

The Effect of Flowering on Stomatal Response to Water Stress in Pearl Millet (*Pennisetum americanum* [L.] Leeke)¹

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ABSTRACT

Stomata on upper leaves of drought-stressed pearl millet (*Pennisetum americanum* [L.] Leeke) crops were more open in flowering (F) than in pre-flowering (PF) plants. This was not due to differences in leaf water potential (Ψ). Stomata of PF plants closed when Ψ fell to about -1.7 MPa, while on F plants stomata closed only when Ψ approached -2.3 MPa.

Osmotic adjustment did not account for these differences as relations between turgor potential (Ψ_p) and Ψ were similar in F and PF plants. While stomata of PF plants closed as Ψ_p became zero, in F plants stomata remained open even after bulk leaf turgor was lost.

Differences between F and PF plants were not explained by differences in age of leaves sampled. However, leaves of water-stressed PF plants had higher levels of abscisic acid (ABA) than leaves of F plants, despite similarities in water status. From these results and from relationships between g_s and stage of panicle development, it is concluded that the tendency of stomata to remain open despite water stress and loss of bulk leaf Ψ_p is related to the presence of an emerged panicle. Hypotheses which account for this effect are discussed.

Key words: *Pennisetum americanum* [L.] Leeke; Pearl millet; Flowering; Stomata; Water stress; Abscisic acid.

INTRODUCTION

Responses of stomata to water stress depend on a number of factors. Some of these such as frequency, duration, and intensity of stress, reflect variability of environment (Begg and Turner, 1976), whereas others are related to plant ontogeny (Frank, Power, and Willis, 1973; Teare, Sionit, and Kramer, 1982). In a previous report (Henson, Mahalakshmi, Alagarswamy, and Bidinger, 1983a), we presented evidence that during drought, stomata of pearl millet (*Pennisetum americanum* [L.] Leeke), a tropical C_4 cereal, showed much less tendency to close in response to water deficit once panicles had emerged than when at a non- or pre-flowering stage of development. This was so even when leaf water potential (Ψ) of the

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flowering (F) and pre-flowering (PF) plants did not differ. In some instances there were differences in leaf abscisic acid (ABA) contents, with higher ABA levels being present in leaves of water-stressed PF plants, which may have accounted for the greater stomatal sensitivity to water stress by this group (Raschke, 1975). However, the reasons for the differences between F and PF plants in stomatal response and ABA content were not fully defined in our initial study.

Besides flowering, other factors may have been responsible for the contrasting behaviour of stomata on F and PF plants. In particular, differences in age of sampled leaves or in their ability to adjust osmotically to stress require to be considered, as discussed previously (Henson *et al.*, 1983a). The object of this study was, therefore, to characterize more precisely the factor(s) responsible for reduced stomatal sensitivity to water stress observed in plants of pearl millet following flowering. The main factors considered were leaf water status, leaf age, ABA content, and stage of panicle development.

MATERIALS AND METHODS

Pearl millet (*Pennisetum americanum* [L.] Leeke) was grown in the field at ICRISAT Centre, near Hyderabad, India, in the dry season (February to May) of 1983. The soil was a medium depth Alfisol (c. 1.0 m). Plants were grown from seed in rows 0.75 m apart, and thinned to about 0.1 m apart within rows. Irrigations were given initially to ensure plant establishment, the last being applied 19 d after sowing (DAS) by flooding furrows made between rows. Plants received no further water from that date, except for a 12.5 mm rain which fell 44 DAS. As open pan evaporation rates were about 10 mm d⁻¹ this represented less than two days evaporative demand.

An F₁ hybrid, BJ 104, and two F₆ breeding lines were sampled. Plants designated as flowering had the main-stem panicle fully or almost fully emerged from the flag leaf sheath. Because of the delaying effect of water stress on panicle emergence, stigmas were normally exerted at this stage. Pre-flowering plants without emerged panicles were generally sampled before the appearance of the flag leaf. F and PF plants were obtained either by exploiting variability (resulting from stress) in the developmental stage within a population, or by manipulating photoperiod to control flowering. In the latter comparison plants were grown either under normal photoperiods ('short' days of c. 12.5 h light d⁻¹) which promoted flowering, or the daylength was extended to 16 h d⁻¹ from seedling emergence with tungsten filament bulbs mounted about 2.5 m above the ground. These provided an illuminance of 70–110 lx at ground level which effectively retarded floral initiation and panicle emergence.

