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Stomatal Response to Water Stress and its Relationship to Bulk Leaf Water Status and Osmotic Adjustment in Pearl Millet (*Pennisetum americanum* [L.] Leeke)[1]

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ABSTRACT

The water potential (Ψ) at which stomata completed closure ($\Psi_{\text{st min}}$) was determined for pearl millet (*Pennisetum americanum* [L.] Leeke) at two growth stages by monitoring changes in leaf conductance (g_L) and Ψ following shoot detachment. Leaf water status was evaluated concurrently using a pressure-volume (P-V) technique.

In a pot experiment with young vegetative plants, $\Psi_{\text{st min}}$ closely approximated to the estimated Ψ at zero turgor (Ψ_0) both for control and for drought-conditioned plants which had osmotically adjusted. However, for penultimate leaves of field-grown flowering plants, $\Psi_{\text{st min}}$ was found to be 0·61 (irrigated plants) and 0·87 (droughted plants) MPa below Ψ_0. In drought stressed field grown plants, osmotic adjustment (characterized by a decrease in solute (osmotic) potential (Ψ_s) at both full hydration and zero turgor) was insufficient to maintain a positive bulk leaf turgor potential (Ψ_p) once Ψ had declined to below about 1·5 MPa. It is suggested that localized adjustment by the stomatal complex in response to environmental differences, leaf ageing and/or ontogenetic change, is responsible for the uncoupling of stomatal from bulk leaf water status.

Key words: Stomata, Water stress, *Pennisetum americanum*.

INTRODUCTION

The accumulation of solutes by plants under water stress, resulting in a lowering of solute (osmotic) potential (Ψ_s), is one means whereby the concomitant reduction in cell turgor potential (Ψ_p) may be minimized (Turner and Jones, 1980). This osmotic adjustment is generally considered responsible for any maintenance of turgor-dependent processes during water stress (Hsiao, Acevedo, Fereres, and Henderson, 1976). The maintenance of stomatal opening under stress, a characteristic of drought conditioned plants which is usually manifested as a lowered 'threshold' water potential (Ψ) for closure, has generally been explained in terms of osmotic adjustment (Ludlow, 1980).

While there is evidence to suggest that in a wide range of species stomata close once bulk

leaf Ψ_p reaches zero (Turner, 1974), stomatal closure in some species has been observed to occur at a Ψ below that occurring at zero Ψ_p (Beadle, Turner, and Jarvis, 1978; Turner, Begg, and Tonnet, 1978; Jones and Rawson, 1979). Studies of the responses of pearl millet (*Pennisetum americanum* [L.] Leeke) to drought stress in the field (Henson, Alagarswamy, Bidinger, and Mahalakshmi, 1982a; Henson, Mahalakshmi, Bidinger, and Alagarswamy, 1982b) suggested that for upper leaves of flowering plants there was appreciable opening of stomata when bulk leaf Ψ was either at, or below that occurring at the point of zero turgor. All of these studies suffered, however, from Ψ_s being overestimated (i.e. made less negative) because it was measured using expressed sap. Hence, Ψ_p (calculated as $\Psi_p = \Psi - \Psi_s$) was underestimated (Turner, 1981).

The object of the present study was to re-examine the stomatal response of pearl millet to low Ψ and, in addition, to re-evaluate the extent of osmotic adjustment in field grown plants using the more rigorous pressure volume (P-V) technique (Tyree and Hammel, 1972; Cutler, Shahan, and Steponkus, 1979; Wilson, Fisher, Schulze, Dolby, and Ludlow, 1979). The responses of field plants are contrasted with those observed for young vegetative plants grown in a glasshouse.

MATERIALS AND METHODS

Pot experiment

Pearl millet cv. BJ 104 was grown in 9·0 cm diameter pots in John Innes No. 2 compost in a heated glasshouse at Cambridge, U.K. in May 1982. Plants received natural illumination (long photoperiods) and temperatures varied diurnally between 22 °C and 35 °C. Plants were watered using a wick system unless drought was being imposed.

One group of plants was droughted by withholding water from 13–18 d after sowing (DAS). These plants were rewatered in late afternoon (1800 h) to restore a high Ψ prior to measurements the following day. Changes in leaf Ψ and total (abaxial plus adaxial) conductance (g_L) during rapid development of stress, induced by severing the shoot at its base, were measured in both control and drought conditioned plants 16 and 19 DAS respectively. The procedure was as for field-grown plants (described below) except that g_L was measured using a continuous flow diffusion porometer similar in design to that of Day (1977). Sampling was confined to the sixth (youngest fully expanded) leaf. During sampling, irradiance was c. 650 μmol m^{-2} s^{-1} (400–700 nm) and air temperatures ranged from 26 °C to 30 °C. P-V measurements on leaf six were made using six plants of each treatment as described above, but using whole leaves. Droughted plants were rewatered and allowed to rehydrate overnight before measurement. All P-V measurements were performed within ± 2 d of determining relationships between g_L and Ψ.

