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Carbohydrates and water status in wheat plants under water stress

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SUMMARY

Changes in sugar content during water stress and recovery were examined in leaves of two varieties of durum wheat (*Triticum durum*). The drought-resistant Mohamed Ben Bachir (MBB) from Algeria and the drought-sensitive European variety Capdur differed in the time and type of sugar increase during water stress. Glucose accumulated at a rate closely corresponding with decreasing water potential but more rapidly and to a higher concentration in MBB than in Capdur. Sucrose content correlated less well than that of monosaccharides with changes in water potential. Glucose and, to a lesser extent, fructose appeared to play an important role during water stress and to be more sensitive indicators of the degree of stress and of potential tolerance than proline which increased later and to the same extent in both varieties. After rewatering, the amounts of accumulated solutes in leaves of both types fell quickly to normal, coincident with relatively rapid growth.

Key words: Wheat, water stress, carbohydrates, glucose, sucrose, fructose.

INTRODUCTION

Water stress affects many physiological and biochemical processes in plants (Hsiao, Acevedo & Fereres, 1976; Hanson & Hitz, 1982), resulting in the alteration of some metabolic pathways. Among the major effects are those involving carbohydrate metabolism, with the accumulation of sugars and a number of other organic solutes (Iljin, 1957; Kameli, 1990). Carbohydrate changes are of particular importance on account of their direct relationships with such physiological processes as photosynthesis, translocation and respiration.

Sugars have long been known to increase in a wide range of plants grown at low moisture levels and under salinity, e.g. in wheat (Vassiliev & Vassiliev, 1936), pasture grasses (Julander, 1945) and cotton (Eaton & Ergle, 1948). The rate and extent of increase in sugar content depends on the environmental conditions, species, and even on the genotype within the same species. Iljin (1957) pointed out that the majority of xerophytes have the ability to produce a high content of sugars in dry habitats, whereas mesophytes accumulate far less. Other types of organic solutes increase in different organisms under

stress conditions, including glycerol in yeast (Spencer, 1968), polyols in fungi and some green plants (Lewis & Smith, 1967; Hellebust, 1976; Borowitzka, 1981), amino acids, mainly proline (Singh, Aspinall & Paleg, 1972; Bates, 1973), and glycine betaine (Wyn Jones & Storey, 1978; Hanson & Hitz, 1982) in various crop plants.

Although the increase in sugar content in response to water stress is well known, few reports indicate the concentrations of sugars at different levels of stress and recovery. Three principal sugars, glucose, fructose and sucrose (Acevedo *et al.*, 1979; Jones, Osmond & Turner, 1980; Ackerson, 1981) accumulate in crop plants but little information is available as to which of these is most related to water stress. The aim of the present work was to study the relationship between sugar content and water status, during the development of water stress and after rewatering, in two varieties of wheat of differing drought tolerance.

MATERIALS AND METHODS

Growth of plants

The durum wheat (*Triticum durum* L.) varieties, Mohamed Ben Bachir 8037 (MBB) from Algeria and Capdur (Cap) from NIAB Cambridge were selected on the basis of growth analysis and differential responses to water stress (Kameli, 1990) in a

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preliminary screening experiment comparing 12 varieties of wheat.

Seeds were soaked for 24 h and germinated in vermiculite for 6 d. Sets of 4 seedlings were then planted in 10 cm pots of mixed compost and vermiculite (2:1 v:v). The plants were maintained under growth room conditions (PFR 90–100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 16 h day-length, 22 ± 2 day and 18 ± 2 °C night temperatures, relative humidity 70%). After 17 d of growth with normal water supply, stress was applied by withholding water from half of the pots, selected at random. Control plants received full water treatment throughout the period of the experiment.

Extraction and estimation of soluble sugars

The youngest fully-expanded leaf of one plant from each of 4 replicate pots was harvested 3–4 h after the start of the light period and cut into 0.5 cm lengths. Samples of 0.2–0.4 g f. wt were extracted three times with 5 ml 80% ethanol at 70 °C for 15 min. F. wt/d. wt ratios were determined from similar samples, so that solute content could be expressed on a d. wt basis. The volume of extract was then reduced under compressed air to 3–4 ml, cleared by adding a similar volume of aluminium hydroxide ($\text{Al}(\text{OH})_3$; 20%, w/v in water) and deionized with equal weights of Amberlite IR-45 (OH^-) and IR-120 (H^+) ion-exchange resins.

