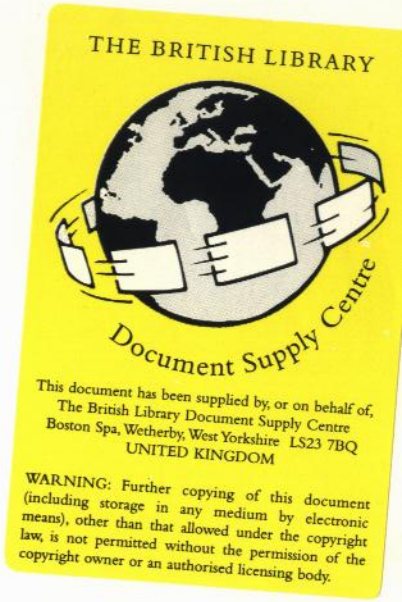
H04 015 AGRO
Bg. 05/98
8600.
015



This document has been supplied by, or on behalf of,
The British Library Document Supply Centre
Boston Spa, Wetherby, West Yorkshire LS23 7BQ
UNITED KINGDOM

WARNING: Further copying of this document (including storage in any medium by electronic means), other than that allowed under the copyright law, is not permitted without the permission of the copyright owner or an authorised licensing body.

Growth and sugar accumulation in durum wheat plants under water stress

By A. KAMELI* and D. M. LÖSEL†

Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK

(*Received 12 August 1994; accepted 21 August 1995*)

SUMMARY

The effect of water stress on growth of *Triticum durum* L. was investigated in relation to sugar accumulation and water status of wheat plants before, during and after a period of water stress. The slight decrease in water potential in the first few days after withholding water had no detectable effect on growth. Inhibition of growth was only apparent when the water content started to decline. Dry weight continued to increase during water stress, even under severe stress (after day 27) which was associated with a sharp rise in sugar content, accounting for 20% of the gain in dry matter between days 27 and 31. The increase in leaf length and leaf area of stressed plants following re-watering, from day 31, was owing to the leaves regaining turgidity after wilting. Growth inhibition coincided with a considerable increase in sugar content. The role of growth inhibition and other factors in sugar accumulation under water stress is discussed. Photosynthesis rather than reserve starch might be the major source of sugar accumulated under water stress in durum wheat.

Key words: Water stress, sugars, growth, wheat.

INTRODUCTION

Soluble sugars have been shown to increase in the leaves of wheat under water stress (Munns & Weir, 1981; Drossopoulos, Karamanos & Niavis, 1987; Kameli & Lösel, 1993). They are also considered to play an important role in osmotic adjustment which is widely regarded as an adaptive response to water stress conditions (Turner & Jones, 1980; Morgan, 1984; Kameli & Lösel, 1993, 1995). Factors which have been suggested to contribute to this increase under water stress include reduced translocation of sugars out of the leaves, slower utilization because of decreased growth and other changes, such as starch hydrolysis. These might contribute individually or together, under different conditions and in different plant species.

The extent to which growth inhibition might be responsible for a rise in sugar concentration under water stress was investigated in this study by comparing the timing of the two responses in the same species. Changes in soluble and insoluble carbohydrates were examined, during and after water stress, in relation to growth.

* Present address: Département de Biologie, École Normale Supérieure, Vieux-Kouba, Algiers, Algeria.

† To whom correspondence should be addressed.

MATERIALS AND METHODS

Seeds of durum wheat (*Triticum durum* L.) variety MBB from Algeria were soaked for 24 h and germinated in vermiculite for 6 d. The seedlings were planted in pots of mixed compost and vermiculite (2:1 v/v), as described previously (Kameli & Lösel, 1993). After 17 d of growth with normal water supply, stress was applied by withholding water from half of the pots, selected in a randomized manner. Stressed plants were again watered from day 31. Control plants received full water treatment throughout the period of the experiment. The plants were grown under fluorescent tubes (Osram white, 65/80 W) with irradiance 90–100 μmol m^{-2} s^{-1}, day and night temperatures 22 ± 2 and 18 ± 2 °C respectively, and relative humidity 70%.

Single plants were harvested nine times during the experiment: three times before the start of stress treatment (days 7, 13 and 17 after germination), four times during the stress period (days 20, 24, 27 and 31 after germination) and twice after re-watering (days 34 and 38 after germination). All measurements were replicated five times, each representing individual plants from five replicate pots.