Field studies

Pearl millet cv. BJ 104 was grown at ICRISAT Centre, near Hyderabad, India (17° 30'N) in the dry season (February–May) of 1982 on a deep (c. 1·0 m) alfisol soil as described previously (Henson et al., 1982a).

Seed was direct sown in plots each accommodating six 4·0 m long rows spaced 0·50 m apart. Plants were thinned to about 0·1 m apart within rows at an early stage of growth. Irrigation was applied by flooding furrows made between rows. Control plots were irrigated regularly to field capacity (at 4–7 d intervals), while drought stress was imposed on other plots by withholding irrigation from 19 DAS.

Climatic conditions were similar to those described previously (Henson et al., 1982a, b) with high maximum day temperatures (35–38 °C), high vapour pressure deficits (> 4·0 KPa) and generally high irradiances (1800 μmol m^{-2} s^{-1}) at mid day. To determine the progress of drought stress in the experiment, the Ψ of the penultimate leaf at mid-day (Ψ_{MD}) was measured at regular intervals using a pressure chamber. Leaves were protected from post-excision evaporative losses by enclosing them in a damp cloth.

Measurements of Ψ, g_L and bulk leaf water relations using a pressure volume (P-V) technique were confined to the penultimate leaf commencing at stem elongation just prior to the boot stage and continuing until flowering and seed set (31–51 DAS). For P-V measurements shoots were detached near their base, placed in water and transferred to a laboratory to rehydrate. Shoots were harvested either at dawn (c. 0600 h) or at noon and left for periods of from 1–6 h to rehydrate before measuring

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Measurements of Ψ, g_L and bulk [leaf water relations using a pressure volume (P-V) technique were] confined to the penultimate leaf [commencing at stem elongation just prior to the boot stage and] continuing until flowering and seed set (31–51 DAS). For P-V measurements shoots were detached near their base, placed in water and transferred to a lab[or]atory to rehydrate. Shoots were harvested either at dawn (c. 0600 h) or at noon and left for periods of from 1–6 h to rehydrate before measuring

(most measurements were, however, commenced within 3 h). Analysis of results for eight paired comparisons indicated no significant effects on Ψ_s at full hydration (Ψ_{s100}) or on Ψ at zero Ψ_p (Ψ_a) of either time of harvest or rehydration time

The P-V procedure used was similar to that described by Cutler *et al.* (1979), using small vials filled with absorbent filter paper to collect sap expelled from the cut leaf end protruding from a pressure chamber. However, because of difficulty in accommodating and sealing whole leaves in the narrow slit of the chamber top, leaves were cut in half longitudinally (excluding the mid rib) while still attached to the plant. The leaf portion to be used for the measurement was enclosed in a plastic sheath, cut off the plant, and the surfaces of the protruding basal portion and exposed vertical edge of the tissue were lightly coated with vacuum grease prior to insertion in the chamber to reduce evaporative losses. Paired measurements were then made of Ψ and expressed sap weight at successive stages of leaf dehydration. Readings were corrected to 100% sap recovery by reference to the total change in fresh weight of the sample from the beginning to the end of the measurement period. Such correction did not generally change the estimate of Ψ_a and had only a small effect (usually <0.05 MPa) on Ψ_{s100}; the two major parameters of interest.

The bulk volumetric modulus of elasticity (ξ) of leaves was calculated as $[\Delta\Psi_p/\Delta RWC] \times 100$ over the *RWC* (relative water content) range from full hydration to zero Ψ_p.

