REGULATION OF ABSCISIC ACID CONCENTRATION IN LEAVES OF FIELD-GROWN PEARL MILLET (*PENNISETUM AMERICANUM* [L.] LEEKE): THE ROLE OF ABSCISIC ACID EXPORT*

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Summary

Diurnal changes in the concentration of abscisic acid (ABA) which occur in leaves of droughted, field-grown plants of pearl millet (*Pennisetum americanum* [L.] Leeke) are not always correlated with changes in bulk leaf water potential (Ψ). A rapid decline in ABA content of the leaves following its rise to a peak level in mid-morning, was observed in several time-course studies despite continued water stress.

The possibility that the reduction in ABA in leaves was due to an elevated rate of its export was examined in two ways: (i) by measuring ABA concentrations in developing panicles (possible sinks for leaf-produced ABA) and in leaves, and (ii) by comparing the amounts of ABA in ungirdled leaves and in leaves heat-girdled at the base of the lamina to block export. ABA concentrations in panicles generally paralleled those in leaves, though the peak level of ABA in the morning in panicles occurred later than in the leaves in some samplings. Although girdling initially increased ABA concentration, it did not prevent a subsequent fall which generally paralleled the decline observed in untreated leaves. The decrease in ABA that occurred despite the block to export and despite continuing stress was, therefore, attributed to changes in the synthesis or metabolism of ABA *within* the leaf.

The probable rate of export of ABA from leaves, calculated from the changes in its concentration due to girdling, was highest at the time of most rapid ABA accumulation and declined thereafter. The percentage export of recently assimilated carbon declined similarly. However, the probable absolute rate of export of photosynthate, computed from stomatal conductance and [^{14}C]-export measurements, was not uniquely related to that of ABA.

Key words: Abscisic acid, leaf water potential, diurnal changes, export, *Pennisetum americanum*.

Introduction

The concentration of the plant hormone abscisic acid (ABA) in leaves is known to be highly sensitive to their water status, increasing markedly as water (Ψ) and turgor (Ψ_p) potentials fall (Walton, 1980; Pierce and Raschke, 1980). Under field conditions, plants frequently experience substantial diurnal changes in the water status of their leaves, and such changes are especially pronounced during drought (Hanson & Hitz, 1982). It might be expected, therefore, that similar diurnal changes should occur in ABA concentration ([ABA]), with the ABA content of the leaves increasing as Ψ falls and *vice versa*.

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Although studies have been conducted with a number of species to examine diurnal changes in [ABA], a simple inverse relationship between [ABA] and Ψ has seldom been observed (McMichael & Hanny, 1977; Quarrie, 1980 and unpublished results; Xiloyannis, Uriu & Martin, 1980; Henson *et al.*, 1982a, 1984; Kannangara, Durley & Simpson, 1982; Burschka, Tenhunen & Hartung, 1983; Loveys and Düring, 1984).

The concentration of ABA in leaves is determined by rates of its synthesis and metabolism, and by rates of import and export. In detached leaves the rise in [ABA] in response to water stress results largely from an increased rate of synthesis (Pierce & Raschke, 1981; Zeevaart, 1980; Murphy, 1984), while during rehydration synthesis is decreased and the rate of metabolism increased (Pierce & Raschke, 1981) leading to a rapid fall in [ABA]. In intact plants there is evidence for significant export of ABA from mature leaves (Goldbach & Goldbach, 1977; Zeevaart, 1977; Setter, Brun & Brenner, 1981; Zeevaart & Boyer, 1984) via phloem-mediated translocation (Hoad, 1973, 1978; Zeevart, 1977; Hoad & Gaskin, 1980; Setter, Brun & Brenner, 1980; Weiler & Ziegler, 1981; Everat-Bourbouloux, 1982; Zeevaart & Boyer, 1984). However, both Hartung (1976) and Hoad (1978) have suggested that the export of ABA may be reduced during water stress.