Both RWC and water potential were measured in order to estimate the water status of the plants. The water potential of the youngest expanded leaf was

determined using a pressure chamber (Scholander *et al.*, 1965) and relative water content (RWC) by the relative turgidity technique of Weatherley (1950), as modified by Barrs & Weatherley (1962).

After dry weight measurements, the whole shoot was ground in a ball mill and a sample of 40–60 mg of ground dried tissue was used for estimation of soluble carbohydrates. Carbohydrates were extracted with 3 × 5 ml hot 80 % ethanol (70 °C) for 15 min. The pooled extract was then reduced under compressed air to 3–4 ml, cleared by adding a similar volume of 20 % aluminium hydroxide $Al(OH)_3$ (w/v in water) and deionized with equal weights (1 g) of Amberlite® IR-45 (OH^-) and IR-120 (H^+).

Soluble sugars were estimated by gas chromatography, after conversion to trimethylsilyl derivatives (Holligan & Drew, 1971). Starch and alpha-glucans remaining in the residue from ethanol extraction were hydrolysed to glucose using amyloglucosidase (AMG), and free glucose then determined by the method of Lloyd & Whelan (1969).

The results were analysed by two-way analysis of variance using the GENSTAT statistical package (Genstat release 4.04B, Lawes Agricultural Trust, Rothamstead Experimental Station).

RESULTS

Although a slight decline in water potential was noted from day 17, when water was withheld, RWC decreased only 10 d later (Fig. 1). Both RWC and water potential fell sharply after day 27 and returned to control levels rapidly after re-watering. The growth parameters measured (Fig. 2) showed similar responses between days 17 and 24 in both treatments, except for leaf number which was slightly lower in stressed plants after day 17 (Fig. 2*a*). Stressed plants, however, showed a rapid recovery in number of leaves after re-watering.

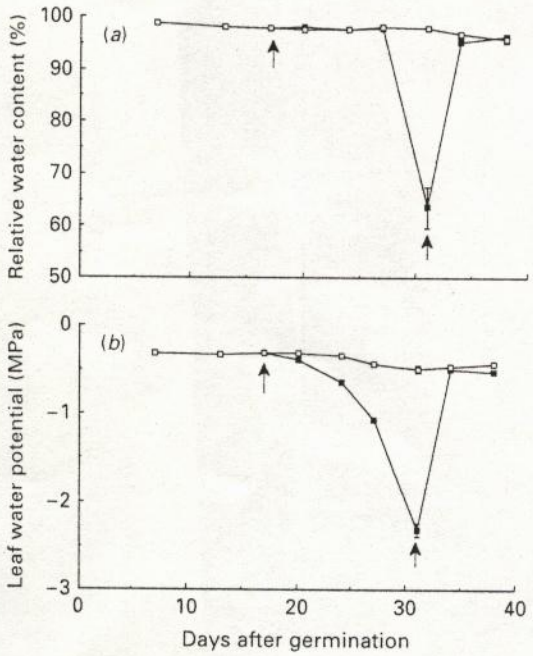


Figure 1. Changes in (*a*) relative water content and (*b*) water potential of leaves in control (□) and stressed plants (■) of *Triticum durum*, before, during and after water stress treatment. Arrows indicate the start and end of stress treatment (means of five replicates).