Plants were sampled for leaf conductance (g_L), leaf water potential (Ψ) and abscisic acid (ABA) content essentially as earlier described (Henson, Alagarswamy, Mahalakshmi, and Bidinger, 1982; Henson *et al.*, 1983a). All these measurements were made on the same leaves using six leaves per treatment. Conductance was measured with an automatic diffusive resistance porometer and Ψ with a pressure chamber. Precautions were taken during the latter measurement to prevent post-excision evaporative losses (Turner and Long, 1980). Leaf samples for ABA analysis were frozen rapidly in liquid nitrogen and subsequently lyophilized. ABA content was determined for the whole leaf using the procedure of Quarrie (1978). For some samples chlorophyll content was determined by measuring absorbance at 653 nm of 90% acetone extracts of the freeze-dried leaves.

Most sampling was done in the mid-part of the day when photosynthetic photon flux density exceeded 1900 $\mu\text{mol m}^{-2} \text{s}^{-1}$, air temperatures ranged from 30 °C to 37 °C, and atmospheric vapour pressure deficit was 3.6–4.9 kPa.

Estimates of leaf turgor potential (Ψ_p) were obtained using a pressure–volume (P–V) technique. The method was as described by Henson, Alagarswamy, Mahalakshmi, and Bidinger, (1983b). Using this technique the relationship between Ψ_p and Ψ is obtained for individual leaves over the range of water status from full hydration to zero Ψ_p (Tyree and Hammel, 1972). The turgors of other leaves from the same population (at their mean Ψ) were inferred from these relationships. P–V measurements were made on four leaves per treatment.

RESULTS

Leaf water relations

Although in a previous study (Henson *et al.*, 1983a) it was considered unlikely that differences in Ψ_p accounted for the differences in leaf conductance between F and PF plants,

Ψ_p was determined only *prior* to flowering on that occasion. Midday measurements of Ψ , Ψ_p and g_L were therefore made (experiment 1) using flowering (panicles just emerged) and pre-flowering (stem elongating, flag leaf in whorl) plants of BJ 104 selected within the same plot. Data presented in Table 1 shows that both groups had similar Ψ , and a Ψ_p at, or close to, zero. Despite this, total leaf conductance was significantly ($P < 0.001$) greater in F than in PF plants.

Further estimates of Ψ_p (experiment 2) were obtained for BJ 104 plants which were flowering, or in which flowering had been delayed by extending the photoperiod. In these plants (Table 2; 47 DAS), Ψ_p was also close to zero and did not differ significantly between treatments. This indicated that the capacity to adjust osmotically in response to water stress was not influenced by panicle emergence, and that bulk leaf turgor was not a factor which could account for the differences in conductance.

Critical Ψ for stomatal closure

To determine more precisely the relative sensitivity of F and PF plants to stress, the threshold water potential for stomatal closure was determined by following changes in g_L and Ψ during rapid water loss induced by shoot excision. The method was as described by Henson *et al.* (1983b), and is similar to the approach used by Turner, Begg, and Tonnet (1978) with sorghum. For F plants closure occurred at *c.* -2.25 MPa Ψ , some 0.6 MPa *below*

TABLE 1. Leaf water status (Ψ , Ψ_p) and leaf conductance (g_L) of flowering (F) and pre-flowering (PF) plants of pearl millet, BJ 104; experiment 1

Measurements *in situ* (Ψ , g_L) were made at mid-day 42 d after sowing; $n = 6 \pm$ s.e. mean. Ψ_p was inferred from pressure-volume measurements on separate samples; $n = 4 \pm$ s.e. mean.

Treatment	Ψ (MPa)	Ψ_p (MPa)	Adaxial g_L	Abaxial g_L (cm s^{-1})	Total g_L
F ^a	-1.63 ± 0.05	0.0	0.47 ± 0.02	0.29 ± 0.06	0.76 ± 0.07
PF ^b	-1.58 ± 0.04	0.04 ± 0.01	0.07 ± 0.01	0.18 ± 0.03	0.25 ± 0.03

^a Flag leaf.

^b Second youngest fully expanded leaf.

TABLE 2. Ψ , Ψ_p , total g_L and ABA content of leaves of flowering (F) and pre-flowering (PF) plants of pearl millet BJ 104; experiment 2

For F plants the flag leaf was sampled; for PF plants (flowering retarded by daylength extension), a lower leaf which emerged on the same day as the flag leaf of F plants was sampled. Data are means of $6 \pm$ s.e. mean except for Ψ_p , where $n = 4$.

