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An Association between Flowering and Reduced Stomatal Sensitivity to Water Stress in Pearl Millet [*Pennisetum americanum* (L.) Leeke]*

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ABSTRACT

Stomatal sensitivity to water stress was investigated in pearl millet [*Pennisetum americanum* (L.) Leeke] in relation to stage of plant development, leaf water status and ABA content by sampling plants at midday. For the same leaf water potential (Ψ), droughted plants with emerged panicles were found to have a greater leaf conductance (g_L), indicative of greater stomatal opening, than plants sampled prior to panicle emergence. The difference between such flowering (F) and non-flowering (NF) plants in Ψ at stomatal closure was estimated to be at least 0·6 MPa. This difference was considered unlikely to be the result of differential bulk leaf osmotic adjustment, and for most samples from both F and NF plants, bulk leaf turgor potential (Ψ_p) was estimated to be zero.

Stomatal closure in NF plants was associated in two genotypes (BJ 104 and line 112) with higher leaf ABA levels. Differences in ABA levels between F and NF plants were, however, smaller or absent in genotypes Serere 39 and B282. These genotypes were at lower Ψ than BJ 104 and line 112 when sampled and showed smaller differences between F and NF plants in conductance.

Lower ABA levels in F plants are ascribed either to effects of leaf ageing or to effects of flowering on ABA content of the leaf. Significant differences in g_L in the absence of differences in ABA content are taken to imply changes in stomatal sensitivity to the hormone or in its access to the stomatal complex.

Key words: *Pennisetum americanum* (L.) Leeke, pearl millet, flowering, stomata, water stress, abscisic acid.

INTRODUCTION

The sensitivity of stomata to leaf water deficits is known to be influenced by both endogenous and environmental factors (Turner, 1974; Begg and Turner, 1976). Stomata of many species show adaptation to water stress. The range of leaf water potential (Ψ) over which stomatal opening is sustained is extended in drought-adapted plants. This stomatal adjustment is often most marked in species which have a high tolerance to dehydration (Ludlow, 1980).

Stomatal sensitivity to water stress may change during plant development. In cereals, stomata become less sensitive to water stress as the plant ages (Frank, Power and Willis, 1973), and sensitivity is lower during reproductive than during vegetative growth (Ackerson and Krieg, 1977; Morgan, 1977; Ackerson, Krieg and Sung, 1980; Teare, Sionit and Kramer, 1982).

Stomatal opening during flowering and grain fill was observed in crops of pearl millet [*Pennisetum americanum* (L.) Leeke] despite severe water stress (Henson *et al.*, 1982*a*).

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In contrast, vegetative millet plants grown in pots (Henson *et al.*, 1981), or in the field (Mahalakshmi and Alagarswamy, unpublished results), closed their stomata rapidly in response to water stress. In addition, stomata on plants in which flowering had been retarded by extending the photoperiod were more sensitive to low Ψ than stomata on flowering plants (Henson *et al.*, 1982*a*). We recently demonstrated (Henson *et al.*, 1983) that stomata of field-grown flowering plants remained open at Ψ considerably below zero turgor potential (Ψ_p), again in contrast to young pot-grown vegetative plants in which stomatal closure was closely linked with loss of bulk leaf Ψ_p.

While these observations strongly suggested a developmental change in stomatal response to water stress, environmental influences could not be discounted. By exploiting normal plant-to-plant variation in developmental rate within open pollinated genotypes direct comparisons can be made between flowering (F) and non-flowering (NF) plants growing together in the same plots and hence subjected to the same environmental conditions and stress history. The results of such an approach are reported here. As there is strong evidence that ABA regulates stomatal behaviour in water-stressed plants (Raschke, 1975; Davies *et al.*, 1981), the possibility that flowering may influence stomatal conductance via an effect on leaf ABA content was also investigated.

MATERIALS AND METHODS

Pearl millet [*Pennisetum americanum* (L.) Leeke] was grown at two field sites at ICRISAT Centre, near Hyderabad, India, during the dry season of February to May in 1980 and 1982. The two sites had the same soil type (alfisol) but different soil depths; site 1 was shallow ($\simeq$ 0·5 m) compared with site 2 ($\simeq$ 1·0 m). Plants were grown from seed in rows 0·5 or 0·75 m apart, and thinned to about 0·1 m apart within rows. Plants were drought-stressed by withholding irrigation (normally given weekly as a surface flooding between rows) following stand establishment.