Leaf conductance (taken to indicate stomatal conductance) was measured using an automatic diffusive resistance porometer (Delta T Devices, Burwell, U.K.) as described previously (Henson *et al.*, 1982a). The g_L of each plant was determined initially (before detachment) to ensure that stomata were open. To determine the Ψ at which stomata closed, shoots were severed at the node below the penultimate leaf node and the inflorescence removed. They were held upright *in situ* and allowed to lose water rapidly. Using a population of such shoots, measurements were taken of g_L and Ψ (pressure chamber) for the penultimate leaf during the course of dehydration. Stomata were designated as 'closed' once the combined g_L for the two leaf surfaces had declined to below about 0.2 cm s^{-1} (c. 13% or less of maximum g_L; see Fig. 3). In one series of measurements shoots from droughted plants were placed in water to rehydrate prior to measurement to extend the range of Ψ to higher values. Six separate series of measurements, three with irrigated and three with droughted plants were made. Sampling took place 50–52 DAS following relief of drought by rain 47 DAS (Fig. 1), and was confined to between 1100 h and 1500 h when irradiance was high (~1800 μmol m^{-2} s^{-1}) and g_L was maximal. The penultimate leaf was approximately 20 d past full leaf expansion at this stage.

RESULTS

Stomatal response and leaf-water relations of young plants

The water potential at stomatal closure, $\Psi_{s,min}$, measured on the 6th leaf of young vegetative plants coincided closely to Ψ_a of the leaf (Table 1). Similar results (not presented) were obtained for plants of the same age grown in controlled environment cabinets. In drought conditioned plants the increased solute concentration (lower Ψ_s) resulting from osmotic adjustment caused a lowering of ψ_a by 0.2 MPa and a similar shift in $\Psi_{s,min}$. Thus in young plants stomatal closure was closely linked to loss of bulk leaf Ψ_p and $\Psi_{s,min}$ was influenced by bulk leaf osmotic adjustment

Leaf-water relations in the field

Withholding irrigation resulted in a substantial reduction in Ψ_{MD} at the commencement of sampling 31 DAS (Fig. 1). Ψ_{MD} of droughted plants further declined between 31 and 45 DAS but stress was relieved on day 47 by a 16 mm rain. No response to rain 43 DAS was detected, probably because the 7 mm received was less than one days evaporative demand (c. 10 mm), and Ψ_{MD} was only measured 2 d after the rain. Irrigated plants suffered stress 37 DAS due to high evaporative demand but otherwise maintained Ψ_{MD} relatively high (generally > −1.0 MPa). Further increase in Ψ_{MD} of irrigated plants was generally prevented by evaporative demand until 49 DAS, when such demand was temporarily reduced due to rain.

As estimated from P-V curves, Ψ_a changed little in irrigated plants (c. ±0.1 MPa), and, except for 37 DAS, positive turgor was maintained at all times. In droughted plants, apart

TABLE 1. *The relationship between leaf water status and stomatal behaviour for watered and droughted plants of pearl millet cv. BJ 104*

All data are in MPa.

Treatment	Ψ_{s100}[a]	Ψ_{s100}[b]	Ψ_a[c]	$\Psi_{s,min}$[d]	$\Psi_a - \Psi_{s,min}$
(a) Vegetative plants, pot experiment, leaf 6, c. 16–19 DAS[e]					
Watered	0.17	0.83	1.02	0.95	+0.07
Droughted	1.61	1.01	1.22	1.25	+0.03
Significance of difference (*P*)	<0.001	<0.01	<0.05		
(b) Flowering plants, field experiment, penultimate leaf, 50–52 DAS[e]					
Watered	0.87	1.15	1.39	2.00	+0.61
Droughted	1.93	1.30	1.60	2.47	+0.87
Significance of difference (*P*)	0.001	<0.01	<0.01	<0.05	

[a] The minimum mid day Ψ measured during the experiment. For (a) $n = 6$; (b) $n = 3$.
[b] Ψ_s at full hydration and Ψ (= Ψ_a) at zero Ψ_p respectively. Data obtained from P-V measurements.
[c] $n = 6$.
[d] Ψ at which stomata closed ($g_L = 0.2$ cm s^{-1}). Estimated using populations of detached shoots; one per treatment for (a); three per treatment for (b) for which data are means.
[e] Days after sowing.

from the first sampling 31 DAS and those at 49–51 DAS, Ψ_{MD} was always below Ψ_a. Thus, turgor was zero at mid day in these plants. Two further features are evident from Fig. 1. Firstly, the decline in Ψ_{MD} was generally paralleled by the decline in Ψ_a. Secondly, there was no dramatic increase in Ψ_a of droughted plants as a result of rehydration following rain.

The relationships between Ψ_{s100}, Ψ_a and Ψ_{MD} are shown in Fig. 2 in which data for irrigated and for droughted plants prior to rehydration only, are plotted. Both Ψ_{s100} and Ψ_a declined linearly with Ψ_{MD} over the range −1.5 to −1.93 MPa. Above a Ψ_{MD} of −1.5 MPa there was no significant change in either Ψ_{s100} or Ψ_a. As noted above, the decrease in Ψ_a with Ψ_{MD} (0.86 MPa MPa^{-1}), was insufficient to compensate for the decline in Ψ_{MD}.

