

DROUGHT RESISTANCE OF *Sorghum bicolor*. 6. CHANGES IN ENDOGENOUS GROWTH REGULATORS OF PLANTS GROWN ACROSS AN IRRIGATION GRADIENT

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KANNANGARA, T., SEETHARAMA, N., DURLEY, R. C. AND SIMPSON, G. M. 1983. Drought resistance of *Sorghum bicolor*. 6. Changes in endogenous growth regulators of plants grown across an irrigation gradient. *Can. J. Plant Sci.* **63**: 147–155.

A gradient of water stress was created among sorghum plants with a line-source sprinkler irrigation system. Changes in endogenous growth regulators, leaf water potential (ψ_w), solute potential (ψ_s), leaf conductance, leaf temperature, leaf senescence, leaf area and plant height were monitored. Abscisic acid (AbA) levels were increased and phaseic acid (PA) levels were reduced under stress. IAA levels could not be related to stress. Leaf ψ_w , leaf ψ_s and leaf conductance were reduced and leaf temperature was increased by water stress. Leaf area development was more sensitive to stress than stem elongation. Linear correlations between irrigation, AbA, leaf ψ_w , leaf ψ_s and plant height were high ($r > 0.9$). The correlation between AbA and grain yield ($r = -0.65$) was similar to that between irrigation and grain yield ($r = 0.68$), but opposite in sign. Leaf AbA content measured during early growth could thus be used to predict grain yield under a given irrigation regime.

Key words: Abscisic acid, phaseic acid, indole-3-acetic acid, water stress, grain yield, sorghum.

[Résistance à la sécheresse du *Sorghum bicolor*. 6. Changements dans les régulateurs endogènes de croissance de plantes cultivées sur un talus d'irrigation.]
Titre abrégé: Résistance à la sécheresse du sorghum

Un gradient de contrainte hydrique a été provoqué chez des plantes de sorgho irriguées par un système d'aspersion à canalisation centrale. On a observé les changements affectant les régulateurs de croissance internes, le potentiel hydrique foliaire (ψ_w), le potentiel osmotique (ψ_s), la conductance, la température et la sénescence des feuilles, l'indice foliaire et enfin la hauteur des plantes. Les concentrations en acide abscisique (AAb) ont augmenté en conditions de stress hydrique, tandis que les concentrations en acide phaséique (AP) diminuaient. Les concentrations en acide indole-acétique (AIA) n'ont pas été touchées. Le stress hydrique a entraîné une chute du potentiel hydrique, du potentiel osmotique et de la conductance foliaires, ainsi qu'un accroissement de la température foliaire. L'évolution de la surface foliaire était plus sensible au stress que l'allongement de la tige. Les corrélations linéaires obtenues entre l'irrigation, AAb, ψ_w , ψ_s et la hauteur des plantes étaient élevées ($r > 0,9$). La corrélation entre AAb et le rendement grainier ($r = 0,65$) était semblable à la valeur obtenue entre l'irri-

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gation et ce même rendement ($r = 0,68$), mais de signe opposé. Les concentrations en AAb de la feuille mesurées en début de croissance pourraient donc servir de prédicteur du rendement grainier dans un régime de culture sous irrigation.

Mots clés: Acide abscisique, acide phaséique, acide indole-3-acétique, contrainte hydrique, rendement grainier, sorgho, irrigation par canalisation centrale

Water deficit stress alters the growth and development of plants (Hsiao 1973). Levels of abscisic acid (AbA), indole-3-acetic acid (IAA) and ethylene increase (Beardsell and Cohen 1975; Hall et al. 1977) and cytokinins decrease (Itai and Vaadia 1971) under water stress. AbA, in particular, is considered to play a role in drought resistance of plants. For example, more AbA accumulated in the leaves of drought-resistant sorghum and maize than in the drought-susceptible plants of the same species (Larqué-Saavedra and Wain 1974). AbA reduced stomatal conductance (Jones and Mansfield 1970), raised the permeability of plant cells to water (Glinka and Reinhold 1971) and improved water-use efficiency of the plant (Mizrahi et al. 1974). Furthermore, the external application of AbA to well-irrigated plants produced responses similar to those found in water-stressed plants (Quarrie and Jones 1977). Studies of water stress effects on growth regulators have been carried out in growth chamber or greenhouse conditions because, until recently, hormonal changes were difficult to evaluate in plants grown under field conditions.