Sampling occasion (DAS)	Treatment	Ψ (MPa)	Ψ_p (MPa)	Total g_L (cm s^{-1})	ABA content (ng g^{-1} dry weight)
47	F	-1.93 ± 0.04	0.02 ± 0.01	0.67 ± 0.06	241 ± 6
	PF	-1.62 ± 0.03	0.07 ± 0.04	0.17 ± 0.04	311 ± 22
50	F	-1.99 ± 0.08	—	0.54 ± 0.05	192 ± 25
	PF	-1.69 ± 0.03	—	0.14 ± 0.04	339 ± 20

the Ψ at which turgor became zero (Fig. 1A). This effect was observed previously (Henson *et al.*, 1983b). Conductance of PF plants was less well related to Ψ (Fig. 1B), but the lower limit of Ψ for closure (*c.* -1.7 MPa) appeared to coincide with the point at which turgor became zero.

Leaf age and ABA

In the previous study (Henson *et al.*, 1983a) the leaves of F and PF plants differed in age at the time of sampling, as comparisons were mainly made using the penultimate leaf of F plants and the most recently *fully expanded* leaf of PF plants. The leaf sampled on F plants would have emerged before that sampled on PF plants. This bias was partially corrected in a further experiment by using the *second* most recently expanded leaf of PF plants (Table 1) or by sampling leaves of PF plants which had emerged at the same time as the flag leaves sampled from F plants (Table 2). The results show that differences in conductance were still obtained even when the sampled leaves were all of a similar age with respect to time of ligule emergence. The lower conductances of PF plants were, however, associated with significantly higher leaf ABA concentrations (Table 2).

Influence of the panicle

The above results suggested that the change in stomatal behaviour was related to the stage of growth or development of the panicle (possibly via an effect on leaf ABA content; see below). To obtain more information on this aspect leaf conductance was related to panicle size using a population of drought-stressed BJ 104 which included plants at various developmental stages (as a result of the stress). The Ψ and g_L of the youngest fully emerged leaf (regarded as the main 'source' leaf for the panicle) were measured together with the length and dry weight of the panicle or vegetative apex. For all plants ($n = 54$) mean leaf Ψ was -1.99 ± 0.02 MPa. It was slightly lower for flag leaves of plants with emerged panicles (-2.11 ± 0.05 MPa, $n = 13$) or with panicles in the boot stage (-2.10 ± 0.05 MPa, $n = 14$) than for plants at earlier stages of panicle development (-1.91 ± 0.05 MPa, $n = 27$). Increased conductance was associated with panicles which were over 19 cm long and 3.0 g or more in dry weight (Fig. 2A, B). In all cases these panicles were emerged (and plants were hence regarded as flowering). When panicles were still in the 'boot', *i.e.* enclosed within the flag leaf sheath, flag leaf conductances were similar to leaf conductances of plants at earlier stages of panicle development and could not be related to variation in panicle size.

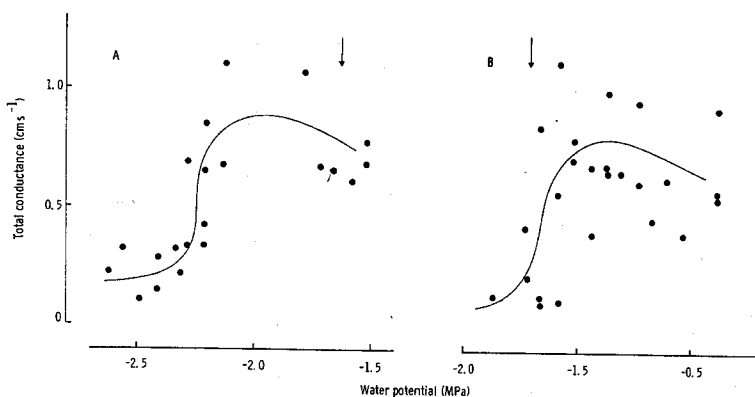


FIG. 1. Relationship between total g_L and bulk leaf Ψ during rapid drying induced by shoot detachment of droughted plants of BJ 104. (A) flowering plants, (B) pre-flowering plants. Curves were fitted by eye. Arrows indicate Ψ at zero Ψ_p as determined from pressure-volume curves.