The above decrease in Ψ_a did not arise due to changes in cell wall elasticity (Turner and Jones, 1980) and therefore was probably fully accounted for by solute accumulation. No significant difference in the bulk volumetric elastic modulus (ξ) was detectable between 'adjusted' and 'non adjusted' plants; i.e. those with $\Psi_{MD} <$ and $> −1.5$ MPa respectively (mean $\xi = 9.00$ and 9.08 MPa for the two groups). The corresponding difference in Ψ_{s100} was significant at $P < 0.01$.

Stomatal response in the field

Stomatal opening at low leaf Ψ was observed as on previous occasions (Henson *et al.*, 1982a). Plants in droughted plots with Ψ at or below zero Ψ_p 51 and 52 DAS (Fig. 1), still exhibited appreciable conductances when sampled mid-day to early afternoon ($g_L = 0.97 \pm 0.09$ cm s^{-1} 51 DAS, and 1.02 ± 0.05 cm s^{-1} 52 DAS compared with 1.59 ± 0.08 cm s^{-1} and 1.60 ± 0.10 cm s^{-1} for the control plots 51 and 52 DAS respectively).

To determine the bulk leaf Ψ at stomatal closure ($\Psi_{s,min}$) shoots were detached and allowed to dehydrate rapidly. After an initial transient opening phase, stomata responded to declining Ψ by closing abruptly at a critical 'threshold' Ψ (Fig. 3). From the results of six separate series it was clear that for both irrigated and drought-conditioned plants $\Psi_{s,min}$ was
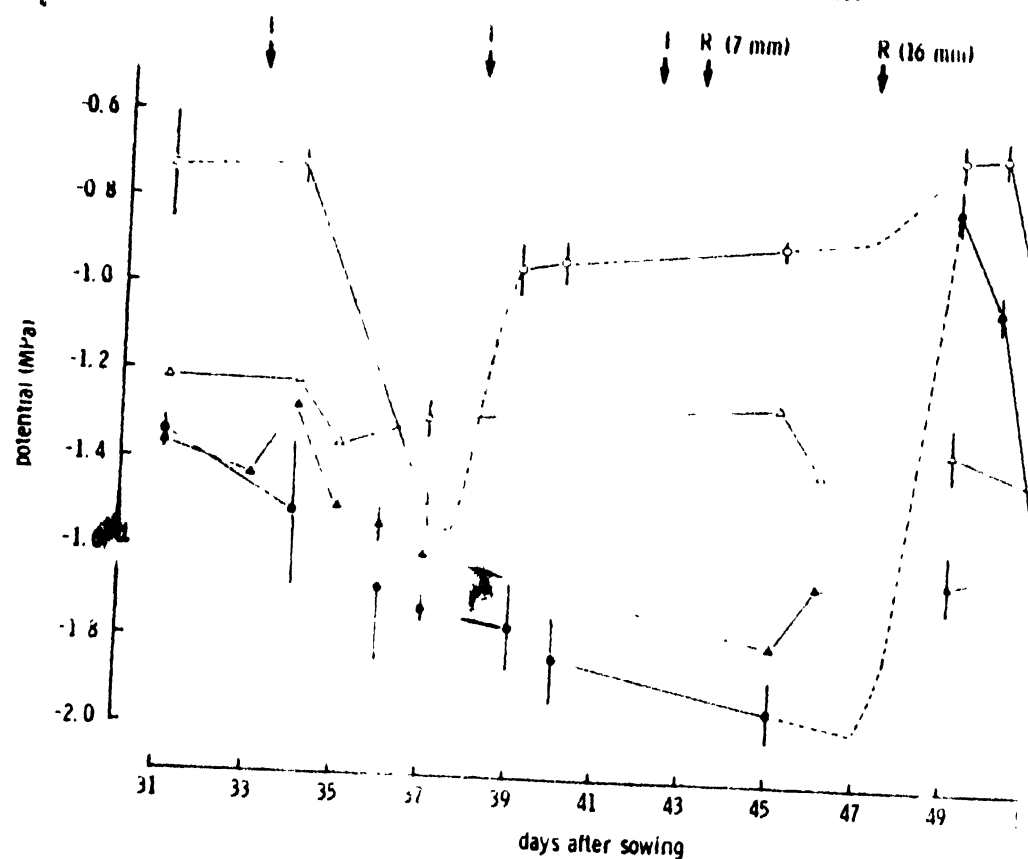
FIG. 1. Seasonal changes in Ψ_{MD} measured with a pressure chamber (O, ●) and in Ψ_π derived from P-V measurements after shoot rehydration (△, ▲), for irrigated (O, △) and droughted (●, ▲) plants grown in the field. Arrows indicate times of irrigation (I) or rainfall (R). Vertical bars on points indicate 2 × s.e. mean, where n = 2 to 6. Points without bars represent single plants.