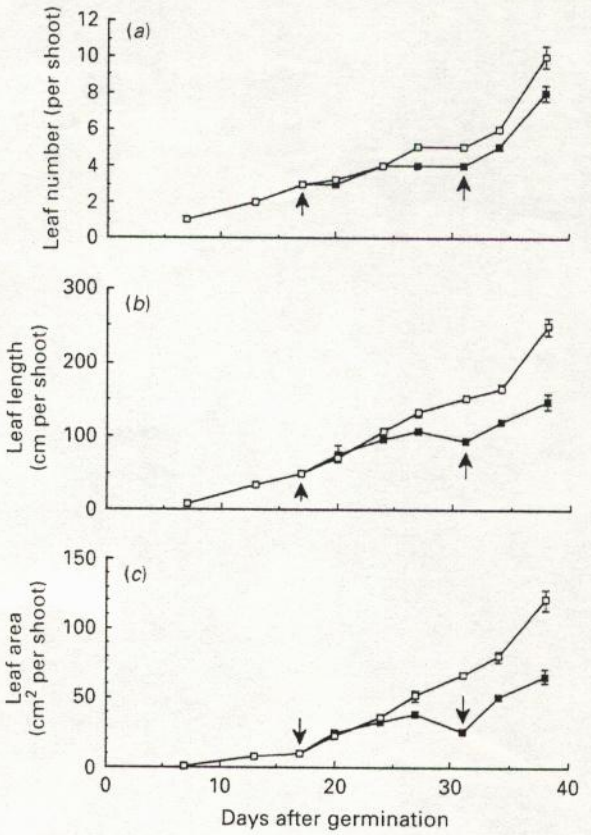


Figure 2. Changes in (*a*) leaf number (*b*) leaf length and (*c*) leaf area in control (□) and stressed plants (■) of *Triticum durum*, before, during and after water stress treatment. Arrows indicate the start and end of stress treatment (means of five replicates).

Leaf length, leaf area and fresh weight measurements (Figs 2, 3*a*) showed similar responses, ceasing to increase after day 24 and decreasing significantly after day 27 (i.e. after 10 days of stress) which coincided with a considerable loss of water. These parameters recovered slightly after re-watering but still showed a significant deficit 7 d later. Dry weight measurements (Fig. 3*b*) increased in both treatments at similar rates until day 24, after which the rate of increase was lower in stressed plants, but continued even after day 27. After re-watering on day 31, rapid recovery in dry weight was noticeable by day 34. This parameter was the least affected by water stress among those measured.

Both soluble and insoluble carbohydrates, which were high at the early stages of seedling development, declined until 10–11 d after germination, when the carbohydrate reserve in the seeds was exhausted, as indicated by an empty pericarp. Soluble sugars increased slightly in stressed plants after day 17

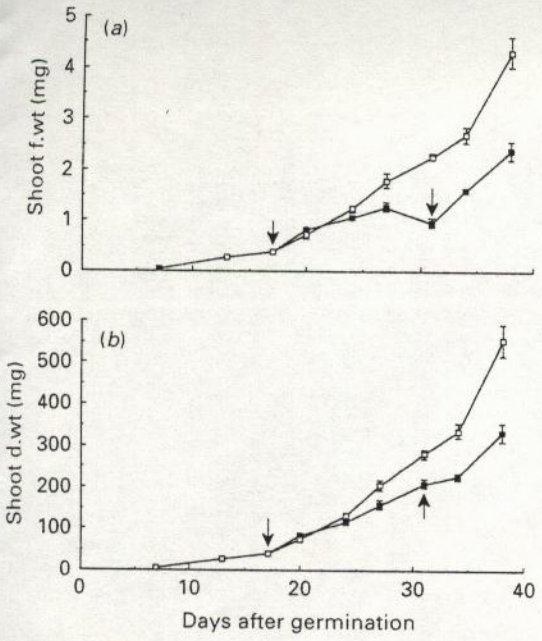


Figure 3. Changes in (*a*) fresh and (*b*) dry weight in control (□) and stressed plants (■) of *Triticum durum*, before, during and after water stress treatment. Arrows indicate the start and end of stress treatment (means of five replicates).