In this paper, we report a further investigation into the short-term temporal changes occurring in [ABA] in fully-expanded leaf blades of pearl millet (Henson *et al.*, 1982a). Such leaves are primary sources of photosynthetic assimilates for meristematic and other sinks in the plant and, being major sites of ABA production, they may also serve as sources of ABA for such sinks (King & Evans, 1977; Zeevaart, 1977; Morgan, 1980). The export of ABA from the leaf may, therefore, be a significant process in determining the temporal changes in [ABA]. Export has been suggested to account for the lack of correlation on a diurnal basis between [ABA] and Ψ in leaves of grape vines (Loveys & Düring, 1984). In pearl millet an important role for export in regulating leaf [ABA] was suggested by results of phloem-girdling experiments (Henson, 1984; Henson & Mahalakshmi, 1985). Consequently, further studies were undertaken to determine whether certain diurnal changes in [ABA] in the leaves of millet, known to be unrelated to changes in Ψ, could be explained by changes in the extent of ABA export from the leaf.

MATERIALS AND METHODS

Plant culture

Pearl millet (*Pennisetum americanum* [L.] Leeke) cvs BJ 104 and B282, an F_1 hybrid and an inbred line respectively, were grown in the field at ICRISAT Center, near Hyderabad, India, during the dry seasons (February to May) of 1983 and 1984. Maximum day temperatures during sampling in March and April were approx. 35 to 37 °C, atmospheric water vapour pressure deficits were up to 3 kPa and photosynthetic photon flux density at mid-day was approx. 2000 μmol m^{-2} s^{-1}. Rainfall during crop growth was infrequent, and generally contributed less than 2 to 4 d evaporative demand during the life of the crop. Consequently it was easy to impose drought by withholding irrigation.

Plants were grown from seed on a medium depth (about 1 m) alfisol soil. The rows were 0·75 m apart and the plants were thinned to 0·1 m apart within rows. Initially, frequent irrigation from overhead sprinklers ensured a good crop stand, but once the crop was established, water was applied by flooding furrows made

between the rows. The last irrigation was generally given about 14 d after sowing (DAS) though some plots continued to be irrigated for longer periods.

Girdling treatments

Leaves were heat-girdled to block transport via the phloem using a resistive wire heating clamp applied for 10 to 20 s just above the ligule, simultaneously to both leaf surfaces. Alternatively, a heated soldering iron was used. Girdling resulted in a zone of dead cells approx. 2 mm wide across the leaf and was shown to be effective in preventing export of [^{14}C]-labelled assimilates from the leaf (Henson & Mahalakshmi, 1985).

Measurements

Plants were sampled at two stages of growth; (i) prior to flowering when the panicles were 10 to 50 mm long and still enclosed within flag leaf sheaths, and (ii) after flowering during early grain set. At stage (i) [ABA] was determined for both leaves (youngest fully-expanded) and panicles. At stage (ii) only the flag leaf was sampled. Main shoots were used in all experiments with cv BJ 104 but with B282 [Fig. 1(c, f)] both the main shoots and the primary tiller shoots were sampled. However, as the results for main shoots and tillers were very similar, only the pooled results are presented.

Stomatal conductance (g_s) was measured at the mid-point of the leaf on both surfaces using a steady-state porometer (Li-1600; Licor, Lincoln, Nebraska, USA) which maximized boundary-layer conductance. Leaf water potential was determined using a pressure chamber, taking appropriate precautions to minimize post-excision evaporative losses. Panicles and leaves in which the concentrations of ABA were to be determined were rapidly frozen in liquid nitrogen; the former directly after harvest, the latter immediately after Ψ determination. Dry weights were obtained after lyophylization. Concentrations of ABA in leaves and panicles were determined using the method of Quarrie (1978).

The percentage loss from flag leaves of recently assimilated photosynthate was determined following a brief exposure of the leaf to $^{14}CO_2$, as previously described (Henson & Mahalakshmi, 1985). Loss of radioactivity during the first 60 min after exposure was determined in two ways. In the first method a Geiger-Müller tube mounted under the truncated tip of the leaf was used to monitor the loss of radioactivity directly in the field (Henson & Mahalakshmi, 1985). In the second, two portions of leaf, each 10 mm wide on either side of the midrib, were sampled from each leaf, one immediately after exposure to $^{14}CO_2$ and the other 60 min later. The samples were immediately frozen in liquid nitrogen, subsequently lyophylized and ground to a fine powder. Radioactivity was measured by planchette counting at infinite thickness using a Geiger system. The difference in count rate between the two samples was taken to indicate the extent of $^{14}CO_2$ export. Control samples taken immediately after exposure to $^{14}CO_2$ indicated no significant differences between the two sides of the midrib in the amount of assimilated radioactivity.

