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Leaf Abscisic Acid Content and Recovery from Water Stress in Pearl Millet (*Pennisetum americanum* [L.] Leeke)[1]

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ABSTRACT

Water potential (Ψ) and abscisic acid (ABA) content were measured in leaves of drought-stressed, field-grown plants of pearl millet (*Pennisetum americanum* [L.] Leeke) during rehydration, initiated either in response to a diurnal night-time reduction in evaporative demand, or to irrigation. Overnight rehydration, manifested as a substantial increase in Ψ, was not accompanied by any reduction in ABA levels. In contrast, an increase in Ψ following irrigation resulted in an appreciable reduction in ABA content. Such reduction was, initially, only partial when field plants were rewatered at dusk, but was rapid and complete within 3 h when irrigation was applied mid-afternoon.

The possibility that light or temperature changes might have prevented loss of ABA during night-time rehydration was investigated in pot experiments. At similar air temperatures, young pot-grown plants rehydrated more rapidly, and ABA levels fell more quickly, in darkness than in light. The onset of rehydration and loss of ABA in darkness were delayed by low (20 °C) compared with high (28 °C) temperature, though after an initial lag, rates of both processes at 20 °C were similar to rates at 28 °C. Neither light nor temperature affected the relationship between ABA content and Ψ.

Key words: Abscisic acid; Rehydration; *Pennisetum americanum*.

INTRODUCTION

One of the best documented biochemical changes in plants subjected to water stress is the increase in concentration of the hormone abscisic acid (ABA) (Wright, 1978; Walton, 1980). This increase, which has been most commonly studied in leaves, is generally reversible, with levels of ABA often declining rapidly (within hours) following relief of water stress (Hiron and Wright, 1973; Bengtson, Falk, and Larsson, 1977; Dörffling, Streich, Kruse, and Muxfeldt, 1977; Ludlow, Ng, and Ford, 1980; Zeevaart, 1980, 1983; Pierce and Raschke, 1981). In pearl millet (*Pennisetum americanum* [L.] Leeke), the reduction of leaf ABA content induced following rewatering of intact, drought-stressed pot-grown plants was completed within 6 h and accompanied the rise in leaf water potential (Ψ) (Henson, 1981).

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As leaf ABA levels are so sensitive to bulk leaf water status, the question arises as to whether changes in ABA content normally accompany daily cyclic changes in the state of hydration of the plant. It is common, particularly under field conditions and with droughted plants, to find large changes in leaf Ψ during a 24 h period as a consequence of variation in both the evaporative demand of the atmosphere and in the conductance of leaves to water vapour (Turner and Begg, 1981; Hanson and Hitz, 1982). Even severely water-stressed plants can rehydrate and increase Ψ to high levels during the night, when evaporative demand is minimal and stomata have closed in response to darkness.

Field studies of ABA levels in leaves of water-stressed pearl millet (Henson, Alagarswamy, Mahalakshmi, and Bidinger, 1982), showed that while there were differences in ABA content between plant groups which could be related to the severity of previous water stress, there was no close relationship between ABA content and diurnal changes in Ψ. During the night ABA content of droughted plants remained essentially constant despite leaf Ψ increasing from −1·24 MPa at 1800 h to −0·52 MPa at 0600 h the following day. However, an increase in Ψ following rainfall *was* accompanied by a significant reduction in ABA content. ABA content was thus affected differently by the two types of recovery from water stress. The effect on ABA of rainfall, in contrast to the lack of effect of overnight rehydration, may have been accounted for by one of several factors. These include: (i) the maximum Ψ attained during rehydration (higher after rainfall than during overnight rehydration?), (ii) the time available for recovery from stress (longer after rainfall), and (iii) the rate at which Ψ increased during rehydration (faster after rainfall?). We here present data which confirm the initial observations, and examine the basis for the contrasting response to different modes of recovery from water stress.

MATERIALS AND METHODS

BJ 104, a commercial F_1 hybrid pearl millet (*Pennisetum americanum* [L.] Leeke), was used in all experiments.

Field experiments

These were conducted at ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) Centre, near Hyderabad, India, in the dry seasons (February to May) of 1982 and 1983.