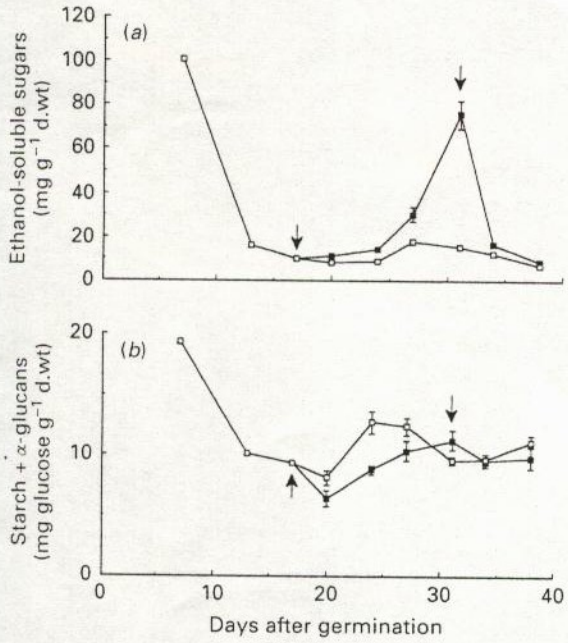


Figure 4. Changes in (*a*) ethanol soluble carbohydrates (mainly glucose, fructose and sucrose) and (*b*) ethanol insoluble carbohydrates (starch and α-glucans) in shoots of control (□) and stressed plants (■) of *Triticum durum*, before, during and after water stress treatment. Arrows indicate the start and end of stress treatment (means of five replicates).

(Fig. 4*a*) but the increase became significant only after day 24. The sugar content then rose rapidly up to day 31 then dropped sharply after re-watering but still remained slightly higher in stressed plants than in controls. Lower amounts of ethanol-insoluble carbohydrate (starch and α-glucans) were found in stressed plants between days 17 and 24 but the differences from control plants were not significant after day 27 (Fig. 4*b*).

DISCUSSION

Growth and water status

Although a slight decrease in water potential occurred in the first days after withholding water and accelerated during the period of stress, no significant inhibition of growth was apparent until the water content fell (from day 27). Leaf expansion growth (leaf length and leaf area) is often reported to be extremely sensitive to changes in water potential (Boyer, 1968, 1970; Acevedo, Hsiao & Henderson, 1971; Cutler, Shahan & Steponkus, 1980; Van Loo, 1992), resulting in the inhibition of growth even at relatively higher water potential.

At each time interval in the present experiment, RWC and growth measurements were made on the same plants which were then harvested for the estimation of dry weight as well as sugar content. This destructive method might, however, fail to detect small changes in leaf elongation because different sets of plants were used at each harvesting time. Nevertheless, inhibition of expansion growth, as indicated by leaf length and leaf area, is clearly seen at water potentials lower than −1 MPa. Diurnal and short term changes in leaf elongation rate can best be detected by continuous measurements on the same plant (non-destructive method) but the analysis of solutes or sugar content can only be done on different plants.

The growth inhibition (Figs 2, 3), observed at the time when sugar content increased markedly, supports the suggestion that reduction in growth is the main cause of sugar accumulation and might indicate that the slight rise in sugars in the first 6 d of stress also resulted from changes below the level detectable using this method. From continuous measurement of leaf elongation in maize plants under water stress, Volkenburg & Boyer (1985) concluded similarly that solute accumulation in the growing cells occurred after the elongation rate was reduced.

Although the high sensitivity of expansion growth to low water potential seems the likely cause of the increase of solutes under water stress, it is not clear what causes this sensitivity. The dependence of cell enlargement on hydrostatic pressure inside the cell (turgor) as the driving force (Matthews, Volkenburgh & Boyer, 1984) suggests that any decrease in growth results from reduction or loss of turgor, as found by Meyer & Boyer (1972), Sharp & Davies (1979), Van Loo (1992) and Kutschera & Köhler (1994).

Turgor maintenance

Consistent observations of inhibition of growth while turgor is maintained (Cutler *et al.*, 1980; Matsuda & Riazi, 1981; Michelena & Boyer, 1982; Boyer, Cavalieri & Schulze, 1985; Van Loo, 1992) point to the involvement of other factors in the regulation of leaf growth. The yield threshold (i.e. the minimum turgor required for cell expansion) might be high, so that small changes in turgor would lead to growth inhibition. An alteration in the sensitivity of leaf elongation owing to stress acclimation was reported to result from a decrease in the yield threshold (Boyer *et al.*, 1985), allowing growth to continue at lower turgor pressure. Similar effects were found in leaves of re-watered maize plants after water deficit (Acevedo *et al.*, 1971).

In the present study, the loss of turgor indicated by a considerable decrease in RWC occurred only after increases in both leaf length and leaf area were inhibited (i.e. growth had ceased before any noticeable decrease in RWC). The continuation of normal growth during the first 7 d of stress, despite a slight decrease in water potential, is likely to result from osmotic adjustment, already demonstrated in this variety of durum wheat, which enables the plants to maintain turgor and water content (Kameli & Lösel, 1993, 1995). The observation that leaf water potential declined during the first 6 d after withholding water, without a noticeable change in RWC, suggests a degree of osmotic adjustment in these plants preventing water loss in the first days of treatment, when water stress was not severe.