Results and Discussion

Changes in ABA content during the photoperiod

The concentration of ABA in leaves underwent a substantial increase in mid-morning, only to fall again equally rapidly [Fig. 1(d to f)]. Thereafter [ABA]

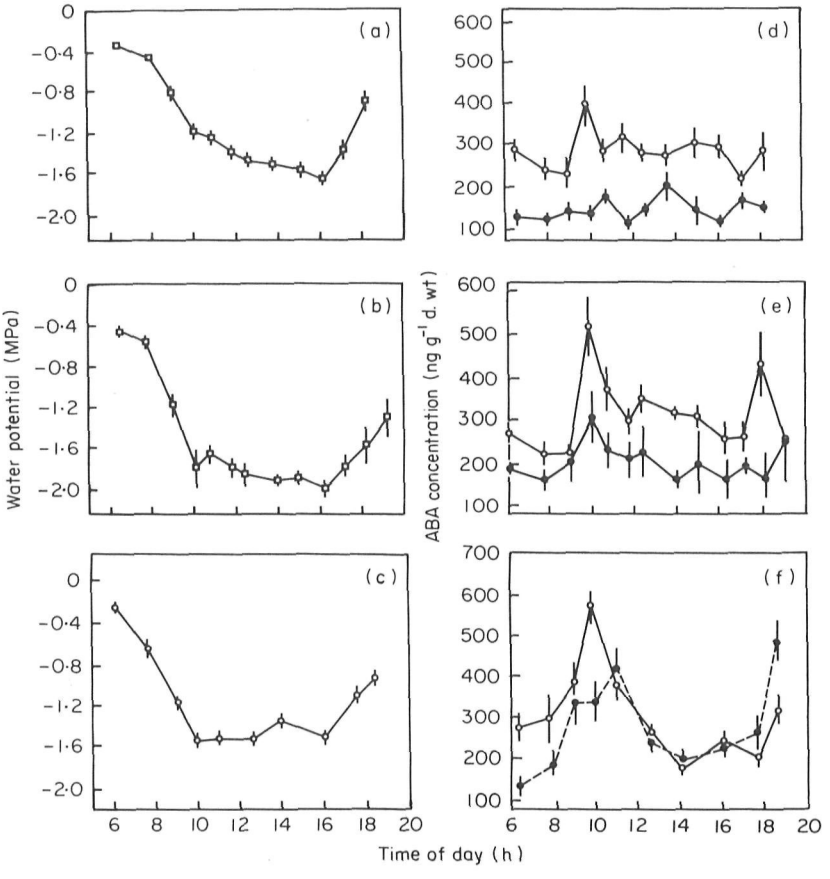

Fig. 1. Changes with time of day in leaf water potential (a, b, c) and ABA concentration of leaves (○) and panicles (●) (d, e, f) of droughted plants of two pearl millet cultivars: BJ 104 was sampled in 1983 40 (a, d) and 48 DAS (b, e); B282 was sampled in 1984 66 DAS (c, f). Data for BJ 104 are for main shoots and are means of 6–7 (a, d) or 3–4 (b, e) samples. Data for B282 are pooled results for main shoots and tillers and are means of 10 to 12 samples. Vertical bars indicate 2 × SE mean.

remained low apart from an increase towards dusk in some samples. This pattern is essentially similar to that observed in an earlier study (Henson *et al.*, 1982a). The rapid rise in [ABA] during mid-morning coincided with a phase of rapidly declining leaf Ψ [Fig. 1(a to c)]. The amplitude of the ABA peak increased with the intensity of water stress as characterized by the extent of the reduction in Ψ. However, the subsequent reductions in [ABA] occurred despite a further fall in Ψ and/or its remaining low for much of the day. Furthermore, a rise in [ABA] near the end of the photoperiod [Fig. 1(e, f)] actually accompanied a *rise* in Ψ.