FIG. 2. Relationships between (A) $\Psi_{s,\,w}$ and Ψ_{MD} and (B) Ψ_π and Ψ_{MD} for irrigated (O) and droughted (●) plants grown in the field. Lines are fitted linear regressions: (A) for a, r 0·87 ($P < 0·001$), y 0·032 + 0·794x; for b, r 0·28 (not significant), y −1·1 + 0·044x; (B) for a, r 0·797 ($P < 0·01$), y 0·078 + 0·864x; for b, r 0·26 (not significant), y 1·27 + 0·061x.

substantially below Ψ_π as estimated by P-V measurements (Table 1; Fig. 3) on plants sampled at the same time. $\Psi_{s,\,min}$ was lowest and differed most from Ψ_π in droughted plants.

DISCUSSION

The use of the P-V technique confirmed the ability of pearl millet leaves to adjust osmotically in response to drought stress in the field (Figs 1, 2). However, as previously concluded (Henson *et al.*, 1982b), adjustment was insufficient to prevent loss of turgor in droughted plants. Bulk leaf turgor reached zero once Ψ_{MD} declined to about 1·5 MPa, which is close to the value of −1·44 MPa for BJ 104 reported previously (Henson *et al.*, 1982b). The data (Fig. 2) suggest that significant adjustment did not occur *until* Ψ_{MD} had reached −1·5 MPa. However, this impression may be illusory. Although there was no evidence that irrigated plants adjusted to any extent over the Ψ_{MD} range of −0·66 to 1·5 MPa, this may be explained by the fact that the range of Ψ_{MD} was largely a result of transient changes involving both increases and decreases in Ψ, and was not due simply to a gradual decline in Ψ. Unless Ψ declines gradually a positive relationship between Ψ_{MD} and adjustment cannot be expected. However, the relatively low $\Psi_{s,\,min}$ (and Ψ_π) of irrigated, flowering plants compared with that of young pot-grown plants (Table 1) is suggestive of *some* prior adjustment, though it is unclear as to whether environmental or purely developmental factors are responsible for this. On no occasion, even with the high Ψ attained after rewatering, was it possible to obtain
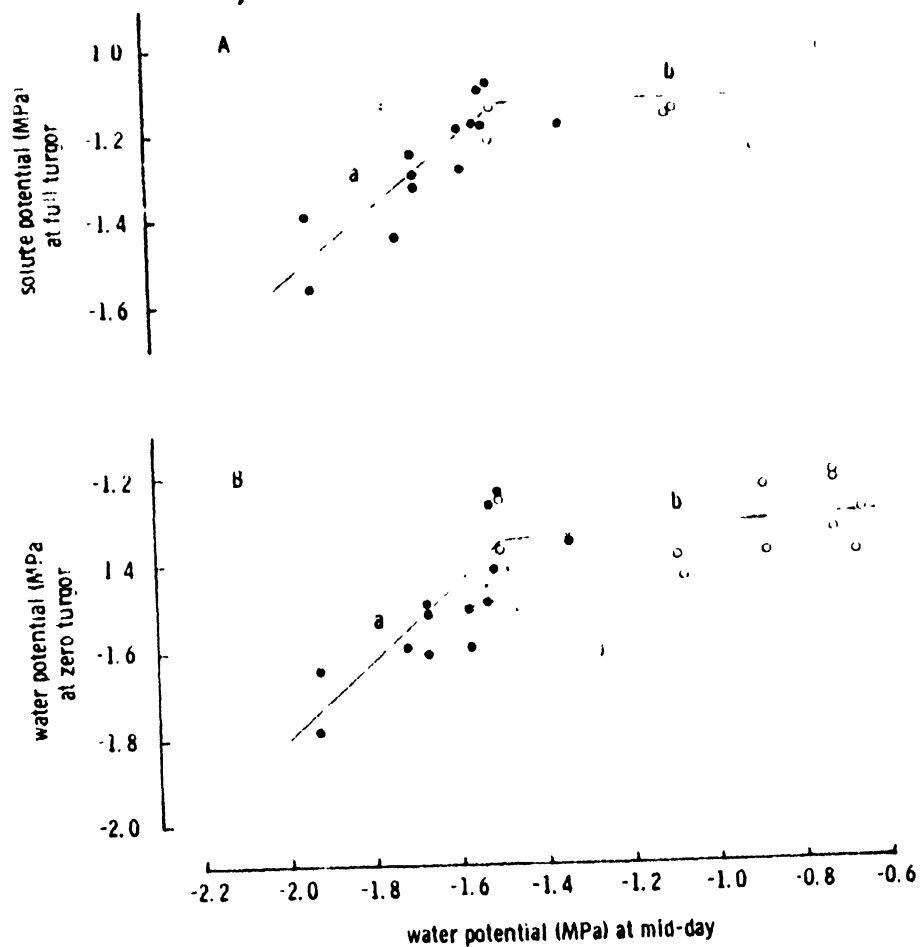
values of $\Psi_{s,\,min}$ for flowering plants higher than −1·02 MPa, whereas values for emerged leaves of young watered pot-grown plants were generally about −0·8 MPa (Table 1; Henson, 1982).