In 1982 plants were grown on a deep (1·0 m) Alfisol soil in plots each accommodating six 4·0 m long rows spaced 0·5 m apart. Plats were irrigated by surface flooding to restore field capacity at intervals of 4–7 d. To impose drought, irrigation was withheld from some of the plots from 19 d after sowing (DAS). The other plots continued to be regularly irrigated, the last irrigation prior to sampling being 38 DAS. Plants were sampled 39 and 40 DAS. On day 39 two of the droughted plots were re-irrigated by surface flooding, one at 1530 h and one at 1800 h (dusk) Indian Standard Time.

In 1983 plants were grown on a medium deep (0·7 m) Alfisol soil in rows 0·75 m apart. They received no irrigation or rainfall from 15 DAS until 42 or 43 DAS when some rows were re-irrigated by surface flooding at *c.* 1500 h. Plants were sampled at dawn 43 or 44 DAS.

Sampling was confined to the first (1982) or second (1983) leaf below the flag leaf on the main stem of healthy, representative, plants. The leaf was enclosed in moist muslin cloth, detached just above the ligule, and Ψ measured using a pressure chamber. The muslin wrapping was left in position during measurement of Ψ to minimize evaporative losses and temperature changes during pressurization and depressurization. The leaf was then rapidly frozen using liquid nitrogen, stored at below −20 °C, and freeze-dried for subsequent ABA analysis. During sampling, periodic measurements were made of air temperature using a screened mercury thermometer.

Bulk leaf turgor potentials were assessed in the 1983 experiment using a pressure–volume method, as previously described (Henson, Alagarswamy, Mahalakshmi, and Bidinger, 1983). Leaf relative water content (*RWC*) was assessed using the standard formula (*RWC* = [fresh weight − dry weight]/[turgid weight − dry weight]). After determining fresh weight, leaves were incubated in distilled water for 4 h at 28 °C in diffuse light (*c.* 3·0 μmol m^{-2} s^{-1} photosynthetic photon flux density) before measuring turgid weight. Dry weight was determined after drying at 85 °C for 24 h.

Pot experiments

In experiment 1 (effect of light) seeds were sown (on 15 September 1982), two per 9·0 cm diameter pot, in John Innes No. 2 compost in a heated glasshouse (minimum 23 °C night temperature; natural photoperiod) in Cambridge, U.K. Plants were subsequently thinned to one per pot. Plants were watered using a wick system as described earlier (Henson, 1981). Drought was imposed by withdrawing wicks from the water supply 13 DAS.

Twenty-one DAS, when the Ψ of droughted plants had fallen to about −1·8 MPa, plants were rewatered shortly after dawn and allowed to rehydrate either in the light or in the dark (by placing plants inside cardboard boxes). The fifth main-stem leaf (the youngest fully-emerged leaf on droughted plants at the start of sampling) was sampled for Ψ and ABA as described for the field experiments. Leaf fresh weight was determined immediately after sampling and leaves were then frozen at below −20 °C and subsequently freeze-dried.

During sampling the natural light was supplemented from 0800 h to 1800 h with light from 400 W high pressure sodium lamps. Cloud cover was variable and irradiance levels ranged from 277–917 μmol m^{-2} s^{-1} (400–700 nm). Air temperatures measured by a thermograph were between 26 °C and 34 °C (Fig. 3A), and were similar (±2 °C) both inside and outside the boxes. Vapour pressure deficit (vpd) ranged from 0·93–2·43 kPa.

In experiment 2 (effect of temperature) plants were grown in a controlled environment cabinet. Cultural procedures were as for experiment 1. Conditions in the cabinet were as described earlier (Henson, 1981). The drought treatment was imposed in the same way as for experiment 1. However, drought was terminated by rewatering plants at the *end* of the 12 h photoperiod. Just prior to this, plants were divided into two groups, one of which remained in the cabinet to rehydrate in the dark at 28 °C, while the other group was placed in a similar cabinet, also in the dark, but at 20 °C. Leaf five was sampled regularly from both groups to determine Ψ and ABA content.