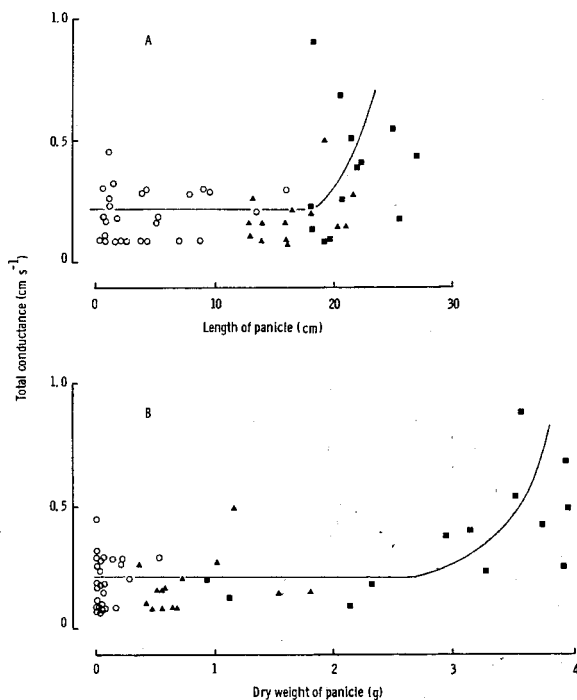


FIG. 2. Relationship between total g_L of the youngest fully-expanded leaf (flag leaf where fully emerged) and (A) length of panicle; (B) dry weight of panicle, for droughted plants of BJ 104. Plants were classified according to stage of development as either: stem elongating, flag leaf not emerged (○), flag leaf emerged, panicle enclosed within flag leaf sheath (△), or panicle emerged from flag leaf sheath (■). Curves were fitted by eye.

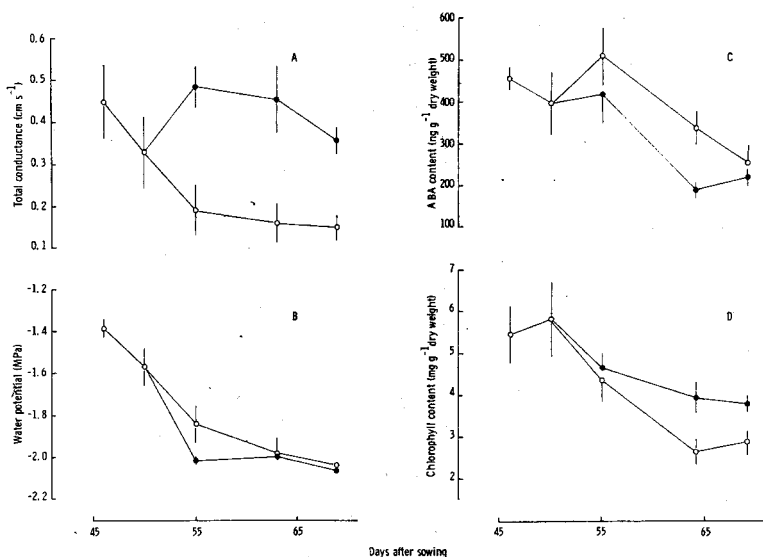


FIG. 3. Seasonal changes in g_L (A), Ψ (B), ABA content (C) and chlorophyll content (D) of leaves of droughted PF (○) and F (●) plants of an F_6 line of pearl millet. Leaves, sampled at mid-day, were the youngest fully-expanded leaf of PF, and the penultimate (flag-1) leaf of F plants. Data are means of four leaves, each sampled from a separate plot. Vertical bars indicate \pm s.e. mean where these exceed the size of the symbols. No F plants were available for sampling before 55 DAS.

Seasonal trends in g_L , Ψ , ABA and chlorophyll content

The influence of flowering on g_L , Ψ , ABA and chlorophyll content during the development of water stress is shown in Fig. 3 for one of the F_6 breeding lines. In plants which did not flower, a gradual reduction in Ψ was accompanied by a similar decrease in g_L , whereas in plants with emerged panicles g_L first increased and was then maintained at a moderately high level despite an equal or slightly greater reduction in Ψ . F plants tended to have lower levels of ABA and higher levels of chlorophyll in the sampled leaves than did PF plants. Similar results were obtained for the second F_6 line investigated (results not presented), except that the differences in chlorophyll content were more marked than those shown in Fig. 3.

DISCUSSION

The present results support the earlier conclusion (Henson *et al.*, 1983a) that differences in bulk leaf water status are unlikely to account for the greater tendency of stomata to open under stress conditions when plants are flowering (i.e. when panicles have emerged). In the absence of emerged panicles leaf conductances of water-stressed plants in the field were low, and the indications were that closure occurred with the attainment of zero Ψ_p in the leaf. In this respect such plants were similar to young vegetative plants grown in pots in a glasshouse (Henson *et al.*, 1983b), in which stomatal closure was concomitant with the loss of bulk leaf turgor. Stomata no longer closed immediately Ψ_p became zero once panicles had emerged (Fig. 1A; Henson *et al.*, 1983b).