There is evidence (Pierce & Raschke, 1980; Davies *et al.*, 1981) that levels of Ψ_p rather than Ψ determine ABA accumulation. No measurements were made in the present experiments, but previous work (Henson *et al.*, 1982b) has shown that diurnal changes in Ψ_p parallel those of Ψ and similarly do not account for the rapid changes in [ABA] in the leaf (Henson *et al.*, 1982a).

[ABA] in developing panicles (major sinks for phloem-mobile compounds from the expanded leaf) was either similar to, or lower than, that in leaves and generally

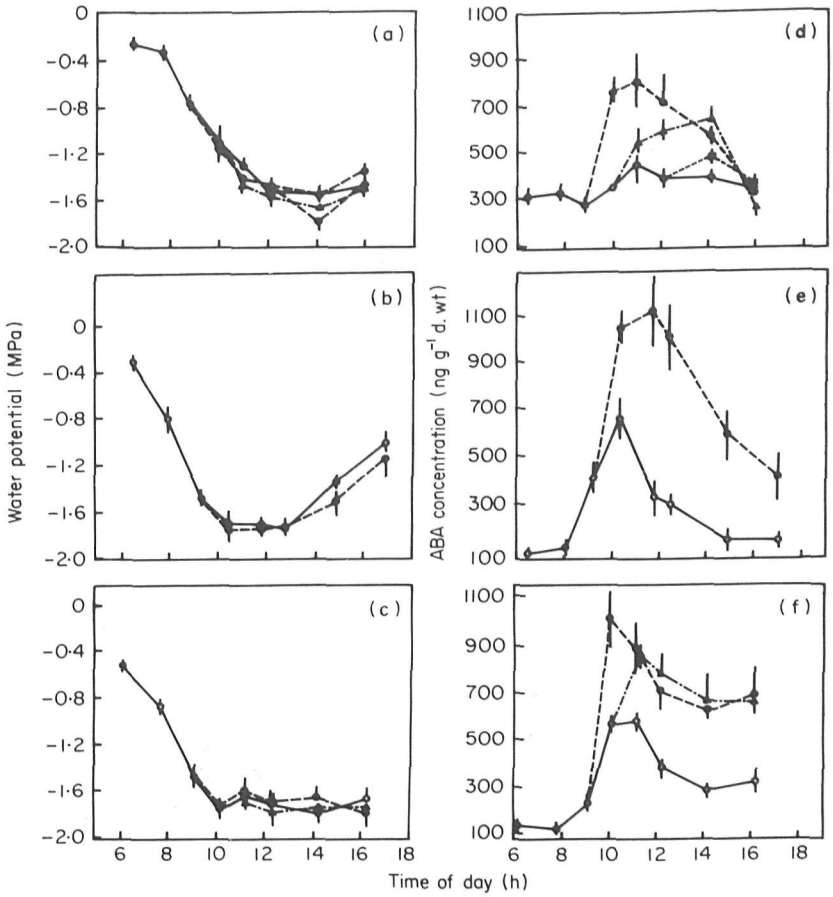

Fig. 2. Effects of girdling on the leaf water potential (a, b, c) and ABA concentration (d, e, f) of flag leaves of three populations of cv BJ 104. All populations were sown on the same day in the same field in 1984 but represent different treatments within the field. Group I (a, d), last irrigation 13 DAS, was sampled 51 DAS. Group II (b, e), last irrigation 49 DAS, was sampled 58 DAS. Group III (c, f), last irrigation 13 DAS, was sampled 58 DAS. Leaves sampled were untreated (○) or girdled at about 0900 h (●), 1000 h (▲) or 1200 h (■). Data are means of six leaves; vertical bars indicate 2 × SE mean.

followed similar time trends [Fig. 1(d to f)]. The temporal shift in the ABA peak in the panicles on two occasions [Fig. 1(d, f)] suggested, however, that the increase in panicle [ABA] might have been linked to the decrease in the leaves.