For droughted, flowering plants there was no substantial, rapid loss of adjustment following rewatering (Fig. 1). This also contrasts with young plants in which adjustment is largely lost within 24 h or 48 h of rewatering (Henson 1982). In the latter study, adjustment persisted longest in emerging leaves and it was suggested that developmental effects on adjustment (via reduced cell size) were responsible. The slow development of stress under field conditions should favour structural adaptations favourable to adjustment (Cutler, Rains, and Loomis, 1977) and hence, its persistence.

The results reported here confirm the earlier conclusion (Henson *et al.*, 1982a) that flowering plants of pearl millet grown under the conditions of high evaporative demand in the semi arid tropics are capable of sustaining stomatal opening even with bulk leaf Ψ at or below zero turgor. This contrasts with the behaviour of young vegetative plants grown in a protected environment (Table 1). In these plants stomatal closure was closely associated with the loss of bulk leaf turgor, whereas in flowering plants in the field closure occurred at water potentials ($\Psi_{s,\,min}$) considerably below zero Ψ_p.
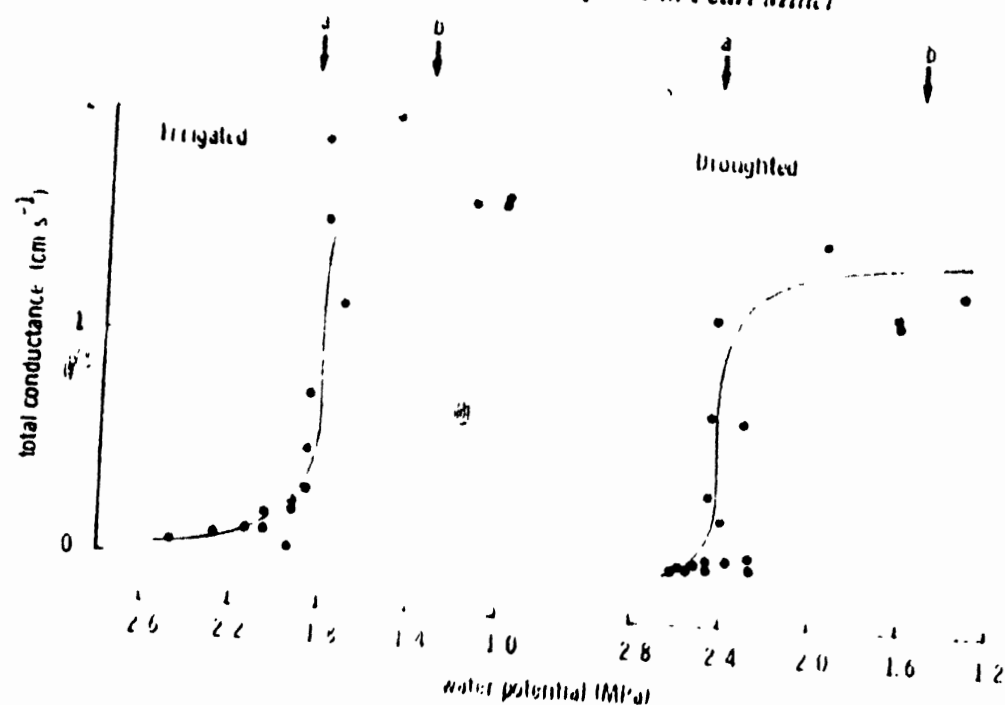
FIG. 3. Examples of the relation between total conductance g_L and bulk leaf Ψ during rapid drying induced by shoot excision of plants grown with or without irrigation in the field. Measurements were made at flowering 51 DAS when air temperature was 35–36 °C, vapour pressure deficit 4.2–4.6 kPa and irradiance (400–700 nm) ~1800 μmol m^{-2} s^{-1}. Curves were fitted by eye. Arrows indicate approximate Ψ at stomatal closure (a) and at zero Ψ_p (b), the latter estimated by P-V measurement.