The objective of this study was to examine how hormones change along a gradient of water stress applied to a sorghum cultivar grown under field conditions. The stress gradient was imposed by a gradient of irrigation applied to plants of the same age which were later harvested for grain yield. A further objective was to compare any changes in hormone levels with other drought-sensitive parameters particularly grain yield.

MATERIALS AND METHODS

The experiment was conducted at ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) near Hyderabad, India (17°

32'N Lat 78° 16'E Long) during December-March 1980/1981 on medium-deep alfisol (red soil). The sorghum cultivar CSH8 was sown in a level plot area in 22 rows each 4 m long oriented north to south with 75-cm row spacing. Plants were spaced 10 cm apart within rows. The plots were irrigated three times (10-day intervals) by a furrow irrigation system for seedling establishment. After seedling establishment, a line-source irrigation system (Hanks et al. 1976) equipped with equally spaced sprinkler heads 170 cm above ground level was used to supply a gradient of irrigation on two occasions at 14-day intervals. The plots were located to the east and west of the line-source which ran parallel to and 38 cm from the first row of each plot. The rows were numbered 1 to 22 away from the line-source, corresponding to the decrease in application of water. Applied water for both plots was monitored by collection funnels placed at the canopy level of each row. In the west plot 3 days after the second irrigation (52 days from planting) measurements were made of leaf water potential (ψ_w), solute potential (ψ_s), AbA, PA, IAA, leaf senescence, leaf area and plant height from six plants within each row (excluding the end rows 1 and 22). In the same plot leaf conductance and leaf temperature were measured from five rows along the moisture gradient prior to harvest of leaf tissue for hormones. Leaf conductances were measured in two plants and leaf temperature in four plants from each of the five rows. Following the above measurements, plants were allowed to reach maturity after four further irrigations (each 10 days apart). Equal amounts of water were given at each irrigation. Total grain yield of each row was recorded. Four plants from both ends of each row were excluded from the grain yield determinations. The plant heights of the east plot were recorded. The slope of the relationship between irrigation and plant height in the east plot was compared with that of the west plot to ensure that irrigation, rather than a soil gradient or other unknown factors, was the primary cause of variation. The slopes were not significantly different from each other (Fig. 1).

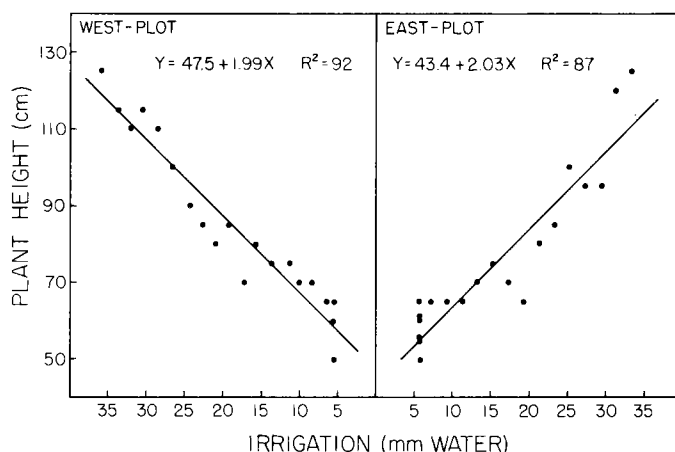


Fig. 1. The relationship between plant height and irrigation in the plots located east and west of the irrigation line-source.

Leaf ψ_w

Leaf ψ_w was measured by the pressure chamber method (Scholander et al. 1965). A strip of leaf tissue taken from the mid-point to the leaf tip from one side of the youngest fully expanded leaf (ligule visible — 12th leaf) was used in each measurement.

Leaf ψ_s

Tissue from the leaf, on the other side of the main vein, adjacent to the area sampled for leaf ψ_w was frozen overnight in a 5-mL disposable syringe. After thawing, the solute potential of the expressed liquid was determined by a Wescor Vapor Pressure Osmometer (Model 513, Logan, Utah).