Sugars were estimated using the GLC method of Holligan and Drew (1971). A known volume of the cleared and deionized extract was dissolved in 0.85 ml of anhydrous pyridine, silylated with 0.1 ml hexamethyldisilazane and 0.05 ml trimethylchlorosilane and left overnight. Volumes of 1–5 μl were analyzed, using a PYE UNICAM series 204 GLC, on two coiled glass columns, 150 cm length, 4 mm internal diameter, containing Chromasorb WHP, 100–200 mesh size as support phase, coated with 2% SE 52 silicone gum as stationary phase. The temperature of the column was set from 140 to 290 °C at 4° per minute with injector and detector temperatures 260° and 350° respectively. Glucose values were recorded as the sum of the α and β peaks.

Estimation of proline

Free proline was determined by the method of Bates (1973) from samples (0.2–0.5 g f. wt) of leaf tissue, using the youngest fully expanded leaf (leaf 4) of another plant from the same pots as the plants taken for sugar estimation.

Water status

Water potential (ψ) was measured using a pressure chamber (Scholander *et al.*, 1965). Relative water

content (RWC) was determined by the relative turgidity technique (Weatherley, 1950), as modified by Barrs & Weatherley (1962).

RESULTS

Differences in water status between stressed and unstressed plants became clear only after 8 d of withholding water, when ψ and RWC began to decrease in stressed plants of both varieties (Figs 1 and 2). These values continued to fall until day 14, at which time the plants were rewatered. Two days

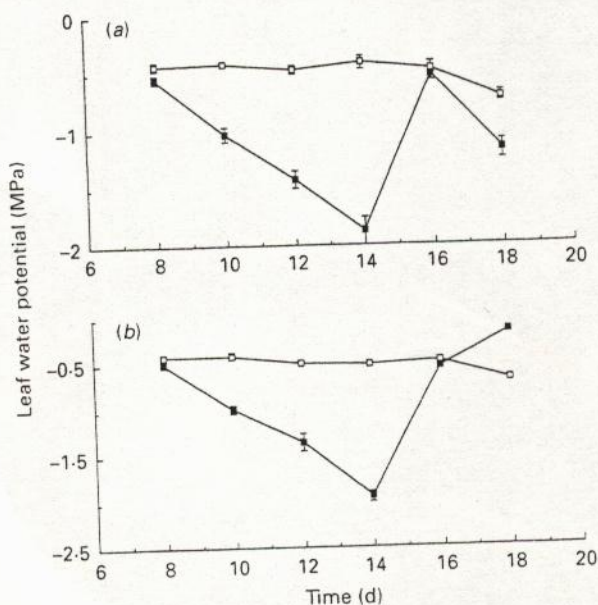


Figure 1. Leaf water potential of stressed (■) and control (□) plants of durum wheat varieties (a) MBB and (b) Capdur from 8 d after withholding water and following rewatering on day 14 (means of 4 replicates).

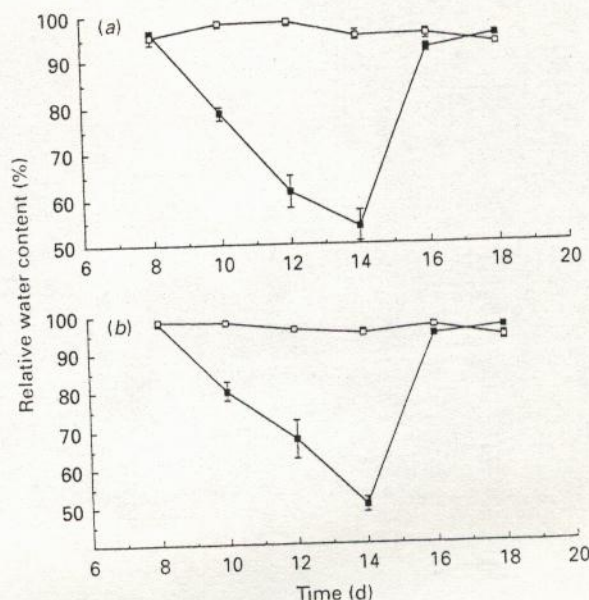


Figure 2. Relative water content of leaves from stressed (■) and control (□) plants of (a) MBB and (b) Capdur from 8 d after withholding water and following rewatering on day 14 (means of 4 replicates).

