



## Physiological Responses of Cotton to Water Deficit in Pakistan

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### ABSTRACT

*In Pakistan most cotton is grown under irrigation. Between 1994 and 1997 a series of experiments were conducted at two sites, The Postgraduate Agriculture Research Station of the University of Agriculture, Faisalabad and the Central Cotton Research Institute in Multan, to examine the effects of reduced irrigation on the yield and physiology of local cultivars. In most cases reducing the frequency of irrigation resulted in lower yields of seed cotton, although occasionally mild water stress increased yields. Chlorophyll fluorescence analysis revealed no significant change in Fv/Fm ratios under moderate to severe water deficit, indicating that PSII electron transport was not impaired. Leaf chlorophyll contents, as measured with a Minolta SPAD meter, increased under water deficit. Stomatal conductance, measured either with a porometer or an infra-red gas analyser, was reduced by water deficit, with consequent reductions in gas exchange parameters (net photosynthesis, transpiration and water use efficiency) and an increase in leaf temperature (measured with an infra-red thermometer). Concentrations of Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>++</sup> and Ca<sup>++</sup> in leaf sap were not affected much by mild water deficit, but increased under severe water stress. Of the anions, Cl<sup>-</sup> and SO<sub>4</sub><sup>-</sup> showed the greatest increases in concentration under reduced irrigation. Concentrations of the quaternary ammonium osmoprotectant glycinebetaine increased to about 100 mol m<sup>-3</sup> in leaf sap under severe water deficit. None of the physiological parameters alone could account for varietal differences in yield under reduced irrigation.*

### Introduction

In the Southern Punjab and Northern Sindh cotton growing region of Pakistan, most of the crop is grown with about eight flood irrigations per season. The growing population and changing lifestyle in the cities will increase the demand for water for domestic use and for power generation, as well as for food crops. This report covers the physiological aspects of experiments designed to evaluate cotton production under reduced irrigation.

### Material and Methods

Between 1994 and 1997 we conducted a series of experiments at two sites to examine the effects of reduced irrigation on the yield and physiology of local cultivars. Cultivation followed normal practices for the area, except that at Faisalabad there was no irrigation in part of the experiment after the first irrigation (25-30 days after sowing). In Multan irrigation was applied when xylem water potentials, measured with a pressure chamber, reached 1.9, 2.4 or 3.0 MPa for control, moderate and severe drought treatments respectively. Physiological measurements were made at both sites during September or early October.

Chlorophyll fluorescence analyses were performed in the field using dark-adaptation leaf clips and either a Morgan CF-1000 fluorometer or an OS-100 modulated

fluorometer. The former was only used in Faisalabad in 1994.

A Minolta SPAD-502 chlorophyll meter was used to quantify chlorophyll contents of leaves. This instrument measures the absorbance by chlorophyll of light at 650 nm shone through the leaf, and corrects for non-specific absorbance at about 940 nm. Results are given as SPAD instrument readings.

Gas exchange measurements were made on young expanded leaves in the morning with a CIRAS-1 infra-red gas analyser (PP Systems Ltd.) and a broad-leaf chamber at saturating photosynthetic (400-700 nm) photon flux densities (>1,500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Dry air supply to the leaf chamber at 350  $\mu\text{mol mol}^{-1} \text{CO}_2$ .

Further stomatal conductance measurements were made with an AP4 porometer (Delta-T Devices Ltd.). The instrument was carefully calibrated each morning, and used with the limits specified for this porometer. Both abaxial and adaxial surfaces were measured. Individual leaf temperatures were measured at close range with various infra-red thermometers.

Fresh leaf samples were frozen in microcentrifuge tubes and taken to Bangor, Wales, for chemical analysis. Sap was extracted by centrifugation after thawing the samples, and inorganic ions measured by ion chromatography. Quaternary ammonium compounds (mainly glycinebetaine) were measured using modified periodide or reineckate assays, and the

results compared with data obtained on selected samples by HPLC on a Na<sup>+</sup>-form cation exchange column (Gorham, 1996).

## Results

Since the extent of the drought stress, both throughout the season and at the time of measurement, varied with year and location, data from different years and sites are presented below to give a balanced picture of responses to drought. As far as seed cotton yields are concerned (Table 1), moderate stress gave yields higher than 90% of the fully irrigated values. Except for Faisalabad in 1996, where climatic and biotic stress factors favoured the drought treatment relative to the irrigated treatment in terms of yield, severe stress reduced yields by as much as 38 %.