ABA analysis

Freeze-dried leaves were coarsely ground, and extracted individually in acetone:water (9:1 v/v) using 1·0 ml per 20 mg dry weight. Aliquots of the extract supernatant were analysed for free ABA using the method of Quarrie (1978).

RESULTS AND DISCUSSION

Field experiments: Overnight rehydration versus re-irrigation mid-afternoon

The 1982 field experiment was designed to test earlier observations (Henson *et al.*, 1982) of the different effects on ABA content of droughted plants of overnight rehydration due to decreased evaporative demand and rehydration following an increased soil water supply. Samples were taken over a 24 h period from droughted, re-irregated, and regularly (*c.* weekly) irrigated plants, the latter serving as controls. Plants were re-irrigated either at 1530 h (to simulate the rainfall of the previous year; Henson *et al.*, 1982), or at 1800 h (dusk) to more closely approximate conditions to those of overnight rehydration. The response to the mid-afternoon re-irrigation versus overnight rehydration will first be compared.

As shown in Fig. 1, leaf Ψ of both control (regularly irrigated) and droughted plants increased substantially during the afternoon and evening hours and reached their maxima at the end of the dark period. Although Ψ of droughted plants attained a high level (−0·33 MPa) it remained below that of controls and fell more steeply than control Ψ after dawn. As before (Henson *et al.*, 1982), ABA levels did not decline in droughted plants as a result of nocturnal rehydration (Fig. 2); rather there was a tendency for levels to increase.

While it was possible that Ψ did not rise high enough in droughted plants to initiate ABA decline (though Ψ of droughted plants was only 0·13 MPa below Ψ of controls at the end of the night), when droughted plants were rewatered mid-afternoon, ABA content declined to the level of controls within 2·5 h (Fig. 2), although Ψ increased during this period to only −0·54 MPa. This was less than both Ψ of control plants and that eventually attained nocturnally by droughted plants (Fig. 1). These results suggest that neither maximum Ψ reached nor time for recovery from stress accounted for the lack of decline in ABA content in droughted plants during overnight rehydration.