From experimental evidence that osmotic adjustment can reduce growth sensitivity to water stress (Cutler *et al.*, 1980) or allow growth to proceed at a slower rate under water stress (Meyer & Boyer, 1981) by maintaining turgor, it can be concluded that the continuation of growth at lower water potential is a result of turgor maintenance, whereas the inhibition of growth is not entirely dependent on turgor (BassiriRad & Coldwell, 1992).

In contrast to other growth parameters, dry weight increased during water stress, even under severe stress (after day 27), coinciding with a sharp increase in sugar content which accounted for 20 % of the rise in dry matter between days 27 and 31.

Recovery phase

The increase in leaf length and leaf area in stressed plants, immediately after re-watering, was owing to the leaves regaining their turgidity after wilting (Fig. 1). There was no indication in this experiment of higher rates of leaf expansion than in control plants. Stressed plants still had lower rates of leaf area and leaf length increase than did control plants (35 % that of controls) between 3 and 7 d after watering, indicating a continuing effect on growth. Although turgor is regained quickly after re-watering, other factors might continue to limit growth, depending on the degree of water stress to which the plants were exposed. The rate of dry weight increase was less affected than leaf expansion but was still only 50 % of that of controls, a deficit which is likely to affect the final yield of the plants.

Photosynthetic capacity depends on leaf area, which was reduced considerably under stress. Part of this reduction is irreversible, owing to leaf senescence induced by water stress, as was also noted by Boyer (1976). Leaf area in stressed plants as 55 % of that of control plants, whereas the increase in leaf area was only 35 % of that in controls, 7 d after watering. This 20 % gap could be explained by effects on the photosynthetic apparatus itself, resulting from the severity of water stress applied. Other workers (e.g. Gates, 1955) concluded that growth can return to normal after exposure to mild water stress, which suggested that processes necessary for growth, such as photosynthesis, were not affected.

The role of starch in sugar accumulation

Changes in amounts of sugars and starch from the same tissue did not support an important role of starch in the observed increase in sugars under water stress. Although the decline in starch and α-glucans in the early days of stress (days 17–24), when growth was still not affected, might have resulted in a slight increase in sugars, no such decrease was noticed later when sugar accumulation was high (days 24–31). Thus in *Triticum durum*, at least, starch might not be an important source for sugar accumulation. The stability of starch in stressed plants was not significantly different from well-watered plants, as was observed by Barlow, Munns & Brady (1980). A comparison between the amounts of sugars accumulated and starch, especially in the growing tissue, suggests that during prolonged water stress starch is not of great value in providing substantial amounts of sugars for osmotic adjustment, as was also concluded by Munns, Brady & Barlow (1979).

Osmotic potential was not measured in this experiment, but was previously determined for similar plants (Kameli & Lösel, 1995). On the basis of a tissue water constant consistently close to 90 % of fresh weight in leaves of this stage of development, the range of sugar content per unit dry weight indicated in Figure 4 would be equivalent to between 4 and 47 mmol kg^{-1} tissue water, if all the soluble sugars were monosaccharides (mol. wt. = 180). The real contribution to osmotic pressure will be lower, however, since a significant part of the soluble sugars is usually present in the form of disaccharides (mol. wt. = 342).

In a separate experiment (Kameli, 1990), it was shown that free fructose, after hydrolysis of existing

fructans, was much higher in the growing than in the expanded leaves. However, the increase due to the stress treatment was significant only in the growing tissue. There was no indication of fructans being hydrolysed to fructose, since the amount of fructose was not lower in the growing leaves of stressed than of control plants and the amount was even higher in the growing leaves under water stress.