Net photosynthetic rate, transpiration and stomatal conductance were all reduced by severe water deficits (Table 2). Under more moderate conditions (irrigation at 1.9 MPa plant water potential) the reductions in these parameters were small. Internal CO<sub>2</sub> concentrations were not, or only slightly, affected by water deficits, indicating parallel effects on stomatal conductance and mesophyll resistance. Instantaneous water use efficiency was slightly lower in the severe treatment than in the controls. Leaf chlorophyll contents (as measured *in situ* with the SPAD meter) increased in both water-stress treatments, although the contribution of increasing leaf thickness was not quantified.

In the two years where direct comparisons are possible, stomatal conductance values measured with a porometer (Table 3) were slightly higher than those calculated from the gas exchange data (Table 2), but the same pattern of decreasing conductance with increasing water deficit was observed. Adaxial conductance values were higher than abaxial values in some cases, whereas in others (notable Multan in 1995) abaxial stomatal conductances were higher. Adaxial and abaxial measurements were made consecutively for each leaf, and no other factors were obviously responsible for these differences in adaxial/abaxial ratios, although similar observations were made in Spain (Gorham *et al.*, 1998) and Turkey (Gorham, unpublished observations).

The decreases in stomatal conductance with water deficit, and hence in transpirational cooling, resulted in higher leaf temperatures, both derived from the gas exchange data and energy balance calculations, and measured directly with infra-red thermometers (Table 4). The highest leaf temperatures were recorded in Faisalabad in 1995 (38.7 °C) and in Multan in 1996 (37.0 °C), when maximum seed cotton yields were recorded (Table 1).

Chlorophyll fluorescence analysis indicated little direct effect of drought on electron transport associated with photosystem II (Table 5), although there was a

general reduction in Fv/Fm ratios at midday in Multan in 1996.

The increases in sap solute concentrations shown in Table 6 for Multan in 1996 are representative of data from other experiments both at Multan and Faisalabad. The increases can only partly be explained by a reduction in leaf water content (data not shown). Substantial increases in concentrations of potassium, calcium, sulphate and glycinebetaine were recorded. In Faisalabad the concentrations of sodium, chloride and magnesium were higher than at Multan. Except for the lack of and increase in malate concentrations, the data are in agreement with similar observations in Spain in 1996 (Gorham *et al.*, 1998).

## Conclusions

Differences between the cultivars for physiological parameters were small (data not shown) compared with the large differences between the irrigated and water deficit treatments. In general, seed cotton yields were not closely correlated with physiological parameters, although there was a relationship between high mean leaf temperature and the percentage reduction in yield in different experiments. Reduced yield was not caused primarily by reduced photosynthetic capacity on a leaf area basis. Increases in glycinebetaine, potassium and calcium concentrations contributed to osmotic adjustment. Mild water stress did not result in a substantial decrease in yield.

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## References

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**Table 1. Seed cotton yields. Values in parentheses are % of control yields. The terms control, moderate and severe refer to irrigation at 1.9, 2.4 or 3.0 MPa plant water potential at Multan, and to full irrigation, or one irrigation 50 days after sowing, or one irrigation 30 days after sowing respectively at Faisalabad.**

| Location   | Year | Units   | Vars. | Control | Moderate  | Severe    |
|------------|------|---------|-------|---------|-----------|-----------|
| Faisalabad | 1994 | g/plant | 18    | 42      | 43 (102)  | 38 (90)   |
| Faisalabad | 1995 | g/plant | 18    | 58      |           | 36 (62)   |
| Faisalabad | 1996 | g/plot  | 10    | 842     |           | 967 (115) |
| Multan     | 1995 | g/plant | 4     | 103     | 93 (90)   | 85 (83)   |
| Multan     | 1996 | kg/ha   | 4     | 1689    | 1574 (93) | 1325 (78) |
| Multan     | 1997 |         |       |         |           |           |