Effect of girdling on the ABA content of leaves

Heat girdling blocks the export of [^{14}C]-labelled assimilate (Henson & Mahalakshmi, 1985) and causes accumulation of solutes and ABA within the leaf (Henson, 1984). If the reduction in [ABA] of millet leaves in late morning was due to export, it should be prevented by girdling.

Figure 2(d to f) shows the effect of girdling at different times on leaf [ABA] in three different groups of plants of cv BJ 104. The first group [Fig. 2(d)] had only low levels of ABA in ungirdled leaves and no pronounced peak, although levels increased between 0900 and 1000 h. Girdling resulted in a substantial increase in [ABA] without greatly affecting leaf Ψ [Fig. 2(a)]. However, the [ABA] in girdled

leaves subsequently declined to that present in ungirdled leaves in spite of the fact that Ψ remained low.

The second group of plants [Fig. 2(b, e)] sampled 7 d later, 58 DAS, developed lower Ψ and showed a pronounced peak in [ABA] at about 1000 h. Girdling again resulted in a marked increase in [ABA] followed by a decline. Leaf Ψ was changed (lowered) by girdling only in late afternoon.

The third group of plants was also sampled 58 DAS but had been droughted for longer than group two. Effects of girdling on [ABA] were similar to those of the other groups, with [ABA] of girdled leaves changing in parallel with those of untreated leaves [Fig. 2(f)]. Again, girdling had little effect on changes in leaf Ψ [Fig. 2(c)].

Table 1. *Rates of accumulation of ABA (ng g^{-1} d. wt h^{-1}) in ungirdled and girdled leaves of cv Bȝ 104 following different times of girdling*

Plant group*	Approximate time of girdling	Ungirdled	Girdled	Difference
	(a) averaged over 1 h from time of girdling			
I	0900	76	470	394
	1000	95	199	104
II	0900	250	645	395
III	0900	347	786	439
	1000	22	248	226
	(b) averaged over 2 h from time of girdling			
I	0900	77	244	167
	1000	18	128	110
	1200	0	45	45

* Groups referred to are those in Fig. 2.

In all these experiments, therefore, girdling caused a substantial increase in [ABA] while having little effect on leaf Ψ. This increase in ABA can thus be attributed to a block to export, assuming that rates of ABA synthesis and metabolism were not affected directly by girdling (Setter, Brun & Brenner, 1981).

The rate at which [ABA] increased following girdling appeared to depend on the time of girdling (Fig. 2). The differences between rates of change in [ABA] in girdled and ungirdled leaves provide a measure of the probable rate of ABA export from the leaf, and the present data (Table 1), imply that rate of ABA export *decreased* during the morning, being higher between 0900 and 1000 h than subsequently. If this was so, then a sudden *increase* in export rate is unlikely to account for the rapid decline in [ABA] during late morning. Furthermore, this decline occurred in both girdled and in ungirdled leaves (Fig. 2; Table 1). If this decline were not due to export it must have resulted from internal changes in rates of ABA synthesis and/or metabolism in the leaf. Similar changes in [ABA] with time have been found in water-stressed, *detached* millet leaves (Henson & Quarrie, 1981). Clearly, direct measurements of the metabolism and synthesis of ABA in the leaf are needed to resolve this question.

Milborrow (1981) has suggested that ABA may restrict its own biosynthesis by a feed-back inhibition. An alternative is that ABA may stimulate its own metabolism as demonstrated in barley aleurone layers by Ho & Uknes (1982). Either of such processes could underlie the changes in [ABA] shown in Figs 1 and