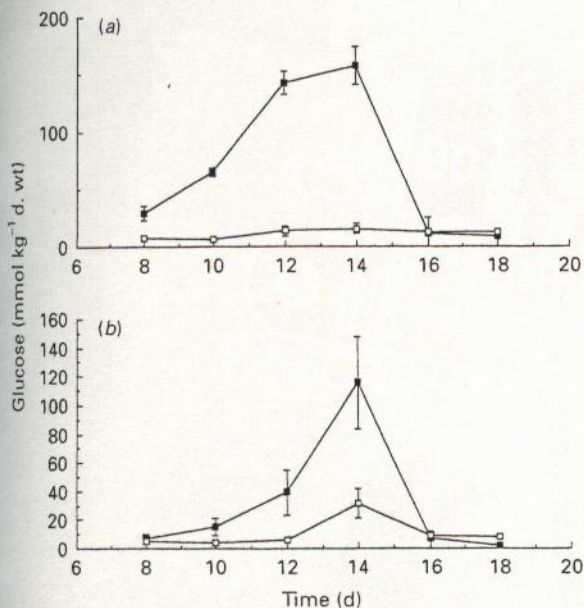


Figure 3. Glucose content of leaves from stressed (■) and control (□) plants of (a) MBB and (b) Capdur from 8 d after withholding water and following rewatering on day 14 (means of 4 replicates).

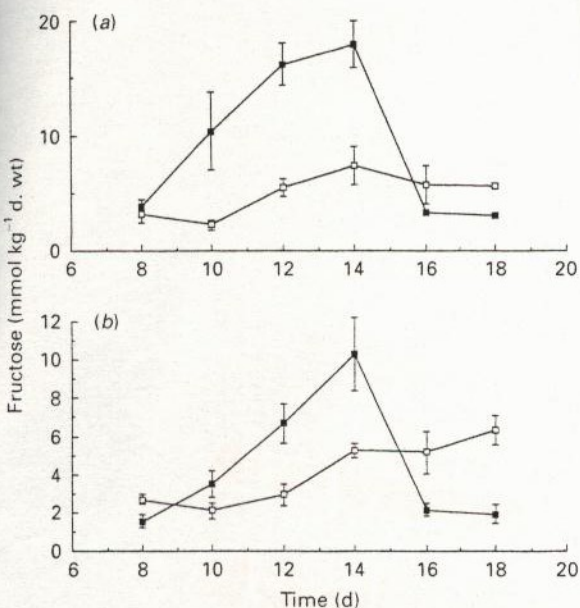


Figure 4. Fructose content of leaves from stressed (■) and control (□) plants of (a) MBB and (b) Capdur from 8 d after withholding water and following rewatering on day 14 (means of 4 replicates).

later, the water status of stressed plants was the same as that of control plants. Plants of Capdur lost slightly more water after day 12, reaching a lower RWC than MBB by day 14. Control plants showed no major changes in either ψ or RWC throughout the period of the experiment.

The two varieties exhibited differences in the time of increase of sugars, especially glucose, relative to water status. Glucose increased dramatically with decreasing water potential (Fig. 3), commencing before day 8 without water in MBB and from day 8

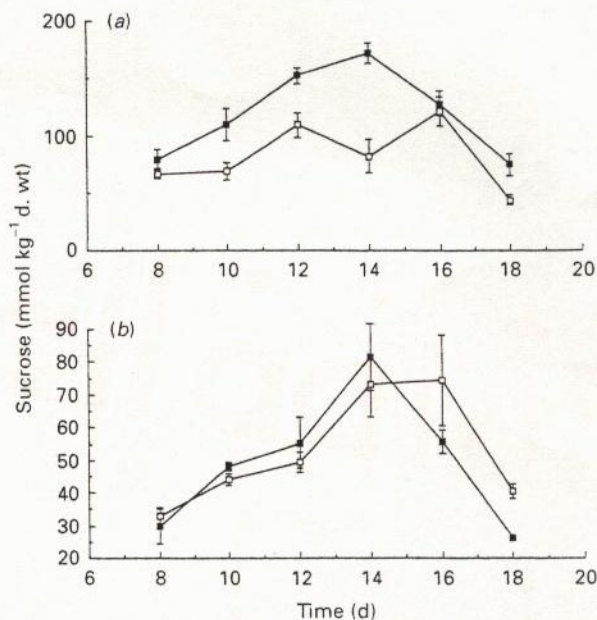


Figure 5. Sucrose content of leaves from stressed (■) and control (□) plants of (a) MBB and (b) Capdur from 8 d after withholding water and following rewatering on day 14 (means of 4 replicates).

in Capdur. The rate of glucose accumulation as well as the amount of glucose recorded throughout the period of water stress was higher in MBB. After rewatering, the glucose concentrations in stressed plants of both varieties fell rapidly, reaching the values of the control plants within 2 days. The pattern of fructose changes (Fig. 4) was similar to that observed with glucose but at concentrations only about one tenth of those of glucose.