**Table 2. Photosynthetic gas exchange parameters measured with a CIRAS-1 infra-red gas analyser.**

| Location   | Year | Vars | Control | Moderate | Severe |
|--|------|------|---------|----------|--------|
| Net Photosynthesis ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )                |      |      |         |          |        |
| Faisalabad   | 1996 | 10   | 24.4    |          | 15.9   |
| Multan   | 1997 | 4    | 28.1    | 27.0     | 19.0   |
| Transpiration ( $\text{mmol m}^{-2} \text{s}^{-1}$ )                       |      |      |         |          |        |
| Faisalabad   | 1996 | 10   | 15.7    |          | 10.9   |
| Multan   | 1997 | 4    | 12.9    | 12.8     | 9.7    |
| Stomatal conductance ( $\text{mmol m}^{-2} \text{s}^{-1}$ )                |      |      |         |          |        |
| Faisalabad   | 1996 | 10   | 503     |          | 262    |
| Multan   | 1997 | 4    | 546     | 536      | 337    |
| Internal CO <sub>2</sub> concentration ( $\mu\text{mol mol}^{-1}$ )        |      |      |         |          |        |
| Faisalabad   | 1996 | 10   | 237     |          | 226    |
| Multan   | 1997 | 4    | 236     | 239      | 237    |
| Water use efficiency ( $\mu\text{mol H}_2\text{O mmol}^{-1} \text{CO}_2$ ) |      |      |         |          |        |
| Faisalabad   | 1996 | 10   | 1.55    |          | 1.44   |
| Multan   | 1997 | 4    | 2.18    | 2.11     | 1.95   |
| Chlorophyll content (SPAD units)   |      |      |         |          |        |
| Multan   | 1996 | 4    | 52.0    | 54.1     | 55.8   |
| Multan   | 1997 | 4    | 45.9    |          | 50.0   |

**Table 3. Stomatal conductance (gs,  $\text{mmol m}^{-2} \text{s}^{-1}$ ) measured by porometry.**

| Location   | Year | Parameter       | Control | Moderate | Severe |
|------------|------|-----------------|---------|----------|--------|
| Faisalabad | 1995 | Adaxial gs      | 327     |          | 131    |
| Faisalabad | 1996 | Adaxial gs      | 315     |          | 133    |
|            |      | Abaxial gs      | 294     |          | 146    |
|            |      | Total gs        | 609     |          | 279    |
|            |      | Adaxial/Abaxial | 1.07    |          | 0.91   |
| Multan     | 1995 | Adaxial gs      | 335     | 251      | 239    |
|            |      | Abaxial gs      | 668     | 493      | 453    |
|            |      | Total gs        | 1003    | 744      | 692    |
|            |      | Adaxial/Abaxial | 0.50    | 0.51     | 0.53   |
| Multan     | 1996 | Adaxial gs      | 672     | 157      | 86     |
|            |      | Abaxial gs      | 532     | 151      | 84     |
|            |      | Total gs        | 1203    | 308      | 170    |
|            |      | Adaxial/Abaxial | 1.45    | 1.55     | 1.30   |
| Multan     | 1997 | Adaxial gs      | 349     | 234      | 102    |
|            |      | Abaxial gs      | 391     | 268      | 71     |
|            |      | Total gs        | 740     | 502      | 173    |
|            |      | Adaxial/Abaxial | 0.89    | 0.87     | 1.44   |

**Table 4. Leaf temperature (infra-red thermometry).**

| Location   | Year | Vars. | Control | Moderate | Severe |
|------------|------|-------|---------|----------|--------|
| Faisalabad | 1995 | 18    | 30.9    |          | 38.7   |
| Faisalabad | 1996 | 10    | 26.6    |          | 32.6   |
| Multan     | 1995 | 4     | 30.6    | 32.3     | 31.9   |
| Multan     | 1996 | 4     | 32.4    | 34.6     | 37.0   |
| Multan     | 1997 |       | 27.4    | 27.6     | 29.0   |

**Table 5. Chlorophyll fluorescence analysis (Fv/Fm ratios).**

| Location   | Year | Time  | Control | Moderate | Severe |
|------------|------|-------|---------|----------|--------|
| Faisalabad | 1994 | 10.00 | 0.7833  |          | 0.7841 |
| Faisalabad | 1996 | 10.00 | 0.7301  |          | 0.7224 |
| Multan     | 1996 | 12.00 | 0.7101  | 0.7343   | 0.7284 |
|            |      | 16.00 | 0.7867  | 0.8115   | 0.8066 |

**Table 6. Solute concentrations (mol m<sup>-3</sup>) in sap of cotton plants grown at Multan in 1996.**

| Parameter      | Control | Moderate | Severe  |
|----------------|---------|----------|---------|
| Sodium         | 43 ± 3  | 51 ± 4   | 56 ± 5  |
| Potassium      | 119 ± 5 | 153 ± 8  | 179 ± 7 |
| Magnesium      | 63 ± 3  | 75 ± 4   | 91 ± 6  |
| Calcium        | 139 ± 5 | 164 ± 6  | 200 ± 6 |
| Chloride       | 76 ± 3  | 89 ± 3   | 97 ± 4  |
| Nitrate        | 8 ± 1   | 7 ± 1    | 8 ± 1   |
| Malate         | 59 ± 3  | 70 ± 3   | 62 ± 3  |
| Sulphate       | 95 ± 3  | 109 ± 4  | 129 ± 4 |
| Glycinebetaine | 39 ± 3  | 66 ± 4   | 79 ± 5  |