It is possible that the method of estimating $\Psi_{g, min}$ by following rapid changes in g_L and Ψ gave rise to underestimates of $\Psi_{g, min}$ due to a time lag in the response of the guard cells to the fall in bulk leaf Ψ. Such a lag could arise if there were a large enough resistance to water flow between epidermal and mesophyll tissues. Such a resistance has been implicated in the direct response of stomata to changes in atmospheric vapour pressure deficit (Sheriff, 1979). Pearl millet stomata do show a humidity response (Black and Squire, 1979; Squire, 1979; Henson, unpublished results), although this is reported to be absent in water-stressed plants (Squire, 1979). If a resistance to water movement were influencing $\Psi_{g, min}$ then the extent of this resistance must change with age and/or environment and be increased by water stress to account for the differences between watered and droughted field plants observed in $\Psi_{g, min}$ and its relationship to Ψ_{a}. That the occurrence of $\Psi_{g, min} < \Psi_{a}$ was not entirely an artefact of the method of determining $\Psi_{g, min}$ using detached shoots is furthermore shown by the observations, both in the present and previous (Henson *et al.*, 1982a) studies, of high conductances by *intact* plants with Ψ at or below zero Ψ_p.

Stomatal closure at Ψ below zero Ψ_p has been observed previously in other species (Beadle *et al.*, 1978; Turner *et al.*, 1978; Jones and Rawson, 1979). In these reports, however, Ψ_p was obtained from the difference between Ψ and Ψ_s measured with a pressure chamber and dewpoint hygrometer respectively, and it was considered (Beadle *et al.*, 1978; Turner *et al.*, 1978) that measurement errors accounted for stomatal closure associated with Ψ below zero turgor. The P-V technique used here is considered to be a more rigorous method of evaluating Ψ_{a} and the large difference between Ψ_{a} and $\Psi_{g, min}$ found in field-grown plants constitutes strong evidence for the 'uncoupling' of stomatal behaviour from bulk leaf water status. The extent of this 'uncoupling' is seen to be variable depending on plant or leaf age and/or environmental conditions.

The data indicate that while shifts in $\Psi_{g, min}$ were not wholly accounted for by bulk leaf osmotic adjustment, such adjustment probably contributed to the differences observed in $\Psi_{g, min}$ between plants in the two studies and also between irrigated and droughted plants in the field. Begg and Turner (1976) in their review pointed out the differential responses of plants grown in the field and in controlled environments. Jordan and Ritchie (1971) found that stomata of leaves on cotton plants grown in the field remained open even when leaf water potentials approached −3.0 MPa, whereas stomatal closure occurred at −1.6 MPa in phytotron grown plants. Altered stomatal response has also been observed in relation to ontogenetic changes. Fully irrigated apple trees bearing fruits had greater stomatal opening than non fruiting trees (Hansen, 1971). Ackerson, Krieg, and Sung (1980) found the response of sorghum stomata to be independent of bulk leaf water potential in flowering, but not in vegetative, plants. Leaf age effects may, however, have been confounded with ontogenetic changes in that study. Syvertsen (1982) observed in citrus higher $\Psi_{g, min}$ for recently expanded leaves than for mature leaves. Such observations suggest that environmental differences, leaf age, and developmental changes may all be involved in changes in the relationship between g_L and Ψ. Further research to evaluate the relative influence of these three factors in millet is in progress.

The independence of stomatal behaviour from bulk leaf water relations rules out the use of $\Psi_{g, min}$ as proposed by Turner *et al.* (1978) to measure the degree of osmotic adjustment in field grown plants of pearl millet. The mechanisms whereby stomatal opening is sustained despite zero bulk leaf Ψ_p have still to be resolved though it seems possible that localized osmotic adjustment by the guard cells may be involved. That the difference between $\Psi_{g, min}$ and Ψ_{a} in pearl millet at flowering (Table 1) was most pronounced in droughted plants where bulk leaf adjustment was most favoured) is consistent with this idea. Brown, Jordan, and Thomas (1976) observed differential osmotic adjustment by abaxial and adaxial guard cells of cotton which correlated with stomatal response to water stress, though this adjustment alone was insufficient to account wholly for the difference in stomatal response to stress. Other mechanisms of stomatal adjustment have been considered briefly by Ludlow (1980), though there is little relevant experimental data concerning these. There is thus a need for investigations to elucidate the processes responsible for stomatal adaptation and hence changes in its relation to bulk leaf water status.