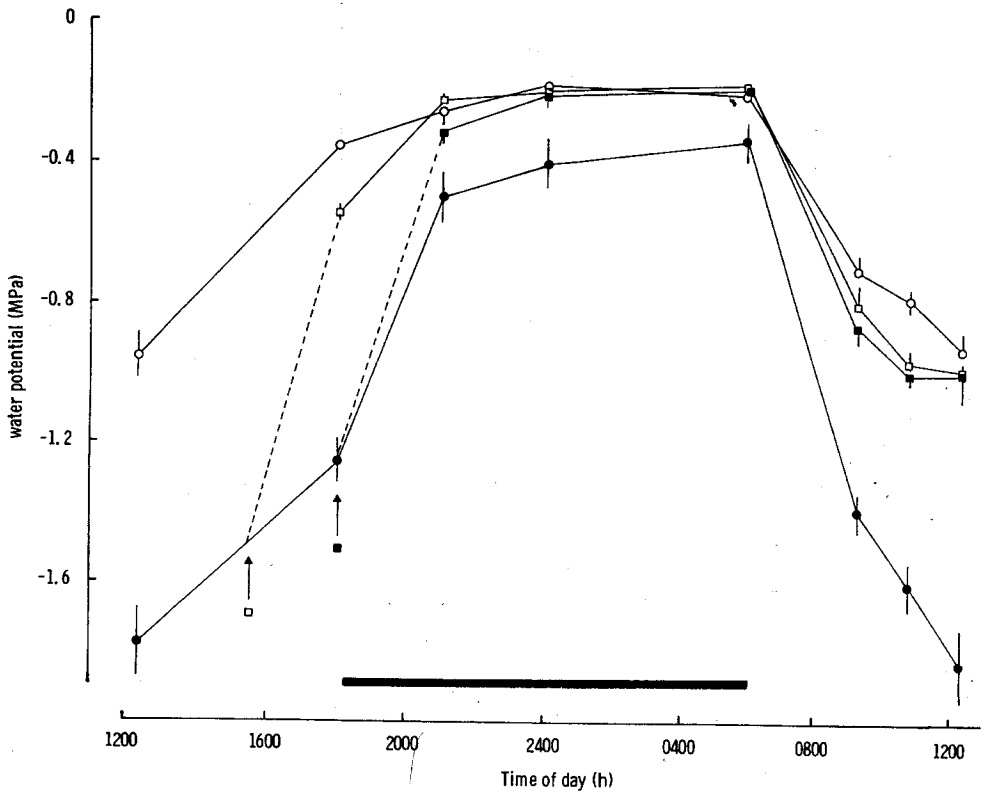

FIG. 1. Changes in leaf Ψ of field-grown plants over a 24 h period from 1200 h 39 d after sowing. Data, means of six, are for the penultimate leaf. Vertical bars indicate 2 × s.e. mean. Duration of dark period is indicated by black bar at base of figure. Treatments were: (i) regularly irrigated control (○), (ii) droughted (●), (iii) droughted → re-irrigated at 1530 h (□), (iv) droughted → re-irrigated at 1800 h (■). Arrows show when irrigation was applied.

Recent evidence suggests that turgor potential (Ψ_p) rather than Ψ controls both ABA accumulation during water stress and ABA loss during rehydration (Pierce and Raschke, 1980, 1981). Therefore, in the 1983 experiment both Ψ and Ψ_p were determined in nocturnally rehydrated and re-irrigated plants. The plants were at a similar developmental stage and degree of water stress to those sampled in 1982. The re-irrigated treatment was watered at *c.* 1500 h either 42 or 43 DAS. Prior to this, Ψ at mid-day (−1·89 MPa) was close to the 1982 value (Fig. 1), while Ψ_p was close to zero (0·04 MPa). As in 1982, the nocturnally rehydrating plants had reached a high Ψ by dawn (−0·32 MPa), though this was still significantly below that of re-irrigated plants, as was *RWC* (Table 1). Ψ_p, however, did not differ significantly between treatments at dawn yet ABA levels differed substantially. Thus, failure to regain turgor was not a reason for maintenance of high ABA levels in nocturnally rehydrated plants.

Overnight rehydration versus re-irrigation at dusk

A major contrast between nocturnal rehydration and rehydration following irrigation or rainfall during the day, is that, due to light and greater evaporative demand by the atmosphere, the latter treatments are associated with greater water flux through the plant and with assimilation of CO_2 as stomata re-open. Such differences were minimized when plants were

