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Physiological Changes in Cultured Sorghum Cells in Response to Induced Water Stress[1]

II. SOLUBLE CARBOHYDRATES AND ORGANIC ACIDS

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ABSTRACT

Eight cultivars *Sorghum bicolor* (L.) Moench were grown as callus cultures under induced, prolonged water stress (8 weeks), with polyethylene glycol in the medium. Concentrations of soluble carbohydrates and organic acids in callus were measured at the end of the growth period to determine differences in response to prolonged water stress. Sucrose, glucose, fructose, and malate were the predominant solutes detected in all callus at all water potentials. All cultivars had high levels of solutes in the absence of water stress and low levels in the presence of prolonged water stress. However, at low water potentials, low levels of solutes were observed in drought-tolerant cultivar callus and high solute levels were observed in drought-susceptible cultivar callus. Estimated sucrose concentrations were significantly higher in water-stressed, susceptible cultivar callus. Large solute concentrations in susceptible cultivar callus were attributed to osmotic adjustment and/or reduced growth during water stress.

Recently, a correlation between responses to drought in the field and responses to drought in culture has been established in sorghum (11). Subsequently, we observed that in callus cultures proline levels were increased in response to prolonged, moderate to severe, water stress, especially in one cultivar whose growth was very susceptible to drought; conversely, low proline levels were associated with a cultivar which sustained growth during water stress (1).

In the present investigation we analyzed soluble carbohydrate and organic acid levels by GC in sorghum callus cultures when water stress was induced by additions of PEG to the culture medium. As in our previous investigation (1), the objective was to determine if there are inherent differences at the cellular level between cultivars during prolonged, sustained water stress. This might indicate what metabolic changes occur and whether or not any of these changes confer a significant advantage to any cultivar in response to drought.

One of the metabolic changes in response to water stress that confers an advantage through turgor maintenance and sustained growth is osmotic adjustment whereby solutes accumulate (2, 6). Reducing sugars contribute most to the change in osmotic potential and osmotic adjustment in cultured tomato cells adapted to low water potentials (4). However, in this study the cells had been adapted to continuous, low external water potential which had persisted for hundreds of generations prior to measurements of internal solutes (4). In contrast to this approach, we wanted to relate internal solute levels to cell growth during water stress in cells not previously adapted to a desired water potential. Furthermore, because the cell cultures of interest were derived from drought-susceptible and drought-tolerant cultivars whose growth under prolonged water stress was decidedly different (11), we wanted to characterize the internal solutes relative to their final growth. This information would be most useful in selection of genotypes for drought-tolerance. The data reported here indicate that sucrose was significantly lower in tolerant cultures which sustain growth during prolonged water stress and higher in susceptible cultures whose growth is reduced.

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MATERIALS AND METHODS

Callus Cultures. Callus cultures were initiated from sorghum seeds by the procedure of Smith *et al.* (10). Seeds of *Sorghum bicolor* (L.) Moench varieties B35, RTx7078, RTx7000, RTx432, BTx3197, BTx623, 1790E, RTx430, R9188, and BTx378 were used. The callus was subcultured at 4-week intervals until enough callus material was obtained to initiate the experiment (11). All calli were less than 6 months old at the start of the water stress. Both callus initiation and subculture medium contained two soluble carbohydrate sources, sucrose (20 g L^{-1}) and inositol (100 mg L^{-1}) (10).

Water Stress. PEG was added to the growth medium to concentrations of 5, 10, 15, 20, and 25% (w/v) before the pH was adjusted to 5.7. The medium composition before addition of PEG was the same as the callus initiation and subculture medium. Twenty-five ml of medium were distributed into each culture tube, and Heller supports (5), made with Whatman No. 2 filter paper, were placed on the medium. The water potential of the medium after autoclaving was −0.2 MPa for the control without any added PEG, and −0.35, −0.5, −0.75, −1.15, and −1.62 MPa for 5, 10, 15, 20, and 25% PEG, respectively.

Culture Conditions. Callus pieces were aseptically weighed and placed on Heller supports. All cultures were grown at 28°C with a photoperiod of 16 h at 30 μE (PAR) m^{-2} s^{-1}. Ten replicates were used at each stress level, and callus fresh weight was measured after 8 weeks. Eight weeks provided a prolonged water stress interval that allowed adequate time for observing differences in growth between cultivars (10). The calli were then frozen in liquid N_2 and lyophilized. The dry weights were recorded, and the samples were processed for solute analysis.