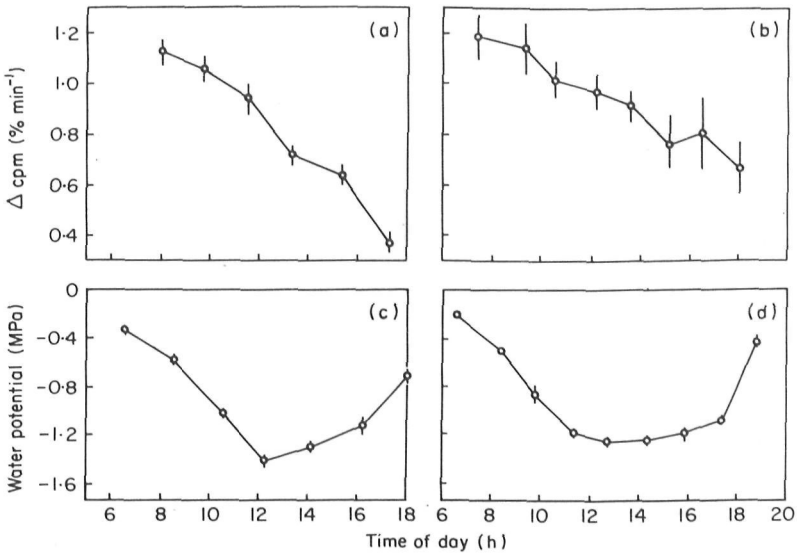

Fig. 3. Changes with time in the proportion of recently assimilated ^{14}C lost from flag leaves of cv BJ 104 (a, b) and in leaf water potential (c, d). ^{14}C loss was determined either *in situ* (a) or after destructive sampling (b) for later laboratory analysis, following dosing with ^{14}C at the mean times indicated as described in Materials and Methods. Data are mean of eight (a, c, d) or six (b) leaves; vertical bars, where they exceed the size of the symbols, indicate 2 × SE mean.

Table 2. *Leaf water potential and the rate of [^{14}C]-labelled assimilate movement (Δ cpm) in the flag leaf of cv BJ 104 at two growth stages**

Growth stage	Treatment	Ψ (MPa) at midday	Δ cpm (% min^{-1})
Panicles just emerged	Irrigated	−0·85 ± 0·05	0·68 ± 0·06
	Droughted	−1·68 ± 0·03	0·64 ± 0·12
Grain present (soft dough)	Irrigated	−0·92 ± 0·06	0·83 ± 0·05
	Droughted	−1·77 ± 0·04	0·73 ± 0·07

* Data are means ± SE; for Δ cpm, $n = 4$ (panicles just emerged) or 5 (grains present); for Ψ, $n = 6$ (both stages). ^{14}C loss was measured between 1300 and 1400 h. At both growth stages, Ψ of irrigated and droughted plants differed significantly at $P < 0{\cdot}001$.

2. A rapid decrease in Ψ in early morning would first stimulate ABA synthesis and promote a rise in levels despite an increased rate of turnover (Milborrow, 1981; Pierce & Raschke, 1981). Later, self-inhibition of synthesis and/or promotion of metabolism at high [ABA] would cause levels to fall. The fact that ABA levels then remain low for several hours implies that a switch mechanism operates, and that there is not a continuous modulation of the rate of synthesis or metabolism.

Relation between ABA and export of photosynthate

Hoad & Gaskin (1980) proposed that the movement of ABA throughout the plant is largely governed by the movement of photosynthate. In millet the percentage of recently assimilated ^{14}C exported during the first hour following uptake was found to decline continuously during the course of the day (Fig. 3). It showed no dependence on leaf Ψ (Fig. 3; Table 2). Both the rate of export of

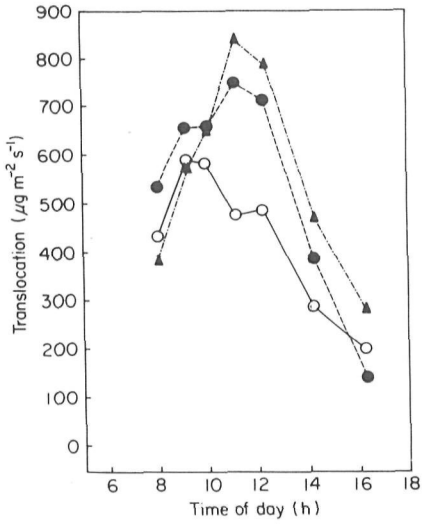

Fig. 4. Changes with time in the estimated rate of translocation of photosynthate from leaves of pearl millet cv BJ 104. Data refer to ungirdled leaves of groups I (▲), II (●) and III (○) shown in Fig. 2. See text for further details.