Four genotypes were used in this work. In 1980, BJ 104 (an F_1 hybrid) was grown at site 2 only. The treatment and sampling of these plants, some of which were given a longer daylength to delay flowering, has been previously described (Henson *et al.*, 1982*a*). Two other genotypes, B282 and Serere 39, were sampled on 12 May 1982 from sites 1 and 2, 68 and 70 days after sowing (DAS) respectively. Line 112, an early flowering F_4 progeny derived from a B282 × Serere 39 cross was sampled on 26 April 1982 from site 1 (52 DAS) from three plots with slightly differing stress levels. Flowering (F, half bloom to early grain fill) and non-flowering (NF, flag leaf emerging) plants were sampled within each plot.

Measurements of leaf conductance (g_L), and Ψ were made on the youngest fully expanded leaf of NF and on the penultimate leaf (i.e. the first leaf below the flag leaf) of F plants as earlier described (Henson *et al.*, 1982*a*). An automatic diffusive resistance porometer was used to measure g_L of both leaf surfaces. Leaf Ψ was determined using a pressure chamber with evaporative losses being minimized by enclosing the leaf in a moist cloth before detachment. All sampling was conducted in the mid-part of the day when irradiance was saturating (> 1800 μmol m^{-2} s^{-1}) and air temperatures were approx. 36–39 °C.

After g_L and Ψ measurements, leaves were frozen using solid CO_2 (1980) or liquid nitrogen (1982), subsequently lyophilized and assayed for ABA using the procedure of Quarrie (1978).

Relationships between Ψ_p and Ψ were evaluated for B282 and Serere 39 in 1982 by a pressure–volume (P–V) method (Henson *et al.*, 1983). Measurements were made mainly on the penultimate leaf or, if this was not fully emerged, on the youngest fully-expanded leaf on the main stem. The plants were sampled between jointing and boot stages (31 to 46 (DAS) from plots at site 2 adjacent to those sampled for g_L, Ψ and ABA.

RESULTS AND DISCUSSION

All plants sampled in 1982 were severely water-stressed, with leaf $\Psi < -1{\cdot}95$ MPa (Fig. 1). While differences in Ψ between NF and F plants within plots were found to be non-significant there were generally much higher conductances in F than in NF plants. Over the Ψ range observed ($-1{\cdot}95$ to $-2{\cdot}60$ MPa), conductances of NF plants were low throughout and stomata could be regarded as closed. In contrast, stomata of flowering plants ranged from being fully open to closed. From Fig. 1 the Ψ at which stomata of the two groups closed was estimated to differ by at least 0·6 MPa.

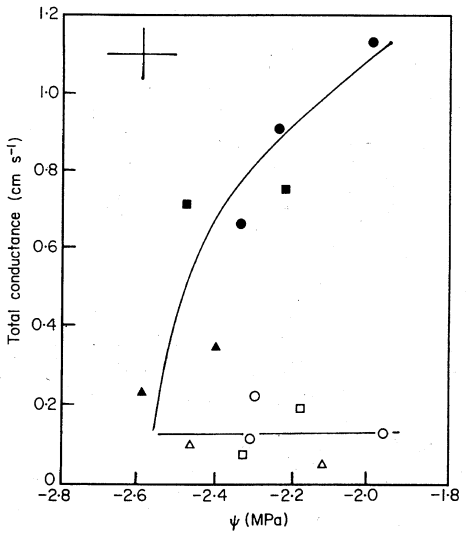

FIG. 1. Relationships between total conductance (abaxial+adaxial) and Ψ in non-flowering (open symbols) and flowering (closed symbols) plants of pearl millet genotypes B282 (□, ■), Serere 39 (△, ▲) and 112 (B282 × Serere 39) (○, ●). Points are means of 6; bars indicate 2 × pooled s.e. mean. Lines were fitted by eye.

A *genotypic* difference was found in the response of stomata of flowering plants to Ψ. Genotypes B282 and Serere 39 had the same Ψ yet the latter had a significantly ($P < 0{\cdot}001$) lower g_L. This was associated with a significantly greater leaf ABA content (see below).