Photosynthesis as a source of sugar accumulation

There is strong evidence implicating photosynthesis as the main source of accumulation of organic solutes under water stress, as was indicated by the cessation of osmotic adjustment in *Centrosema* resulting from stomatal closure (Ludlow *et al.*, 1983). Meyer & Boyer (1972, 1981) showed that cutting the photosynthetic cotyledons from soybean seedlings prevented solute accumulation and osmotic adjustment. From similar experiments with sunflower seedlings, Kutschera & Köhler (1994) demonstrated that, although the turgor pressure driving hypocotyl extension growth was a function of tissue osmotic pressure in intact seedlings, removal of the source of organic solutes by excision of both cotyledons inhibited hypocotyl growth and was accompanied by a significantly greater decrease in turgor rather than in osmotic pressure. Solutes accumulate as long as their uptake exceeds utilization (growth and respiration), resulting in a net gain of carbon, even at severe water stress (McGree, Kallsen & Richardson, 1984).

A number of factors might be involved in the increased content of sugars and possibly other compounds in durum wheat under water stress. The higher sensitivity of expansion growth to changes in water potential relative to other processes such as photosynthesis and translocation (Boyer, 1976) seems to be the primary cause of accumulation of soluble sugars. This could be a result of differences between the turgor levels required for stomatal opening and growth, as concluded by Wright, Smith & Morgan (1983). The present observations are consistent with the view (Hsaio, Acedevo & Fereres, 1976) that the maintenance of turgor by osmotic adjustment might allow stomata to stay open and CO_2 assimilation to continue unaffected while expansion growth is inhibited. While CO_2 assimilation is maintained, the translocation of solutes to sites away from the source of synthesis is likely to continue. There is no strong evidence to support suggestions that hydrolysis of starch provides a significant source of sugars either after photosynthesis stops, as proposed by Munns & Weir (1981), or during early stages of stress, as discussed previously.

Reduced utilization of photosynthate, resulting from inhibition of growth when water stress develops slowly over a relatively long period, as in the present study, could account for the increase in solute concentrations in leaf tissues. However, the osmotic adjustment (accumulation of solutes) observed by some workers, e.g. Kituta and Richter (1988), during rapid water stress (hours), cannot be explained solely by a reduced utilization of solutes and could result from hydrolysis of existing polysaccharides or proteins.

ACKNOWLEDGEMENT

Financial support during the period of this work for A. Kameli from the Algerian Ministry for Higher Education is gratefully acknowledged.

REFERENCES

Acevedo E, Hsiao TC, Henderson DW. 1971. Immediate and subsequent growth responses of maize leaves to changes in water status. *Plant Physiology* **48**: 631–636.

Barlow EWR, Munns RE, Brady CJ. 1980. Drought responses of apical meristems. In: Turner NC, Kramer PJ, eds. *Adaptations of Plants to Water and High Temperature Stress*. New York: Wiley, 191–205.

Barrs HD, Weatherley PE. 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Australian Journal of Biological Science* **15**: 413–428.

BassiriRad H, Caldwell MM. 1992. Root growth, osmotic adjustment and NO_3^- uptake during and after a period of drought in *Artemesia tridentata*. *Australian Journal of Plant Physiology* **19**: 493–500.

Boyer JS. 1968. Relationship of water potential to growth of leaves. *Plant Physiology* **43**: 1056–1062.

Boyer JS. 1970. Leaf enlargement and metabolic rates in corn, soybean and sunflower at various water potentials. *Plant Physiology* **46**: 233–235.

Boyer JS. 1976. Water deficits and photosynthesis. In: Kozlowzki TT, ed. *Water Deficits and Plant Growth*, **4**. New York: Academic Press, 153–190.

Boyer JS, Cavalieri AJ, Schulze ED. 1985. Control of rate of cell enlargement: excision, wall relaxation and growth-induced water potentials. *Planta* **163**: 527–543.

Cutler JM, Shahan KW, Steponkus PL. 1980. Influences of water potentials and osmotic adjustment on leaf elongation in rice. *Crop Sciences* **20**: 314–318.

Drossopoulos JB, Karamanos AJ, Niavis CA. 1987. Changes in ethanol soluble carbohydrates during the development of two wheat cultivars subjected to different degrees of water stress. *Annals of Botany* **59**: 173–180.

Gates, CT. 1955. The response of the young tomato plant to brief periods of water shortage. *Australian Journal of Biological Science* **8**: 196–214.

Holligan PM, Drew EA. 1971. Routine analysis by gas-liquid chromatography of soluble carbohydrates in extracts of plant tissues. II Quantitative analysis of standard carbohydrates and polyols from a variety of plant tissues. *New Phytologist* **70**: 271–279.