Plant Growth Regulators

Immediately after the measurement of physiological parameters the top three leaves from each of six plants in every row excluding rows 1 and 22 were harvested, frozen in dry ice, transported to the laboratory and kept in a freezer at -20° prior to freeze-drying. The top three youngest leaves were chosen for hormone extraction since they contained the highest AbA levels under both irrigated and nonirrigated conditions (Kannangara, Durley and Simpson, unpubl. data). The tissue was freeze-dried below 0°C until most of the water was removed and then allowed to dry fully at ambient temperature inside the freeze dryer for a few hours. The freeze-dried tissue was ground to a powder

and batches of 0.625 g were extracted in aqueous methanol (20:80), purified and analyzed for AbA, IAA and PA by high performance liquid chromatography as previously described (Durley et al. 1982).

Leaf Conductance and Temperature

Adaxial (R_{ad}) and abaxial (R_{ab}) leaf resistance of the youngest leaf fully exposed to the sun was measured. Leaf conductance was calculated according to the formula

$$\frac{1}{R_{\text{leaf}}} = \frac{1}{R_{ad}} + \frac{1}{R_{ab}}$$

where R_{leaf} represents the total leaf resistance. Leaf resistance was measured with a Delta-T autoporometer (Delta-T Devices, Cambridge, U.K.). Leaf temperature was measured with a Telatemp infrared thermometer (Telatemp Corp., Calif.). The number of senesced leaves (50% or more yellow) was recorded. Leaf area of unsenesced leaves was measured with a leaf area meter (Hayashi Denkoh Co. Ltd., Tokyo). Plant height was measured from the soil surface to the topmost ligule visible in the culm.

Statistical Analysis

Individual variances with respect to any given parameter were calculated for each of the 20 rows and were tested for homogeneity. Whenever the variances were homogenous a common pool standard error was calculated and given in

the figure to avoid crowding with individual standard errors.

RESULTS

Plant heights were reduced in both east and west plots in response to the gradient of decreasing irrigation (Fig. 1). The correlations between irrigation and height within and between plots were highly significant ($r > 0.9$). This indicates that the variation of height was due to stress effects from irrigation and not to other factors. Only the data for the west plot are presented in the remainder of the paper. Leaf areas were reduced in response to decreasing irrigation (Fig. 2) from rows 5 to 22. The first four rows suffered some waterlogging which reduced leaf area. Leaf area reductions were due in part to decreased leaf size and in part to increased leaf senescence but not to leaf number, which was unaffected by the treatment. The number of leaves senesced increased to a somewhat stable level and then showed a decline in the rows

furthest from the line source (Fig. 2). Over the stable level about 50% of the total number of leaves senesced.

Leaf temperature and leaf conductance were measured in five rows across the irrigation gradient (Fig. 3). Leaf temperature was above the air temperature in all the rows measured. In general, leaf temperature increased and leaf conductance decreased as the amount of irrigation to the plant rows decreased (Fig. 3). Leaf conductance in the well-irrigated plants was nearly three times that of the severely stressed plants.

Except in the first four rows, where water logging occurred, leaf ψ_w became increasingly more negative with decrease in irrigation water (Fig. 4). The leaf ψ_w values changed about twofold over the irrigation gradient. The leaf ψ_s values also decreased with reduction in irrigation (Fig. 4). Leaf ψ_s varied over a narrower range than leaf ψ_w . The changes in leaf ψ_s were insufficient to maintain a positive turgor

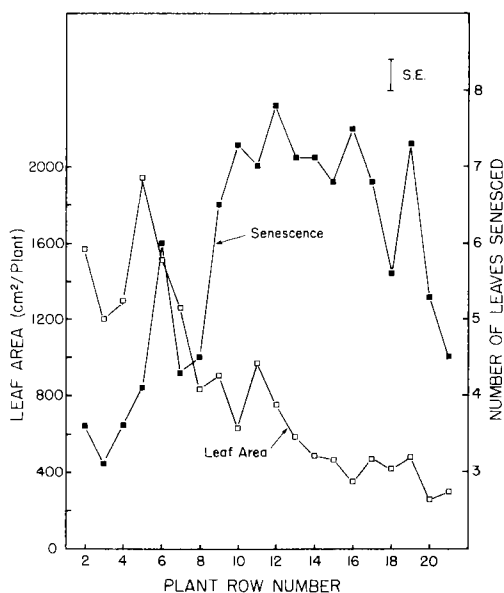


Fig. 2. The relationship between row number and leaf area (excluding three top leaves) per plant (\square — \square) and number of senesced leaves per plant (\blacksquare — \blacksquare) in *Sorghum bicolor*. L. SE represents pooled standard error for senesced leaves.