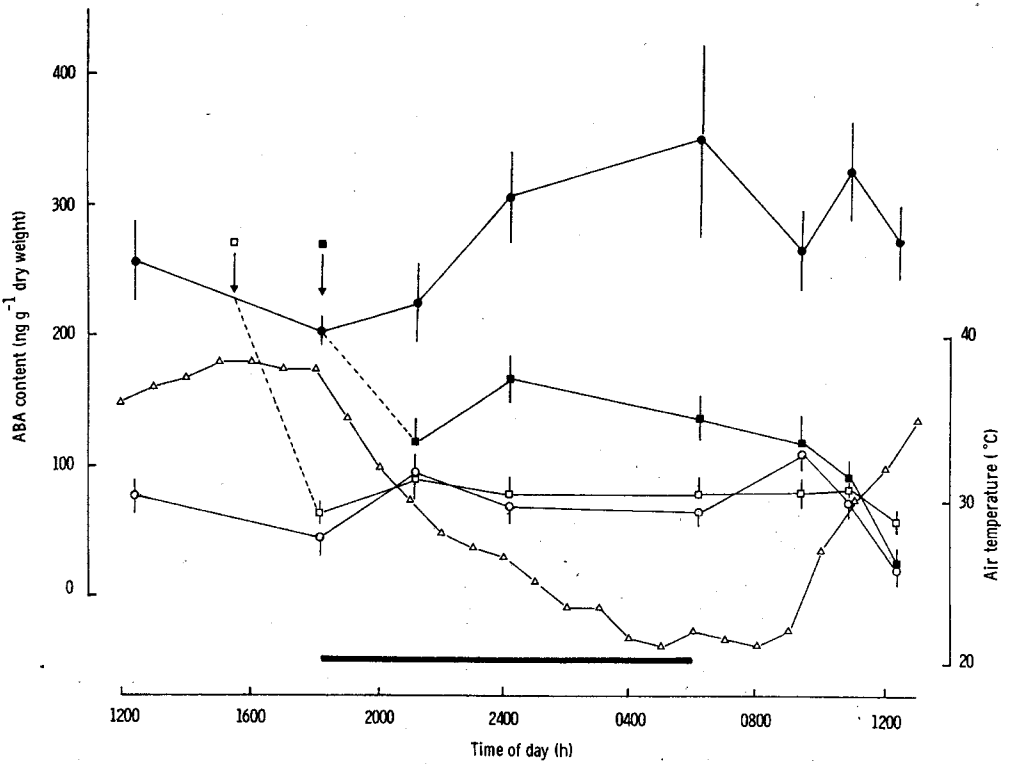

FIG. 2. Changes in ABA content of field-grown plants and in air temperature over a 24 h period from 1200 h 39 d after sowing. △ = air temperature; other details are as in Fig. 1.

TABLE 1. *Leaf water status and ABA content in field-grown plants at dawn* (c. *0615 h*) *43–44 d after sowing* (*DAS*)

The third youngest leaf (i.e. second below the flag leaf) on the main stem was sampled separately for either (a) Ψ and ABA, (b) *RWC*, or (c) estimation of Ψ_p at the mean dawn Ψ by pressure–volume measurements. Ψ and ABA samples were taken both 43 and 44 DAS; data for the two sampling dates were combined; $n = 12 \pm$ s.e. mean. *RWC* was determined 44 DAS; $n = 6 \pm$ s.e. mean. Ψ_p samples were taken both 43 and 44 DAS and are means of 4 ± s.e. Re-irrigated plants were watered by surface flooding *c.* 1500 h on the day prior to sampling.

Treatment	Ψ (MPa)	Ψ_p (MPa)	*RWC* (%)	ABA content (ng g^{-1} dry weight)
Droughted	−0·32 ± 0·016	1·16 ± 0·04	96·7 ± 0·3	200 ± 20
Re-irrigated	−0·20 ± 0·005	1·24 ± 0·05	99·5 ± 0·3	35 ± 7

re-irrigated at dusk. The resulting changes in Ψ (Fig. 1) were similar to those following re-irrigation at 1530 h with maximum Ψ again being greater than during nocturnal rehydration. Despite this, the reduction in ABA content was only partial (Fig. 2) and ABA levels for most of the night were higher than in either controls or plants re-irrigated at 1530 h. These results suggest that environmental conditions during rehydration affected the change in ABA content; a possibility investigated subsequently in pot experiments under controlled conditions.

Pot experiments: Effect of light

In a previous pot experiment with pearl millet, ABA content was reduced from very high to control levels within 6–7 h following rewatering at dawn (Henson, 1981). In that experiment plants were exposed to light, but from experiments with other species using detached leaves there is no evidence of a light requirement for ABA degradation (Zeevaart, 1980; Pierce and Raschke, 1981); rather, ABA disappears faster in the dark than in the light (Bengtson, Klockare, Klockare, Larsson, and Sundqvist, 1978; Zeevaart and Boyer, 1982; Zeevaart, 1983).

As shown in Fig. 3B, following rewatering, droughted millet plants rehydrated initially more rapidly in the dark than in the light (0·39 MPa h^{-1} in dark, 0·34 MPa h^{-1} in light; meaned over the first 3 h). These rates of rehydration were similar to those found in the field (Fig. 1; see below). Plants in light were estimated to have taken 2–3 h longer than darkened plants for