Although Ψ_p was not evaluated in plants sampled at flowering, Ψ of these plants was probably below the point of zero Ψ_p. P–V measurements prior to flowering (Fig. 2) indicated that while osmotic adjustment in response to water stress resulted in a lowering of solute potential (Ψ_s) and hence Ψ at zero Ψ_p, this was insufficient to maintain positive turgor once Ψ had fallen below about $-1{\cdot}44$ MPa. Linear regression of Ψ at zero Ψ_p on Ψ at midday was significant ($P < 0{\cdot}001$) and did not differ between genotypes. For positive Ψ_p to be restored at $\Psi < -2{\cdot}0$ MPa a large increase in the extent of osmotic adjustment (i.e a decrease in Ψ_s additional to that indicated by the regression line in Fig. 2 of, e.g. $> 0{\cdot}35$ MPa at $\Psi = -2{\cdot}0$ MPa, and $> 0{\cdot}6$ MPA at $\Psi = -2{\cdot}4$ MPa) would be necessary. P–V measurements of both vegetative and flowering plants of genotype BJ 104, reported elsewhere (Henson *et al.*, 1983), revealed no such ability. It is probable, therefore, that bulk leaf Ψ_p was zero when comparisons between F and NF plants were made. We have previously shown that stomata of flowering plants remain open when leaf turgor is lost (Henson *et al.*, 1983). It would appear that this ability is absent or at least poorly developed prior to flowering.

Stomatal conductance of F and NF plants was measured only at midday in these experiments. It is probable that some stomatal opening of NF, as well as of F plants, occurred at some time during the day. In a diurnal study with BJ 104 (Henson *et al.*, 1982*a*) even the most severely stressed plants sampled showed some stomatal opening in mid-morning. The detailed diurnal changes in g_L of F and NF plants remain to be compared.

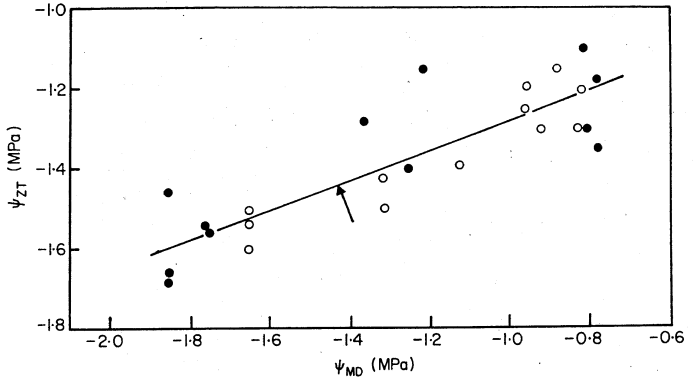

FIG. 2. Relationship between Ψ at zero turgor (Ψ_{zt}) derived from pressure–volume measurements, and Ψ at midday (Ψ_{MD}) for the youngest fully expanded or penultimate leaf of plants of B282 (○) and Serere 39 (●) sampled 31 to 46 days after sowing during the development of water stress. The line is that of best fit for the regression of Ψ_{zt} on Ψ_{MD} where $y = -0{\cdot}91 + 0{\cdot}37x$, and $r = 0{\cdot}85$ significant at $P < 0{\cdot}001$. The arrow indicates where $\Psi_{zt} = \Psi_{MD}$.

While differences in Ψ and Ψ_p failed to account for the difference in total conductance between flowering and non-flowering plants at midday, for line 112 ABA levels were significantly ($P < 0{\cdot}001$) lower in F than in NF plants and, as shown in Fig. 3A, variation in g_L was reflected in variation in ABA content. Water potential was similar in both F and NF plants so the different ABA contents were not apparently due to different levels of water stress (Fig. 4).

In contrast to line 112, ABA content was not significantly influenced by flowering in the parental genotypes. Conductance was weakly related to bulk leaf ABA content in B282 but not in Serere 39 (Fig. 3B, C). Analysis of variance revealed a highly significant effect of genotype ($P < 0{\cdot}001$) on ABA content (mean ABA content in ng g^{-1} d.wt was 517 in Serere 39 and 369 ng in B282) consistent with earlier results (Henson *et al.*, 1981) and with the genotypic difference in g_L (Fig. 1), yet there was no significant effect on ABA attributable to stage of development. Data in Fig. 3C show stomata were generally more closed in NF than in F plants at all ABA contents in Serere 39.