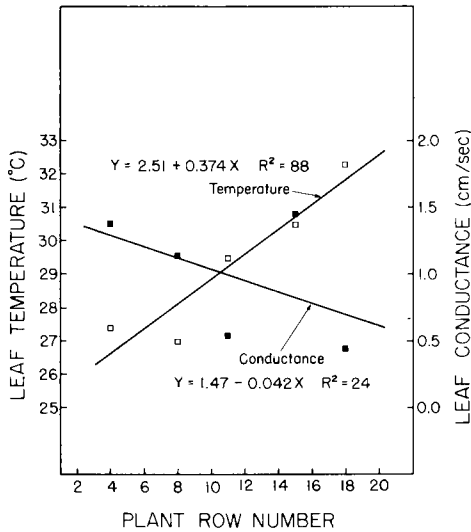


Fig. 3. The relationships between row number and average leaf temperature (□—□) and average leaf conductance (■—■) in *Sorghum bicolor*, L.

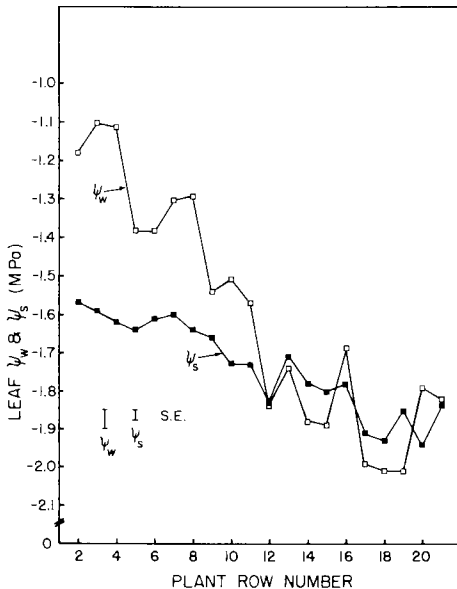


Fig. 4. The relationships between row number and leaf ψ_w (□—□) and leaf ψ_s (■—■) in *Sorghum bicolor* L. SE represents the pooled standard error.

potential ($\psi_w - \psi_s$) in most of the rows beyond row 12.

Leaf AbA levels increased with decreasing irrigation (Fig. 5). Leaf AbA content was low in the first eight rows, then increased rapidly to a relatively stable level in the rows subjected to extreme stress. A very large increase in AbA content occurred between rows 8 to 12. AbA concentration was very sensitive to stress, ranging over the irrigation gradient from 50 to 800 ng/g dry wt.

The levels of PA decreased with increasing water stress (Fig. 5). The changes in PA were not as pronounced as the changes in AbA, changes of IAA levels with irrigation could not be described by simple regressions (Fig. 6). All the correlation coefficients of IAA with other parameters were nonsignificant.

Grain yield was reduced in rows 3 and 4, due to water logging, from the maximum value in row 5. From row 5 to row 22 grain yield decreased with decreasing irrigation. Row 5 also had the tallest plants with the largest leaf area (Fig. 2).

The simple linear correlation coefficients among various parameters are given in Table 1. AbA levels were highly correlated with irrigation (-0.94), grain yield (-0.65), leaf ψ_w (-0.90), leaf ψ_s (-0.93), height (-0.91) and leaf area (-0.86). The correlation between AbA and grain yield ($r = -0.65$) was nearly the same as the correlation between irrigation and grain yield ($r = 0.68$) except opposite in sign. AbA was positively correlated with leaf senescence. Leaf senescence was significantly correlated with parameters of leaf water status. PA was negatively correlated with AbA and the correlation coefficients of PA with irrigation, leaf water status and growth parameters were lower than the corresponding correlation coefficients of those parameters with AbA. PA was not significantly correlated with leaf senescence. Linear correlation coefficients of IAA with other parameters were not significant.