The stomatal responses did not appear to relate to differences in leaf age as leaves of F and PF plants which were matched for time of ligule emergence still differed in g_L . (Such leaves sampled from F and PF plants would, however, have differed in other respects; see below.) When leaves were not matched for ligule emergence (Table 1) the flag leaves sampled on F plants would generally have been older than the leaves sampled from PF plants. Thus, to account for these differences on the basis of leaf age, g_L would have to increase with age; the converse of the trend generally observed (Krizek and Milthorpe, 1973; Davis, van Bavel, and McCree, 1977). Photosynthetic rate of pearl millet showed a general reduction following full leaf emergence (McPherson and Slatyer, 1973), and as stomatal resistance was the main factor governing photosynthetic rate in those experiments, this suggests that the conductance of the leaves also declined with age.

The leaves sampled from F and PF plants would have differed in ways other than age. When selected to be of the same age, the leaves sampled from PF plants were less exposed to light (being partially shaded by younger leaves), though such differences were minimized by the wide row spacing and the high irradiance at the times of sampling. However, the fact that stomatal conductance of even the fully exposed flag leaf remained low unless panicles had emerged (Fig. 2), indicates that neither leaf age, type, nor position, were dominant factors influencing g_L .

Attention, therefore, focuses on panicle emergence as the factor controlling conductance. The results shown in both Figs 2 and 3 suggest that flowering was associated not just with the maintenance of g_L but with an active promotion of stomatal opening despite low or falling Ψ . In both the F_6 millet lines studied, chlorophyll levels were also higher in leaves of F than PF plants so that at least two factors, g_L and chlorophyll content, were more favourable for continued CO_2 assimilation during water stress in F plants. This is an attribute which would be particularly advantageous during the subsequent phase of grain development.

The mechanisms underlying these observations still have to be elucidated. It is possible that the emerged panicle promotes stomatal opening by virtue of its activity as a strong sink for photosynthates. Rapid grain filling is apparently not an essential prerequisite for such activity as plants were generally sampled before stigmas shrivelled. However, preliminary observations

(not presented) suggest that stomatal opening may be further enhanced if panicles are at more advanced stages of development. Decreases in g_L or transpiration rate of well-watered plants have been observed in a number of species following the removal of sinks (Hansen, 1971; Kriedemann and Loveys, 1974; Rawson, Gifford, and Bremner, 1976; Aslam and Hunt, 1978; Setter, Brun, and Brenner, 1980a). Increases in leaf ABA content following sink removal have also been reported (Kriedemann and Loveys, 1974; Setter *et al.*, 1980b). Setter *et al.* (1981) demonstrated that the increase in ABA content in soybean leaves which followed pod removal or petiole girdling arose because of reduced *export* of ABA from the leaf rather than from effects on ABA synthesis or metabolism. Phloem girdling of millet leaves also results in an increased ABA content which is not due to water stress (I. E. Henson, unpublished results). Therefore, the lower ABA contents in leaves of F plants (Table 2; Fig. 3), might be due to the sink activity of the panicle. That ABA may be transported from flag leaves to the inflorescence in cereals has been demonstrated (Goldbach and Goldbach, 1977).

From their work on soybean, Setter *et al.* (1980a, b) concluded that the sink effects on leaf conductance were mediated by ABA rather than by photosynthesis-linked changes in the concentration of CO_2 in the intercellular space of the leaf (C_i). (This interpretation has, however, recently been questioned; Farquhar and Sharkey, 1982.) Rawson *et al.* (1976) also excluded g_L regulation via C_i but for different reasons. Whether the primary control of conductance by the millet panicle under conditions of water stress is via ABA or C_i , remains to be determined. That g_L is at least partly controlled by factors other than the total levels of ABA in the leaf is evident from Fig. 3, as ABA levels actually *declined* as stress increased, yet there was no corresponding *increase* in g_L . (The decline in ABA levels rather than an increase with continued stress has also been observed in maize by Ilahi and Dörffling (1982), but its cause is unknown.) Furthermore, in our earlier study (Henson *et al.*, 1983a), some differences in g_L were found in the absence of any differences in ABA content. Possibly both C_i and ABA are involved in the regulation of g_L and may well interact (Raschke, 1975).

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