One possible explanation for the contrasting behaviour of F and NF plants of line 112 is that genetic segregation for flowering date and other characters may have continued in this F_4 progeny. Thus, these F and NF plants may have been genetically different. Genetic segregation is, however, unlikely to account for corresponding differences in g_L between F and NF plants in the highly inbred cultivar B282 (Fig. 3B).

Furthermore, differences between ABA contents of F and NF plants of line 112 were not unique. Similar differences were found for the F_1 hybrid BJ 104 between flowering plants and plants whose flowering was delayed by extending the photoperiod [NF(P)] (Fig. 5). As previously reported (Henson *et al.*, 1982*a*), stomata of water-stressed F plants remained open much more than stomata of NF(P) plants at a similar Ψ. The difference between the two groups was greatest for upper leaves and decreased with leaves lower

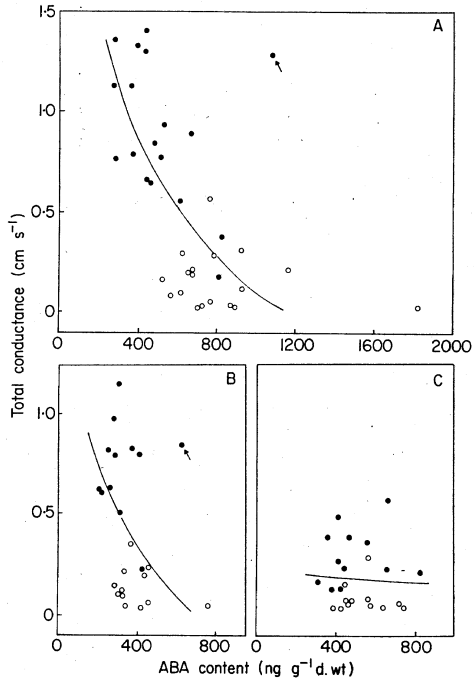

FIG. 3. Relationships between total leaf conductance and leaf ABA content for flowering (●) and non-flowering (○) plants of A, line 112, B, B282 and C, Serere 39. Curves were fitted from linear regressions of semi-logarithmic plots, omitting the outlying data points indicated by arrows. A, d.f. = 33; $r = -0{\cdot}75^{**}$; B, d.f. = 21, $r = -0{\cdot}49^{*}$; C, $r = -0{\cdot}04^{ns}$.

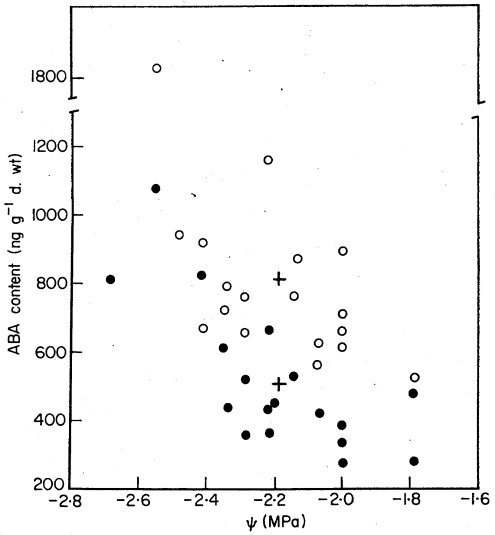

FIG. 4. The relationship between leaf ABA content and Ψ for flowering (●) and non-flowering (○) plants of line 112. Means of data for non-flowering and flowering plants are indicated by upper and lower crosses respectively.

down the canopy. Stomatal opening in upper leaves of droughted flowering plants was equal to that observed in other flowering plants which were irrigated. The difference in g_L between F and NF(P) plants of BJ 104 was, like line 112, related to leaf ABA content (Fig. 5). There was significantly ($P < 0{\cdot}001$) more ABA in leaves of NF(P) plants than in F plants. This difference was greatest for upper leaves, where there was a significant ($P < 0{\cdot}001$) negative correlation between g_L and ABA content (Fig. 5A). Differences in ABA between F and NF(P) plants were less in lower leaves which also showed less difference in g_L (Fig. 5B). Reduced g_L of lower leaves at a given ABA content may be due to lower incident irradiance and/or leaf ageing.