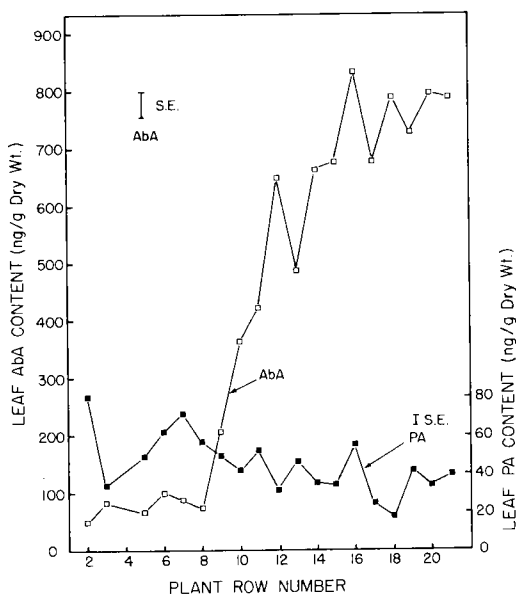


Fig. 5. The relationships between row number and leaf AbA content (\square — \square) and PA content (\blacksquare — \blacksquare) in *Sorghum bicolor* L. SE represents the pooled standard error.

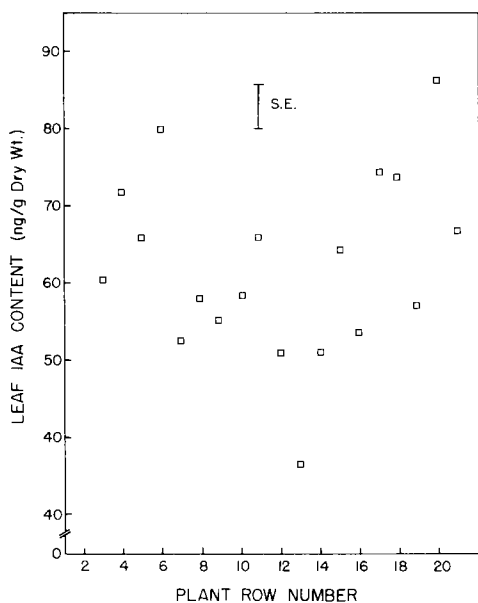


Fig. 6. The relationship between row number and leaf IAA content in *Sorghum bicolor* L. SE represents the pooled standard error.

DISCUSSION

The line-source was effective in creating a gradient of stress under field conditions. The reduction in growth, leaf ψ_w , leaf ψ_s , leaf conductance and grain yield together with increases in leaf senescence and leaf temperature were found to be approximately in proportion to irrigation. The most sensitive of these parameters was leaf area development which was reduced to about one-seventh under stress. The reductions lessened the transpiration area and hence reduced water loss through the plant canopy. The sensitivity of leaf area development to stress is considered to reflect the drought resistant character of a plant (Boyer and McPherson 1975). The irrigation gradient, however, did not alter the phenology of the plants. This is important in relation to the measurement of hormone responses to a changing environment since hormones can be expected to change with phenological changes in the plant. Furthermore, it should be emphasized that all

Table 1. Correlation coefficients of the parameters studied across the moisture gradient

	Irrigation	Leaf ψ_w	Leaf ψ_s	AbA	PA	IAA	Height	Leaf area	Senescence
Leaf ψ_w	0.923								
Leaf ψ_s	0.931	0.913							
AbA	-0.949	-0.907	-0.930						
PA	0.629	0.706	0.768	-0.657					
IAA	-0.296 NS	-0.261 NS	-0.434 NS	0.231 NS	-0.151 NS	-0.124 NS			
Height	0.920	0.836	0.857	-0.912	0.679	-0.056 NS	0.950		
Leaf area	0.876	0.748	0.791	-0.861	0.533	0.126 NS	-0.731	0.706	
Senescence	-0.706	-0.786	-0.646	0.735	-0.439 NS	-0.123 NS	0.507	0.455	-0.419 NS
Grain wt	0.687	0.733	0.723	-0.653	0.613				

NS = not significant at $P = 0.05$.

measurements and leaf harvests were made on the same day and at approximately the same time. This ensured that all plants were of the same age and had been exposed to a similar environment except for moisture. This is important since it is known that hormone levels and leaf water status parameters vary diurnally (Kannangara et al. 1982a) and from day to day in sorghum according to environmental conditions (Kannangara et al. 1982b). It is also known that AbA can vary according to leaf age (Raschke and Zeevaart 1976).