Soluble Carbohydrates and Organic Acids. Extraction of solutes and their analysis have been described previously (7, 12). One hundred mg of lyophilized tissue was extracted with 20 ml hot 80% ethanol and air dried. The dry samples were derivatized with 0.7 ml pyridine, 0.2 ml hexamethyldisilazane, and 0.1 ml trimethylchlorosilane (Pierce Chemical Co., Rockford, IL), placed in a water bath at 55°C for 45 min, and centrifuged at 7000 rpm for 10 min (13). Four μl of sample were injected into a 1.82 m by 2.2 mm i.d. nickel column with a liquid phase of 3% OV-17 supported on Anakrom ABS (90–100 mesh) support. The gas chromatograph (Varian 3700) had a temperature program from 100 to 270°C at an increment of 7°C min^{-1}. The soluble carbohydrates and organic acids identified include fructose, glucose, galactose, sucrose, ascorbate, and malate. A cyanoglycoside, dhurrin, was also identified. Because ascorbate, galactose, and dhurrin contributed only a very small percentage to the total, the individual levels of these solutes are not reported.

RESULTS AND DISCUSSION

In a previous study (11), cultivars BTx623, RTx7000, RTx7078, and RTx430 were shown to be tolerant to drought in the field (9) and to water stress in cell culture; cultivars 1790E, B35, BTx3197, and BTx378 were found to be susceptible to drought in the field (9) and to water stress in cell culture. In culture, RTx430 recorded high relative growth and BTx3197 recorded low relative growth. Two cultivars (RTx432, R9188) were eliminated from the present study because there was not sufficient tissue for analysis. In another study we also observed negative correlations between growth and proline concentrations (1). For example, RTx430 had low levels of proline and high relative growth while BTx3197 had high levels of proline and low relative growth. Because of this, we have chosen to display both the solute and growth data (Figs. 1–6) for: (a) a susceptible cultivar (BTx3197), (b) a tolerant cultivar (RTx430), (c) the mean of four susceptible cultivars (SUSC$\bar{x}$)[2], and (d) the mean of four tolerant cultivars (TOL$\bar{x}$). BTx3197, unlike all other cultivars, was designated as most susceptible due to its demonstrated drought-intolerance in the field and in cell culture (11).

Callus cultures subjected to water stress from −0.35 to −1.6 MPa showed marked reductions in fresh and dry weights (Figs. 1, 2). RTx430 and TOL$\bar{x}$ fresh and dry weights were consistently higher than BTx3197 and SUSC$\bar{x}$ fresh and dry weights over the same range of water potentials (Figs. 1, 2). There was a 30% increase in the dry weight of tolerant cultivar callus at a water potential of −0.5 MPa compared to −0.2 MPa, but dry weight declined thereafter with decreasing water potential (Fig. 2). This increase in dry weight corresponded with the rapid decrease in callus fresh weight at the same water potential (Figs. 1, 2). In susceptible cultivar callus, there was only a slight increase in dry weight at −0.5 MPa (Fig. 2).

Levels of soluble carbohydrates and organic acids were larger in susceptible cultivar callus compared to tolerant cultivar callus cultures when both were water stressed (Figs. 3, 4, 5). Susceptible callus had high levels of sucrose when subjected to water potentials of −0.3 to −1.15 MPa (Fig. 3). Fructose, glucose, and malate levels were especially low in the tolerant cultivar, RTx430, over this same water potential range (Figs 3–5). Similar results with proline were shown in the previous study (1), *i.e.* proline levels were higher at more severe stress levels in susceptible callus tissue. Tolerant callus appeared to increase in dry weight at −0.5 MPa (Fig. 2) perhaps because it was utilizing solutes (Figs. 3–5) for conversion into other cellular constituents needed for the sustained growth which was observed. This inverse relationship between solute levels and growth is most evident in Figure 6

[2] Abbreviations: TOL$\bar{x}$ and SUSC$\bar{x}$ refer to mean of drought-tolerant and drought-susceptible cultivars, respectively.

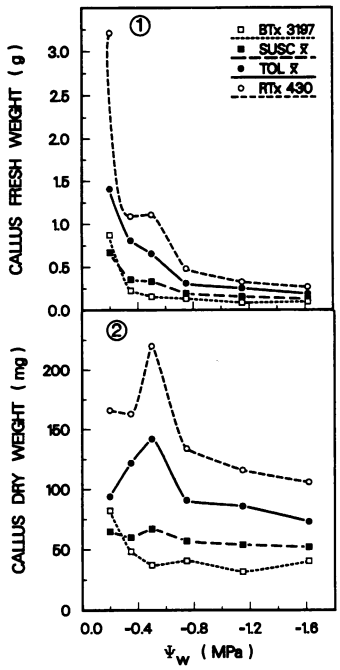

FIG. 1. Callus fresh weight after 8 weeks in response to water stress. (□), BTx3197; (○), RTx430; (●), TOL$\bar{x}$, mean of cultivars RTx430, RTx7000, RTx7078, and BTx623; (■), SUSC$\bar{x}$, mean of cultivars 1790E, B-35, BTx3197, and BTx378.