ABA and the percentage of photosynthate exported from the leaf declined similarly over time. Calculations nevertheless suggest that there may have been little correlation between the export of ABA and the *absolute* rate of export of photosynthate from the flag leaf. Because of variations in amounts of $^{14}CO_2$ offered to leaves, initial levels of radioactivity did not necessarily reflect photosynthetic capacity accurately. However, estimates of the latter, and hence of the absolute rate of translocation, were obtained using measurements of stomatal conductance and a previously established relationship between conductance and rate of CO_2 assimilation (Henson, 1984). While these serve only as an approximation to actual rates of photosynthesis in the field (due to differences in environment and plant ontogeny during measurements), *relative* changes in carbon assimilation during the day should be indicated. From the photosynthetic rates and the data for percentage ^{14}C loss, absolute rates of export of photosynthate from the flag leaves were calculated as described by Pearson (1974), assuming ^{14}C content to decline exponentially (Pearson, 1974; Fussell and Pearson, 1978). The changes in the estimated rate of translocation of photosynthate out of ungirdled leaves of the three BJ 104 populations assayed for ABA (Fig. 2), are shown in Fig. 4. As envisaged by Pearson (1979) there was a peak in carbon translocation during the middle of the day. However, this peak occurred earlier in group three, which were exposed to more water stress. The decline in estimated translocation rate in group three between 0900 and 1200 h contrasts with the increase over this period in the other two groups. Hence, there was no close correlation between the rate of assimilate export and the rate of export of endogenous ABA estimated from girdling treatments (Table 1).

It is suggested that the rate of export of ABA was more related to its rate of synthesis which determined its flux into an exportable, labile pool.

Origin of ABA in developing panicles

The developing panicle is likely to be an important sink for assimilates, and

possibly ABA, from expanded leaf blades. By analogy with wheat (Morgan, 1980; Saini & Aspinall, 1981) developing millet inflorescences may be less prone to water stress than are exposed laminae. Thus, the ABA content of ensheathed panicles may be derived, at least partly, by translocation from the leaves. The parallel relationship between [ABA] of leaf and panicle suggests this (Fig. 1). Data on panicle water relations and tests on ABA production by isolated panicles are however, required before this aspect can be fully resolved.

Conclusions

The following main conclusions are drawn from the data presented in this, and earlier papers (Henson *et al.* 1982a, 1984).

(i) The concentration of ABA in leaves of water-stressed field-grown plants of pearl millet follows a consistent, repeatable daily cycle which is not closely coupled to bulk leaf Ψ or Ψ_p. Similar diurnal changes occur at different stages of development., in different cultivars and in panicles as well as in leaves.

(ii) ABA is exported from leaves but changes in the rate of export are not a cause of the reduction in [ABA] which occurs near mid-day independently of Ψ. The rate of ABA export declines progressively during the morning and appears to be at least partially independent of the absolute rate of export of photosynthate. It may be more dependent on the rate of accumulation of ABA and hence its biosynthesis within the leaf.

(iii) The decline in leaf [ABA] in late morning is the result of internal changes in the rate of ABA synthesis and/or metabolism. These may be a response to the rapid accumulation of ABA triggered by the rapid, early morning fall in leaf Ψ. It is suggested that ABA affects its own synthesis and/or metabolism by feed-back mechanisms.

(iv) Several further questions remain unresolved. The origin of ABA in enclosed, developing panicles of water-stressed plants is presently uncertain, although it seems reasonable to suppose that much, if not all of this, is imported from the leaves. Data are required on the water relations of panicles and their ability to produce and metabolize ABA. Import of ABA via the xylem could contribute to that present in leaves (Loveys, 1984). However, this is unlikely to have been a major source of ABA in millet, because transpiration rates and hence xylem flux would have often been increasing when [ABA] was declining and *vice versa* (see also Henson, 1984). Nevertheless, a recirculation of ABA within the plant probably occurs and should not be overlooked. The increase in [ABA] sometimes observed towards the end of the photoperiod (Fig. 1 this paper and Henson *et al.*, 1982a) is another unexplained phenomenon.

It is apparent from these studies and those of others (see Introduction) that the regulation of ABA concentration in leaves of water-stressed plants is a complex process, and that an understanding of the mechanisms and of their significance in the context of the adaptation of plants to water stress, requires further investigation.

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