Hsiao TC, Acevedo E, Fereres E. 1976. Stress metabolism: water stress, growth and osmotic adjustment. *Philosophical Transactions of the Royal Society of London* B **273**: 479–500.

Kameli, A. 1990. *Metabolic responses of durum wheat to water stress and their role in drought resistance*. Ph.D. thesis, University of Sheffield.

Kameli A, Lösel DM. 1993. Carbohydrates and water status in wheat plants under water stress. *New Phytologist* **125**: 609–614.

Kameli A, Lösel DM. 1995. Contribution of sugars and other solutes to osmotic adjustment in wheat leaves under water stress. *Journal of Plant Physiology* **145**: 363–366.

Kikuta SB, Richter H. 1988. Rapid osmotic adjustment in detached wheat leaves. *Annals of Botany* **62**: 167–172.

Kutschera U, Köhler K. 1994. Cell elongation, turgor and osmotic pressure in developing sunflower hypocotyls. *Journal of Experimental Botany* **45**: 591–595.

Lloyd JB, Whelan WJ. 1969. An improved method for the enzymic determination of glucose in the presence of maltose. *Analytical Biochemistry* **30**: 467–469.

Ludlow MM, Chu ACP, Clements RJ, Kerslake RG. 1983. Adaptation of species of *Centrosema* to water stress. *Australian Journal of Plant Physiology* **10**: 119–130.

Matthews MA, Volkenburgh E, Boyer JS. 1984. Acclimation of leaf growth to low water potentials in sunflower. *Plant Cell and Environment* **7**: 199–206.

Matsuda K, Riazi A. 1981. Stress-induced osmotic adjustment in the growing regions of barley leaves. *Plant Physiology* **68**: 571–576.

McGree KJ, Kallsen CE, Richardson SG. 1984. Carbon balance of sorghum plants during osmotic adjustment to water stress. *Plant Physiology* **76**: 898–902.

Meyer RF, Boyer JS. 1972. Sensitivity of cell division and cell elongation to water potentials in soybean hypocotyls. *Planta* **108**: 77–87.

Meyer RF, Boyer JS. 1981. Osmoregulation, solute distribution and growth in soybean seedlings having low water potentials. *Planta* **151**: 482–489.

Michelena VA, Boyer JS. 1982. Complete turgor maintenance at low water potentials in the elongating regions of maize leaves. *Plant Physiology* **69**: 1145–1149.

Morgan JM. 1984. Osmoregulation and water stress in higher plants. *Annual Review of Plant Physiology* **35**: 299–319.

Munns R, Weir R. 1981. Contribution of sugars to osmotic adjustment in elongating and expanded zones of wheat leaves during moderate water deficit at two light levels. *Australian Journal of Plant Physiology* **8**: 93–105.

Munns R, Brady LJ, Barlow EW. 1979. Solute accumulation in the apex and leaves of wheat during water stress. *Australian Journal of Plant Physiology* **6**: 379–389.

Sharp RE, Davies WJ. 1979. Solute regulation and growth by roots and shoots of water-stressed maize plants. *Planta* **147**: 43–49.

Scholander PF, Hammel HT, Bradstreet ED, Hemmingsen EA. 1965. Sap pressure in vascular plants. *Science* **148**: 339–346.

Turner NC, Jones MM. 1980. Turgor maintenance by osmotic adjustment: a review and evaluation. In: Turner NC, Kramer PJ, eds. *Adaptation of Plants to Water and High Temperature Stress*. New York: Wiley, 87–103.

Van Loo EN. 1992. Tillering, leaf expansion and growth of plants of two cultivars of perennial rye grass grown using hydroponics at two water potentials. *Annals of Botany* **70**: 511–518.

Volkenburgh EV, Boyer JS. 1985. Inhibitory effects of water deficit on maize leaf elongation. *Plant Physiology* **77**: 190–194.

Weatherly 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.

Westgate ME, Boyer JS. 1985. Osmotic adjustment and the inhibition of leaf, stem and silk growth at low water potentials in maize. *Planta* **164**: 540–549.

Wright GC, Smith RCG, Morgan JM. 1983. Differences between two grain sorghum genotypes in adaptation to drought stress. III. Physiological responses. *Australian Journal of Agricultural Research* **34**: 637–651.