favourably situated for photosynthesis would aid water conservation without unduly sacrificing assimilation capacity.

Leaf water status

Drought resulted in large changes in bulk leaf ψ and ψ_p during the day. Negative ψ_p values as found at mid-day with the more stressed treatments (Fig. 5), probably arose due to ψ_s being overestimated following mixing of cell sap and apoplastic water during extraction of sap samples (Tyree, 1976). However, as indicated by severe wilting of the leaves, zero ψ_p was most probably attained at mid-day in these treatments, yet stomata were open (Fig. 6).

The ability of pearl millet stomata to remain open at low ψ and ψ_p suggests that turgor relations between guard and subsidiary cells which govern stomatal aperture were, to a large extent, independent of bulk leaf ψ or ψ_p . Generation of low ψ_s within guard cells would be required to sustain opening in the face of low bulk leaf ψ_p . It is suggested that the guard cells were capable of preferentially accumulating solutes, so maintaining turgor.

As the effects of diurnal changes in water status on g_1 were largely counteracted by the changes in I , the effects of water stress were mainly apparent as differences between treatments, either in the maximum g_1 attained (Figs 2 & 6), in the period over which high g_1 was maintained (Fig. 6), or in the slopes relating g_1 to I (Figs 3 & 7). The observed treatment differences in g_1 were not always explicable in terms of current leaf water status. Thus, at site 2, plants at positions 2 and 3 had similar mid-day ψ and ψ_p (Fig. 5b) yet stomata of the latter group closed to a greater extent as water stress increased (Fig. 6). Furthermore, there was little difference in the maximum total g_1 of moderately stressed plants at site 1 (minimum mid-day $\psi = -1.16$ MPa, $\psi_p = 0.1$ MPa) and that observed for more stressed plants at site 2, position 1 (minimum mid-day $\psi = -1.70$ MPa, $\psi_p = -0.12$ MPa). Stomatal response may, therefore, have been partly conditioned by stress history. Previous stress treatment usually results in a reduction in stomatal sensitivity to water stress. This effect has usually been attributed to osmotic adjustment (Brown *et al.*, 1976; Turner, Begg & Tonnet, 1978), which implies that g_1 is more influenced by bulk leaf ψ_p than by ψ . Osmotic adjustment is, however, small in pearl millet (Henson *et al.*, 1982). Also, examination of the present data shows that the relationship between g_1 and ψ_p differed between sites, and suggests that some adaptation had occurred at site 2 permitting a higher g_1 to be achieved at a lower bulk leaf ψ_p . This adaptation may have involved differences in solute accumulation at the level of the guard cells.

A further explanation for differences in stomatal behaviour, both between and within sites, is that a proportion of the more stressed plants were in the pre-flowering stage and their stomata may have been

more sensitive to stress than were those of plants in full flower (Ackerson & Kreig, 1977; Ackerson, Kreig & Sung, 1980; Morgan, 1977). This factor may also have accounted partly for the greater stomatal closure observed at all positions within the canopy for photo-periodically-retarded plants as compared with flowering plants of the same age and mean ψ (Fig. 8c) (but see note above).

In the present study either the two leaf surfaces responded similarly to water stress (Fig. 6), or the sensitivity of the abaxial (lower) surface exceeded the adaxial (Fig. 2). In general, when greater abaxial than adaxial closure was observed, leaf ψ_p was > -0.1 MPa and $\psi > -1.5$ MPa, while when both surfaces responded similarly to stress, ψ_p and ψ were below these levels. Thus, closure of adaxial stomata appeared to be initiated at lower potentials than closure of abaxial stomata. These results contrast with previous reports (e.g. Kanemasu & Tanner, 1969; Kassam, 1973; Sharpe, 1973; Brown *et al.*, 1976; Turner *et al.*, 1978) of greater adaxial sensitivity to stress in other species. The reasons for the variation in relative response to water stress by the two surfaces in the different experiments with pearl millet are not clear. However, the greater opening of adaxial than abaxial stomata under water stress may have been favoured by the upward folding of the leaf about the mid-rib, a characteristic of water-stressed millet. Leaf folding may have resulted in a more favourable micro-climate at the adaxial surface (e.g. lower lvpd), thus accounting for the lower stress sensitivity of adaxial, compared with abaxial, stomata. Further work is required to resolve both this, as well as other questions raised by the present study.

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