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Inheritance and linkage relationships of a new lobed vexillum mutant in chickpea

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ABSTRACT Inheritance of a new three-lobed vexillum mutant isolated from a chickpea cultivar ICC-7545 is reported A single recessive gene controlled this trait Joint segregation studies revealed linkage with two other marker genes controlling broad leaflets, and double-flowered peduncle The symbol lvx is proposed for lobed vexillum character

WHILE studying the range of morphological variability of the world collection of chickpea (Cicer arietinum L) germplasm, maintained by the Genetic Resources Unit of ICRISAT, during the post-rainy season of 1977 78, a plant with an unusual vexillum (standard petal of the flower) shape was isolated from a broad leaved Ethiopian cultivar, ICC-7545, and found to breed true in subsequent years In contrast to the entire obovate vexillum generally found in chickpeas[9], the new mutant has a three-lobed vexillum (Figure 1) The inheritance of this character and its linkage relationships with two other marker genes of chickpea were studied and its usefulness as a marker is discussed

Materials and Methods

Crosses were made between the lobed vexillum mutant (which also has broad leaflets) and a double-flowered desi (local Indian) cultivar,

FIGURE 1 Flowers of the lobed vexillum mutant (left) and common chickpea (right)

JG-62, with two flowers borne on each peduncle and normal elliptic leaflets, during the 1978 79 season Eight F[1] plants were raised during 1979-80 The F[2] population was grown during the 1980 81 post-rainy season and the observed segregation ratios for the lobed vexillum, broad leaflets, and double flowered peduncle were tested for goodness-of fit to the expected ratios by chi-square[7]

Results and Discussion

All F[1] plants had normal vexillae elliptic leaflets, and single-flowered peduncles, indicating that these traits are dominant A segregation pattern of 3 1 in the F[2] indicated that the lobed vexillum is governed by a single recessive gene Broad leaflets and double-flowered peduncles

Table I Segregation for vexillum, leaflet shape, and number of flowers per peduncle, in a cross between JG-62 and a mutant line of ICC-7545 chickpeas

| Trait | No observed | | x^2 adjusted (3 1) | P |
	normal	mutant*		
Vexillum	365	102	2 569	0 2 0 1
Leaflets	342	125	0 732	0 5 0 3
Flowers	365	102	2 569	0 2 0 1

* Three lobed vexillum broad leaflets two flowers per peduncle

also were found to be monogenic recessives (Table I)

The monogenic recessive inheritance of the double-flowered peduncle obtained here confirms earlier findings[2,4,6] Our studies indicate that broad leaves also are controlled by a single recessive gene similar to several other leaf shapes narrow, obovate, bipinnate and simple studied earlier by various workers[1,5,8]

Joint segregation studies revealed close linkage between lobed vexillum and broad leaflets The double-flowered peduncle, however, appears to be loosely linked to lobed vexillum (Table II)

The occurrence of a lobed vexillum in Cicer arietinum is reported here for the first time The new recessive mutation has no pleiotropic effects and all other characters of the plant essentially remained unaltered The gene symbol lvx is proposed for lobed vexillum

The new mutant is of value to plant breeders as a genetic marker in isolating accidental selfs during hybridization, and in identifying and evaluating marker linked genes affecting specific quantitative traits As a marker it also may be of value in surveying genetic variation in populations and interpopulation variability

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Table II Joint segregation of characters in a cross between JG-62 and a lobed vexillum mutant of ICC-7545 chickpeas

Trait	F[2] phenotypic classes				x^2 adjusted	P
Lobed vexillum (3 1)	normal vexillum normal leaflets	normal vexillum broad leaflets	lobed vexillum normal leaflets	lobed vexillum broad leaflets	154 756	<0 001
vs Broad leaflets (3 1)	318	47	24	78		
Lobed vexillum (3 1)	normal vexillum single flower	normal vexillum double flower	lobed vexillum single flower	lobed vexillum double flower		0 02 0 01
vs Double-flowered (3 1) peduncle	295	70	70	32	6 282	