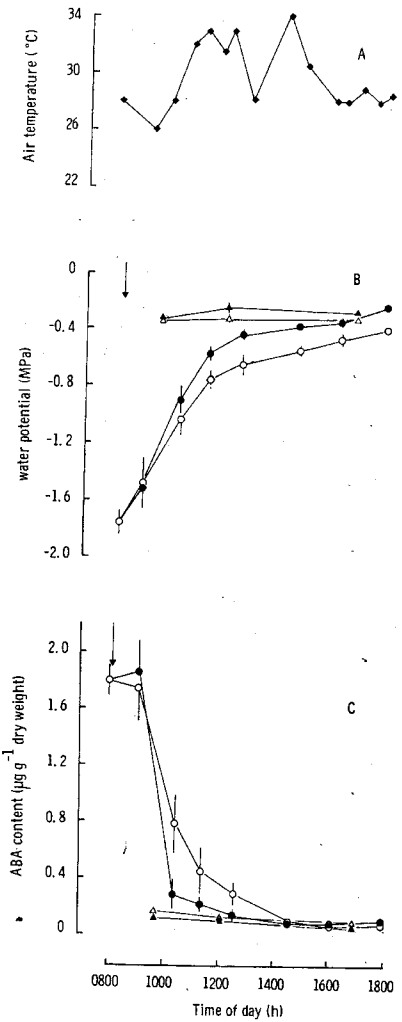

FIG. 3. Changes in air temperature (A), leaf Ψ (B) and leaf ABA content (C) during recovery from water stress of young, pot-grown plants. Plants were either continuously watered (△, ▲), or droughted and rewatered at the time indicated by the arrow (○, ●). In B and C solid symbols refer to plants placed in darkness after rewatering; open symbols represent plants remaining in light. Data in B and C, for leaf 5, are means of six. Vertical bars indicate 2 × s.e. mean where this exceeds the size of the symbol.

Ψ to be fully restored to control levels. The absence of light did not prevent loss of ABA following rewatering (Fig. 3c). Levels actually declined *more* rapidly in darkened plants in accordance with the more rapid rise in leaf Ψ. However, in both groups ABA contents were reduced to control levels by 6 h. There was no evidence of any difference between the two treatments in the relationships between ABA content and Ψ (Fig. 5A). Zeevaart and Boyer (1982) and Zeevaart (1983) have suggested that the more rapid disappearance of ABA observed in stressed *Xanthium* plants rehydrating in darkness as opposed to light may have been due to ethylene stimulation of ABA metabolism. The present results indicate that any difference between light and dark in rates of ABA loss in millet can be attributed directly to differences in rehydration rates. The results also suggest that light was not a factor responsible for treatment differences observed in the field.

Effect of temperature

Following irrigation in the field at 1530 h, air temperatures during rehydration were high (38 °C) and constant, whereas from 1800 h onwards air temperatures were declining, reaching a 21 °C minimum near dawn (Fig. 2). When young droughted pot-grown plants were transferred at the start of the dark period from 28 °C to 20 °C and re-irrigated, there was a lag period of about 1–2 h before maximal rates of either rehydration or ABA decline were attained (Fig. 4). The rates of both processes at 20 °C then matched those at 28 °C, and ABA levels declined to control values within 5 h. The sudden transition from 28 °C to 20 °C initially arrested water uptake but rehydration then proceeded as rapidly as at 28 °C. The reduction in temperature did not prevent ABA loss.

In both the above experiments ABA content declined most rapidly over a narrow range of Ψ (Fig. 5). The 'threshold' Ψ above which the major reduction in ABA level occurred was about −1·2 MPa. This is close to the Ψ at zero turgor found in other experiments with plants grown and treated similarly to those in the present study (Henson, 1982; Henson *et al.*, 1983). These results support the conclusion of Pierce and Raschke (1981) that only a slight restoration of positive turgor suffices to stimulate maximally ABA catabolism.