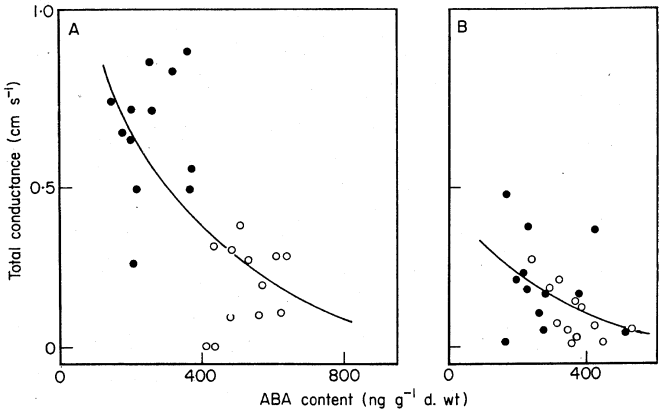

FIG. 5. The relationship between total leaf conductance and leaf ABA content for flowering (●) and non-flowering (○) plants of BJ 104. Non-flowering plants were grown with extended daylength. A, upper leaves (top two fully-expanded leaves on main stem; flag leaf and penultimate leaf of flowering plants); $r = -0{\cdot}66^{***}$. B, lower leaves (3rd and 5th leaf below topmost leaf sampled); $r = -0.43^{*}$. Curves were fitted from linear regressions of semi-logarithmic plots.

The difference in ABA and hence g_L found between F and NF(P) plants could have been linked to differences in Ψ_p as discussed previously (Henson *et al.*, 1982*a*), as leaf Ψ, though similar in both groups, was close to the estimated zero Ψ_p point ($\simeq -1{\cdot}5$ MPa). Small differences in Ψ_p may have been present and were suggested by osmotic measurements on expressed sap (Henson *et al.*, 1982*b*). It is also possible that ABA content was directly influenced by daylength (Zeevaart, 1974). However, lower ABA contents were observed at flowering whether non-flowering was due to stress-related variation in developmental rate, or extended daylength. This suggests a causal association between developmental stage and the changes in ABA and stomatal response.

We do not know whether the changes in ABA were related to flowering *per se*, or were a consequence of leaf position or age. Accumulation of ABA in leaves in response to water stress is reported to be reduced at flowering in soybean (Samet, Cortes and Sinclair, pers. comm.). Similarly, the capacity of millet leaves to accumulate ABA in response to water stress is also known to decrease with age (Quarrie and Henson, 1981). Penultimate leaves on the F plants sampled would have been developmentally older than leaves sampled on NF or NF(P) plants, though whether such a difference in physiological age was responsible for the variation in ABA content remains to be shown.

An alternative hypothesis is that the emerged panicle following anthesis constitutes a strong sink not only for assimilates but also for other compounds such as ABA which can be loaded and transported in the phloem (Zeevaart, 1977). Stomatal conductance is

often increased in the presence of strong sinks such as developing fruits, removal of which may lead to decreases in g_L (Hansen, 1971; Kriedemann and Loveys, 1974; Kriedemann *et al.*, 1976; Setter, Brun and Brenner, 1980*a*) and to increases in ABA and its metabolite phaseic acid (Kriedemann and Loveys, 1974; Kriedemann *et al.*, 1976; Setter *et al.*, 1980*b*). The emerged millet panicle may have sufficiently strong sink activity to stimulate leaf photosynthesis and stomatal opening. The latter could result either from a reduced intracellular CO_2 concentration or a lowered ABA content.

Although the nature of the change in stomatal sensitivity to water stress associated with flowering is uncertain, it appears that pearl millet, probably in common with other C_4 cereals such as sorghum and maize (Ackerson and Krieg, 1977), has a dual strategy for coping with water stress. Thus, water is conserved by midday stomatal closure during the vegetative phases of development, but after flowering, assimilation is maximized at the expense of increased water consumption, due to stomata remaining at least partly open.

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