FIG. 2. Callus dry weight after 8 weeks in response to water stress. Symbols same as Figure 1.

where the ratio of total solute levels to dry weight (μg mg^{-1}DW/gDW) is plotted against water potential. Susceptible cultivar callus had high levels of solutes associated with reduced dry weight growth and tolerant cultivar callus had low levels of solutes associated with increased dry weight growth.

The small sample size in these experiments forced us to pool tissues for solute analysis, thus negating any statistical evaluation of solute levels at each water potential. However, statistical evaluation of mean growth and soluble carbohydrate and organic acid levels in water-stressed callus averaged over the range of all water potentials showed significant differences (Table I). Mean dry weight and fresh weight were significantly higher in tolerant callus and mean sucrose levels were significantly higher in susceptible callus. Although not significant, mean malate levels and mean total solute levels were also higher in susceptible callus.

Because solute concentration determines callus osmotic potential it was meaningful to estimate sucrose concentrations in the callus and relate them to callus water potential. Sucrose concentrations were estimated by using callus water volume (fresh weight minus dry weight). Compared to tolerant callus, sucrose concentrations were higher in the susceptible callus at all water potentials greater than −1.6 MPa (Fig. 7). This increase was due to both higher sucrose levels (Fig. 3) and smaller water volumes in susceptible callus (Fig. 8). High sucrose levels (mg/callus) were associated with low water volumes (ml/callus) in the susceptible callus, BTx3197, and low sucrose levels were associated with high water volumes in the tolerant callus, RTx430 (Fig. 8).

The relationship between sucrose concentration and water potential in Figure 7 is most interesting. It indicates that susceptible cultivar callus may have had lower osmotic potentials (due to higher solute levels and reduced water volumes) as the callus was subjected to water stress. Furthermore, the lowered osmotic potentials would have been associated with reduced growth that was observed in susceptible cultivar callus (Figs. 1, 2). This would suggest that selection for solute accumulation during water stress

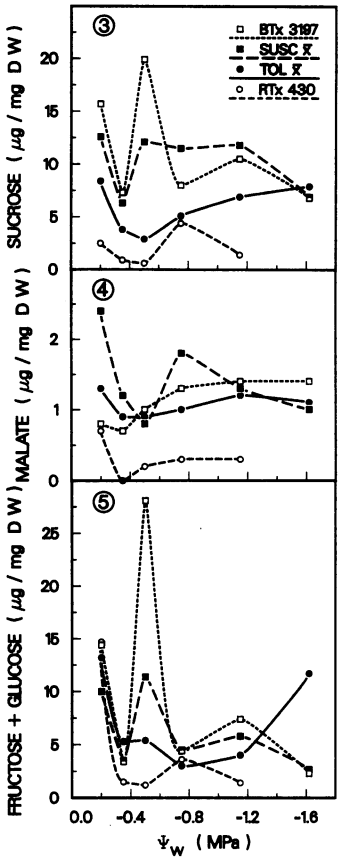

FIG. 3. Sucrose in callus subjected to water stress for 8 weeks. Symbols same as Figure 1.

FIG. 4. Malate in callus subjected to water stress for 8 weeks. Symbols same as Figure 1.

FIG. 5. Fructose plus glucose in callus subjected to water stress for 8 weeks. Symbols same as Figure 1.

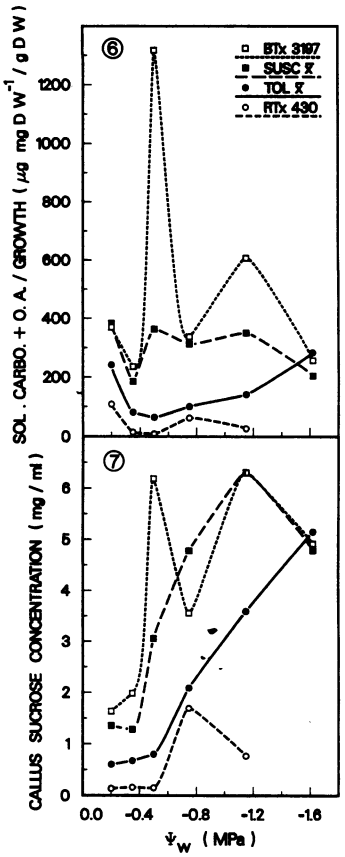

FIG. 6. Ratio of soluble carbohydrates plus organic acids to callus dry weight subjected to water stress for 8 weeks. Symbols same as Figure 1.

FIG. 7. Estimated sucrose concentrations in callus subjected to water stress for 8 weeks. Symbols same as Figure 1.

in sorghum could result in reduced growth potential. A similar observation was noted in cotton, whereby enhanced solute accumulation during water stress was associated with reduced shoot growth in field-grown plants (8).