Sucrose, which was present in much greater amounts than monosaccharides in leaves of unstressed plants, exhibited a smaller response to falling water potentials, especially in Capdur, where the effect of water stress became significant only at day 14 (Fig. 5). Leaves of stressed plants of MBB, however, contained significantly higher amounts of sucrose than unstressed plants from days 10 to 14. During the period of the experiment, the sucrose content of leaves from control plants varied to a greater extent than fructose and glucose, falling sharply between days 16 and 18, a decrease paralleled by that in plants which had recovered from stress.

In leaves of stressed plants of both varieties, proline concentrations rose sharply from day 10 after withholding water (Fig. 6), reaching the same maximum value in MBB and in Capdur by day 14. After rewatering, the level of proline in stressed plants fell very rapidly and, within two days, equalled that of controls. The decreases in sugar and proline content following the relief of stress coincided with a relatively rapid growth, indicated by the greater number of leaves produced. The deficit of two leaves in stressed plants, compared with controls, which resulted from withholding water for 12 d, was compensated within 3 to 4 d after supplying water.

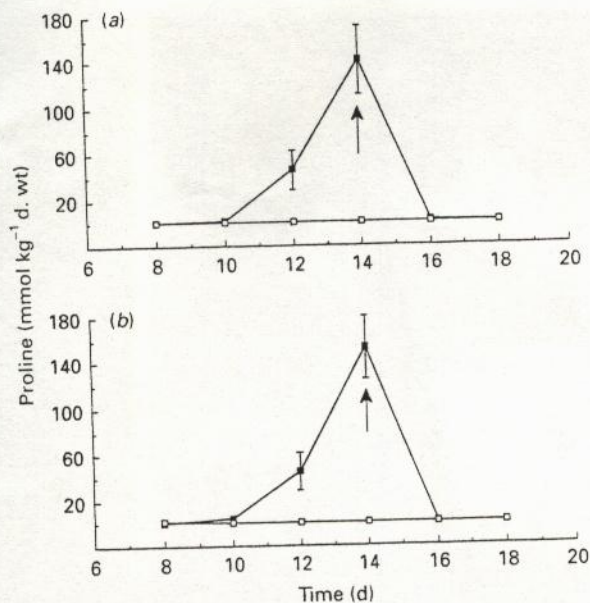


Figure 6. Proline contents of leaves from stressed (■) and control (□) plants of (a) MBB and (b) Capdur from 8 d after withholding water and following rewatering on day 14 (means of 4 replicates).

Table 1. Relationships between sugar concentrations and water potential in leaves of *Triticum durum* varieties MBB and Capdur

	Correlation coefficients	
	MBB	Capdur
Glucose	0.82	0.76
Fructose	0.73	0.82
Sucrose	0.63	0.62

The leaves produced after rewatering were, however, shorter than the corresponding leaves in control plants.

Correlations between soluble sugars and water potential are summarized in Table 1. The correlation coefficient for glucose was higher in MBB ($R = 0.82$) than in Capdur ($R = 0.76$), whereas that for fructose was higher in Capdur ($R = 0.82$) than in MBB ($R = 0.73$). The correspondence between sucrose and water potential was lower than for the monosaccharides and similar in both varieties.

DISCUSSION

The method of applying water stress in this experiment allowed the relatively gradual development of water deficit, as indicated by measurements of ψ and RWC, the two parameters commonly regarded as indicators of the degree of water stress (Hsaio *et al.*, 1976). These values decreased only after water had been withheld for 8 d. The slightly lower values of both ψ and RWC in MBB than in Capdur suggest that Capdur has less ability to retain water as stress intensifies.

In leaves of these two durum wheat varieties, the concentration of glucose increased at a rate closely corresponding to the decrease in water potential, particularly in MBB. Fructose content showed a very similar pattern, also well correlated with water status. The much lower amounts of fructose recorded most likely reflect the synthesis by this species of fructans (Kameli, 1990) which are not included in the analysis of the above experiment. Monosaccharide concentrations which rose above the control relatively early in the stress period, at the time when the fall in water potential first became detectable, are here more sensitive to changes in water status than suggested by the review of Hsaio *et al.* (1976).