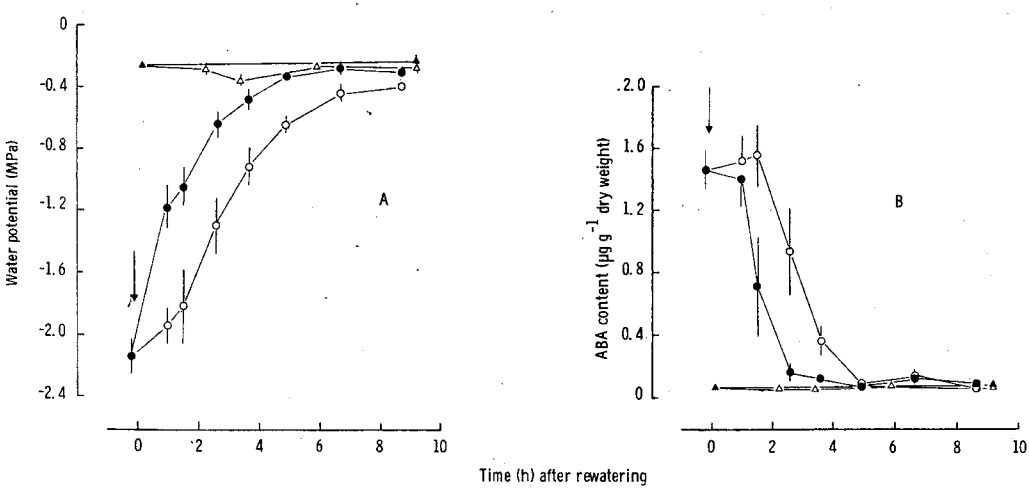

FIG. 4. Changes in leaf Ψ (A) and leaf ABA content (B) during recovery from water stress of young, pot-grown plants rehydrated at either 20 °C (○) or 28 °C (●) in darkness. Arrow indicates time of rewatering. Control plants (not droughted) were also sampled at 20 °C (△) and 28 °C (▲). Data, for leaf 5, are means of six. Vertical bars indicate 2 × s.e. mean where this exceeds the size of the symbol.

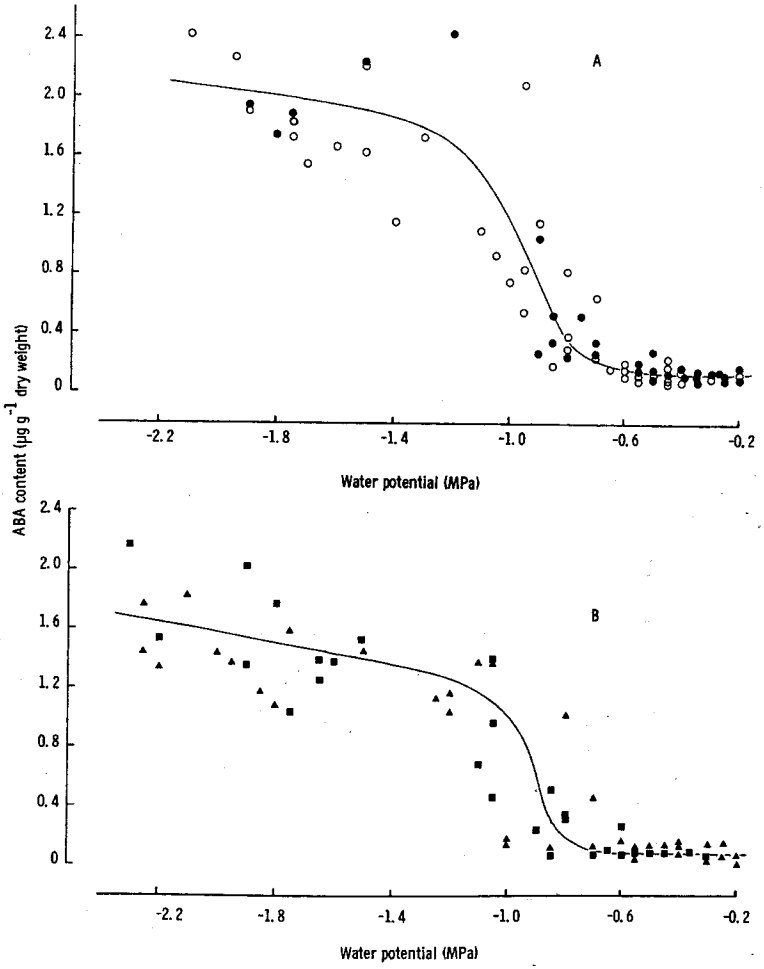

FIG. 5. The relationship between ABA content and Ψ of leaf 5 of young pot-grown plants following relief of drought by rewatering. Points are for individual plants maintained (A) either in light (○) or darkness (●) at 26–34 °C, or (B) in darkness at either 20 °C (■) or 28 °C (▲). Curves were fitted by eye.