It is most apparent from the above results and discussion that callus solute levels and concentrations were different between susceptible and tolerant cultivars during water stress. However, it is not clear whether the observed differences in solute levels were due to cultivar differences in osmotic adjustment or differences in solute utilization during growth. The absence of osmotic potential measurements of callus tissue in this study preclude any definitive assessment of osmotic adjustment phenomena of sorghum *in vitro*. However, the extent of solute accumulation can be addressed by comparing solute levels in stressed and nonstressed callus. If it is assumed that −0.2 MPa represents the nonstressed condition, then it can be observed from Figures 3 through 5 that solute levels in stressed callus were generally lower than nonstressed callus. The exception to this was BTx3197; at −0.5 MPa, callus solute levels were higher than callus at −0.2 MPa. This suggests that BTx3197 callus was adjusting osmotically when subjected to a water potential of −0.5 MPa.

Also, osmotic adjustment was perhaps not as evident in these cell cultures because of the prolonged water stress period of 8 weeks that was used to ascertain differences in fresh weight growth among cultivars (1). Bressan *et al.* (3) have shown that tomato cells growing in the absence of PEG change in osmotic potential from nearly −2.5 MPa after 1 week of growth to only −0.2 MPa after nearly 3 weeks. They attributed this to an increase in cell volume and decreased solute accumulation which could not keep pace with the cell expansion. When tomato cells were subjected to lower water potentials, more negative osmotic potentials resulted after 3 weeks, due to solute accumulation and decreased cell volume. This would suggest that the lower solute levels and larger fresh weights that we observed with drought-tolerant sorghum cell cultures (Figs. 7, 8) may be due in part to larger cell volumes. Conversely, the higher solute levels and smaller fresh weights observed in susceptible cultivars of sorghum (Figs. 7, 8) is perhaps attributed to smaller cell volumes. In addition, the lower soluble carbohydrate levels in drought-tolerant sorghum cultivars may be the result of solute absorption not keeping pace with cell expansion.

Based on our observations and those of Bressan *et al.* (3), it would seem that osmotic adjustment in nonadapted cell cultures would be most evident in the early exponential growth phase; investigations with shortened exposures to water stress are needed before osmotic adjustment phenomena can be compared between sorghum cultivars in cell culture. Osmotic adjustment studies with previously adapted cells may also reveal cultivar differences. The differences in solute accumulation and growth may be indicative of differences which occur at various stages of adaptation, and may not be adaptive characteristics which contribute to drought tolerance. Investigations coupled with cell volume and osmotic potential measurements would be most meaningful in understanding cellular responses to water stress among sorghum cultivars. Furthermore, these studies should be encouraged because sorghum cultivar differences in osmotic adjustment have been previously demonstrated with whole plants (14).

Table I. *Growth and Solute Levels of Water-Stressed Sorghum Callus*

Each value represents the mean resulting from 6 different water potentials ranging from −0.2 to −1.62 MPa after 8 weeks in culture. Total solutes include soluble carbohydrates plus organic acids. Statistical evaluation was performed with analysis of variance.

Characteristic	Cultivar		Cultivars	
	RTx430	BTx3197	TOL$\bar{x}$ (n = 4)	SUSC$\bar{x}$ (n = 4)
Fresh wt (mg)	1080.8[a]	261.9	604.0[b]	305.4
Dry wt (mg)	150.8[c]	46.8	101.2[c]	59.1
Solutes				
Sucrose (μg/mg)	2.0[c]	11.4	5.8[c]	10.2
Fructose + Glucose (μg/mg)	4.5	10.0	7.1	6.3
Malate (μg/mg)	0.3	1.0	1.1	1.4
Total Solutes (μg/mg)	6.8	22.4	14.0	17.9

[a] Significant at $P < 0.10$. [b] Significant at $P < 0.05$. [c] Significant at $P < 0.01$.

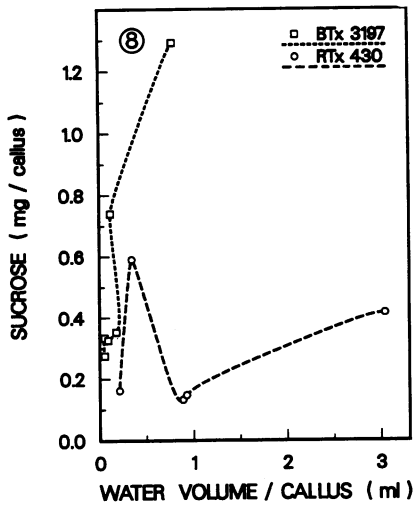

FIG. 8. Sucrose in callus with different water volumes. Symbols same as Figure 1.

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