The differences between the varieties examined here in the rate and amount of glucose accumulation accompanying decreasing water potential may be physiologically important in helping the plants to withstand the effects of reduced water potential and to recover from it after stress is relieved. The correlations observed in the present study, like those found previously between sugars and xerophytic features (Iljin, 1957) or dehydration-tolerance of grass species (Schwab & Gaff, 1986) support a positive role for sugars during water stress.

Relatively few studies have recorded differences in sugar accumulation between individual plant varieties and, in comparisons of different species, little attention has been given to the type of sugar which increased. Ackerson (1981) observed a correlation between glucose and the degree of stress adaptation in cotton plants. Drossopoulos, Karamanos and Niavis (1987), however, found no relationship between either glucose or fructose concentrations and water stress in older plants of *Triticum aestivum* where, at heading (145 d after sowing), sucrose was the only sugar showing a clear correlation with the degree of stress. A number of other studies have found sucrose concentrations to be similar in stressed and well-watered plants (e.g. Timpa *et al.*, 1986).

Sucrose, the principal photosynthate translocated in the majority of higher plants (Thorne & Giaquinta, 1984), was the major sugar present in control plants of *T. durum* in the present study but was less well correlated with water status. Only in the more drought-resistant variety MBB was there a significant increase in sucrose content during stress. At least in young plants of this species, and under the growing conditions employed in the above experiment, glucose, and to a lesser extent fructose, may play a more important role than sucrose during water stress, firstly because their concentrations were clearly higher in stressed plants of the tolerant variety and secondly because their accumulation closely corresponded with plant water status and was the earliest response detected during the development of water stress.

- l'accumulation de la proline au cours du cycle de développement. *Agronomy* 6: 583-590.
- Scholander PF, Hammel HT, Bradstreet ED, Hemmingsen EA.** 1965. Sap pressure in plants. *Science* 148: 339-346.
- Schwab KB, Gaff DF.** 1986. Sugar and ion content in leaf tissues of several drought tolerant plants under water stress. *Journal of Plant Physiology* 125: 257-265.
- Singh TN, Aspinall D, Paleg LG.** 1972. Proline accumulation and varietal adaptability to drought in barley; a potential metabolic measure of drought resistance. *Nature New Biology* 236: 188-189.
- Singh TN, Paleg LG, Aspinall D.** 1973. Stress metabolism: III. Variation in response to water deficit in barley plant. *Australian Journal of Biological Science* 26: 65-76.
- Spencer JFT.** 1968. Production of polyhydric alcohols by yeasts. In: Hockenhull DJD, ed. *Progress in industrial microbiology*, vol. 7. London: Churchill Ltd., 1-42.
- Stewart CR, Hanson AD.** 1980. Proline accumulation as a metabolic response to water stress. In: Turner NC, Kramer PJ, eds. *Adaptation of plants to water and high temperature stress*. New York: Wiley Interscience, 173-189.
- Tan BH, Halloran GM.** 1982. Variation and correlations of proline accumulation in spring wheat cultivars. *Crop Sciences* 22: 459-463.
- Thorne JH, Giaquinta RT.** 1984. Pathway and mechanisms associated with carbohydrate translocation in plants. In: Lewis DH, ed. *Storage carbohydrates in vascular plants*. Cambridge: Cambridge University Press, SEB Seminar Series, 75-96.
- Timpa JD, Burke JJ, Quinsenberry JE, Wendt CW.** 1986. Effect of water stress on the organic acid and carbohydrate composition of cotton plants. *Plant Physiology* 82: 724-728.
- Vassiliev IM, Vassiliev MG.** 1936. Changes in carbohydrate content of wheat plant during the process of hardening for drought resistance. *Plant Physiology* 11: 115-125.
- Waldren RP, Teare ID, Ehler SW.** 1974. Changes in free proline concentration in sorghum and soybean plants under field conditions. *Crop Sciences* 14: 447-450.
- Weatherley PE.** 1950. Studies in the water relations of the cotton plant. I. The field measurement of water deficit in leaves. *New Phytologist* 49: 81-97.
- Wyn Jones RG, Brady CJ, Speirs J.** 1979. Ionic and osmotic regulations in plant cells. In: Laidman DL, Wyn Jones RG, eds. *Recent Advances in the biochemistry of cereals*. London: Academic Press, 63-118.
- Wyn Jones RG, Storey R.** 1978. Salt stress and comparative physiology in the Gramineae. II. Glycine betaine and proline accumulation in two salt- and water-stressed barley cultivars. *Australian Journal of Plant Physiology* 5: 801-816.