Resolving the 'anomalous' behaviour of field-grown plants

Clearly, if the responses to light and temperature by young pot-grown and older field-grown plants were similar, then changes in these variables cannot account for the effects on ABA content of irrigation timing or rehydration mode (i.e. increase in water uptake versus decrease in water loss) observed under field conditions. However, field-grown plants accumulated much less ABA for a given level of stress than did pot-grown plants; a result in agreement with previous findings (Henson, Mahalakshmi, Bidinger, and Alagarswamy, 1981).

Field-grown plants experience large diurnal changes in Ψ which are probably much reduced or absent in droughted plants grown in small pots. When Ψ was determined for pot-grown plants droughted in a controlled environment cabinet, Ψ increased from an initial value of −2·0 MPa to only −1·7 MPa, after 9 h in darkness, whereas in the field, droughted plants underwent an increase in Ψ of nearly 0·9 MPa (−1·25 MPa to *c.* −0·35 MPa) over a comparable period (Fig. 1). Such diurnal changes in Ψ, which would gradually increase

during the course of stress development, may constitute an important factor in 'conditioning' the plant to drought stress.

Differences in the loss of ABA following rehydration in the field may have arisen due to differences in rehydration *rate*. While it was not possible to monitor the rehydration rate in the field continuously, mean rates could be estimated over periods of 2·5–3·0 h (Fig. 1). For droughted plants rewatered at 1530 h and 1800 h, the initial mean rates of increase in Ψ were 0·38 and 0·31 MPa h^{-1} respectively. These changes were associated with decreases in ABA of 66·4 and 26·3 ng g^{-1} dry weight h^{-1}. Between 1800 h and 2100 h, Ψ of droughted plants increased at a rate of 0·25 MPa h^{-1} and there was no reduction in ABA levels (Fig. 2). Although limited, these data suggest that rate of rehydration may have been a factor influencing ABA loss in field-grown plants.

While reduction in ABA levels during recovery from water stress of detached leaves involves a reduction in synthesis and/or an increase in metabolism (Pierce and Raschke, 1981), ABA content of attached leaves is likely, in addition, to depend on import or export of ABA. The relative importance of these processes during rehydration remains to be determined.

Jordan and Ritchie (1971) considered that rehydration in the absence of transpiration after sunset should favour water absorption by mesophyll cells. As much of the ABA in the leaf is located in chloroplasts (Loveys, 1977; Heilmann, Hartung, and Gimmler, 1980), and hence in mesophyll (Singh, Galson, Dashek, and Walton, 1979; Weiler, Schnabl, and Hornberg, 1982), then ABA should be located within rehydrating cells. Furthermore, results obtained by Heilmann *et al.* (1980) and calculations of Cowan, Raven, Hartung, and Farquhar (1982) predict a movement of ABA from chloroplasts into the cytoplasm during darkness due to a decrease in stroma pH. This movement should facilitate ABA degradation as the enzymes responsible for this are considered to be located in the cytoplasm (Hartung, Gimmler, Heilmann, and Kaiser, 1980). The available evidence thus fails to explain why levels of ABA are maintained in plants of pearl millet during nocturnal rehydration.

If the stability of ABA in nocturnally rehydrating leaves were a general phenomenon this may account for the continued inhibition of leaf extension in droughted plants despite regain of turgor during the night (Sharp, Osonubi, Wood, and Davies, 1979; Davies, Mansfield, and Wellburn, 1980; Takami, Rawson, and Turner, 1982). 'Carry-over' of ABA may also account for stomata responding to water stress both on a long-term as well as on a short-term basis (Schulze and Hall, 1981).

CONCLUSIONS

The present results demonstrate that, in pearl millet, water stress-induced accumulation of abscisic acid is largely unaffected under field conditions by the large nocturnal increases in leaf Ψ. Nocturnal recharging of leaf water, unlike rehydration induced by irrigation or rain, does not result in reductions in leaf ABA content. Water-stressed plants may, therefore, accumulate ABA continuously from day to day, which would account for the observation (Henson *et al.*, 1982) that ABA content early in the photoperiod is more closely related to water stress history than to currently prevailing Ψ.

The maintenance of ABA levels in droughted plants overnight did not appear to be due to failure to regain turgor or high Ψ or to insufficient time at high Ψ. The mechanism responsible thus requires further study.

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