Leaf AbA content increased with decreasing levels of irrigation to the plants between rows 8 and 22. A previous study also demonstrated an increase in leaf AbA content in sorghum cultivars subjected to water stress under field conditions (Kannangara et al. 1982b; Durley et al. 1983); however, these increases of AbA to stress included some of the variations due to different environments because measurements were made on different days. In the present study all the physiological measurements were made on the same day and at approximately the same time. This study indicates a distinct threshold at about row 8 where rapid synthesis of AbA commenced at a leaf ψ_w of about 1.3 MPa (compare Figs. 4 and 5). The threshold leaf ψ_w values have been observed in many plant species grown under a variety of conditions (Walton 1980). In pot-grown sorghum plants increased AbA synthesis occurred at a high value of leaf ψ_w (-1.0 MPa) (Beardsell and Cohen 1975). The rapid increase in AbA levels in rows 8-12 corresponded to a marked decline in leaf ψ_w . It is noteworthy that this AbA increase corresponded with a decline in turgor pressure (leaf $\psi_w - \psi_s$) from 0.3 MPa to 0 MPa (Fig. 4). Pierce and Raschke (1980) suggest that AbA synthesis is a turgor-sensitive process. Although stomatal conductivity decreased as stress increased (Fig. 3), there were insufficient values to determine the relationship with AbA.

The increase in AbA levels was also cor-

related with a marked decline in plant height and leaf senescence. The AbA levels were measured in the top three leaves, not in the senescing leaves which contain less AbA than younger leaves (Sivakumaran and Hall 1978; Eze et al. 1981) and also have a low capacity to synthesize AbA (Eze et al. 1981).

While AbA levels increased with increasing water stress, levels of PA, a principal metabolite of AbA, decreased with increasing water stress (Fig. 5). A similar relationship in the relative levels of AbA to PA was found in a diurnal study of hormone changes in sorghum leaves (Kannangara et al. 1982a). The high levels of AbA relative to PA in stressed plants imply that in the short-term AbA is in some way sequestered or not readily metabolized. It is also possible that PA does not accumulate due to immediate conversion to DPA or conjugated forms (Durley et al. 1983).

In the present study with cultivar CSH8 a distinct pattern of change in IAA levels was not observed. However, a previous study reported that IAA levels in leaves of two sorghum cultivars declined with drought stress (Kannangara et al. 1982b). Another study showed a distinct rise with stress in levels of conjugated IAA in a cultivar CSV5 (Durley et al. 1983).

The changes in physiological processes under stress may either directly or indirectly influence the final grain yield. All the physiological parameters except IAA were significantly correlated with grain yield. AbA was the most sensitive parameter to stress as it changed by 16-fold and was also highly correlated with leaf water status. The other parameters; plant height, leaf area, leaf ψ_w , and leaf ψ_s which correlated significantly with irrigation changed proportionately less than AbA under stress. Thus, in terms of these latter parameters, the range over which cultivars of different drought response can be selected from an array is limited. Blum (1974) found leaf ψ_w to be a weak parameter for distinguishing cultivar response to

drought in sorghum. Thus leaf AbA content measured at an early stage of growth might be used to predict grain yield under a given irrigation regime.

ACKNOWLEDGMENTS

The authors thank Dr. J. Peacock and Dr. F. R. Bidinger for the use of facilities at ICRISAT Centre, and Mrs. J. Montgomery and Messrs. A. Ma, D. M. Reddy, K. Reddy and H. Rao for excellent technical assistance. Professor J. A. D. Zeevaert (Michigan State University) is thanked for generously providing a sample of phaseic acid. This research was funded by a grant from the International Development Research Centre, Ottawa.

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