CELLULAR DROUGHT TOLERANCE STUDIES IN LOBLOLLY PINE

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Abstract . - -Drought resistance evaluation and selection in woody, perennial plants often takes several years. Tissue culture technology is being explored as a means of accelerating the selection and tree improvement process and understanding the physiological basis of drought tolerance. The physiological, tolerance response of osmotic adjustment has been demonstrated in vivo and in vitro in a large number of plants. In loblolly pine (Pinus taeda L.), it has been demonstrated only in vivo.

The objective was to determine if loblolly pine callus responded in vitro with osmotic adjustment when subjected to water stress. Water stress was induced by addition of polyethylene glycol to the media, and the callus water status and solute (potassium, organic acids, sugars and amino acids) content were determined over an eight week period. At a media water potential of -0.7 MPa, stressed callus had a reduced: 1) osmotic potential, 2) solute content, 3) water content and 4) cell volume compared to control, nonstressed callus growing on a media at -0.4 MPa. It was concluded that partial turgor and growth maintenance were accomplished in stressed callus by reduction in cell volume rather than osmotic adjustment. Lack of osmotic adjustment in loblolly pine callus in this study could be attributed to rapid callus dehydration.

Additional keywords : Osmotic adjustment, turgor maintenance, polyethylene glycol (PEG), cell volume.

Cell culture has been used to select for herbicide resistance in tobacco (Chaleff and Parsons 1978) and salt resistance in alfalfa (Croughan et al 1978) In vitro selection for drought tolerance is also being explored with grain sorghum (Smith et al 1985), tomato (Bressan et al 1981), and loblolly pine (Newton et al 1985). Callus growth from ten cultivars of grain sorghum subjected to water stress with increasing levels of polyethylene glycol (PEG) added to the medium was highly correlated with field ratings of drought tolerance at the plant level (Smith et al 1985). This suggests that a component of plant drought tolerance may have a cellular basis. Similiarly, correlations between whole plant and cell culture responses have been observed for salt tolerance in grape (Barlass and Skene 1981) and barley (Nabors et al 1980). Therefore, correlations established between cell cultures and whole plants indicate that cell cultures may be effective as an efficient tool for drought tolerance selection.

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Cell culture also can be used to observe physiological responses to environmental stress. Osmotic adjustment, a physiological response observed in a variety of whole plants (Morgan 1984), was observed in salt-stressed tobacco cell cultures (Heyser and Nabors 1981) and water stressed tomato cells in culture (Hands et al 1982). Furthermore, genetic variation in osmotic adjustment in whole wheat plants was correlated with yield (Morgan et al 1986), suggesting that osmotic adjustment could be used as a selection criterion in wheat breeding.

Osmotic adjustment has been demonstrated in whole plants of loblolly pine (Hennessey and Dougherty 1984; Seiler and Johnson 1985; Meier and Newton 1987), but it has not been observed in cell culture. If osmotic adjustment could be shown to occur j vitro as well as in vivo in loblolly pine, this would further substantiate the potential of cell culture (Newton et al 1986) as a technique for drought tolerance selection in tree breeding.

Osmotic adjustment is defined as a net solute accumulation (Jones et al 1981; Morgan, 1984), and it is most often associated with partial or full turgor maintenance (Meyer and Boyer, 1981). Solutes (proline) have been observed to increase in water stressed loblolly pine callus, but the increase was associated with reduced callus growth on media with a water potential below -1.0 MPa, and it appeared that proline was not a significant osmoticum (Newton et al 1986). Solutes which accumulate in most drought stressed plants are low molecular weight products such as carbohydrates, sugar phosphates, organic acids, amino acids and inorganic ions such as potassium and nitrate (Jones et al 1981). It is implied from many studies that osmotic adjustment resides in full or partial turgor maintenance which, in turn, maintains growth, particularly elongation.

The objective of this research was to determine if loblolly pine callus responded with osmotic adjustment when subjected to a moderate water stress (-0.7 MPa). Demonstration of osmotic adjustment was dependent upon the following criteria: 1) callus solute accumulation and 2) a reduction in callus osmotic potential.

MATERIALS AND METHODS

A mixed-orchard source of loblolly pine (Pinus taeda L.) seed was obtained from the Texas Forest Service. Excised cotyledons served as an explant source for callus production. Media and culture conditions have been described previously (Newton et al 1986). Callus was produced on Heller supports immersed in MS (Murashige and Skoog 1962) media. Media water potential and callus osmotic potential were determined psychrometrically (Turner et al 1978). Control, nonstress media had a water potential of -0.4 MPa and the stress media had a water potential of -0.7 MPa (10% polyethylene glycol, PEG).

Sugars, amino acids and organic acids were extracted from lyophilized tissues with 80% ethanol (Newton et al 1986). Sugars and organic acids were determined by gas chromatography (Newton et al 1986). Total amino acids were measured spectrophotometrically according to the ninhydrin method of (Rosen 1957). Potassium levels were determined with atomic emission spectroscopy. All growth data are expressed as a mean of ten replicates. Solute data are expressed as a mean of three replicate extractions from 40 mg of lyophilized tissue. Media water potential and callus osmotic potential values are the mean

of 4 sample measurements. Polyethylene glycol associated with callus was measured spectrophotometrically with the trichloroacetic acid method of Lawlor (1970) and modified by Puryear and Newton (unpublished). Polyethylene glycol levels in the tissue were subtracted from the final tissue dry weight; all dry weight data are expressed without the PEG fraction.

Cell volumes were determined with microscopy using an ocular micrometer.

RESULTS AND DISCUSSION

In order to measure the extent of osmotic adjustment in response to water stress, one must determine if: 1) there is a change in osmotic potential, and 2) the change in osmotic potential is due to an accumulation of solutes and/not a loss of water from the callus cells. To test this, callus growing in MS media (-0.4 MPa) for two weeks was transferred to either a new volume of MS media at -0.4 MPa or was placed in MS media with PEG added to obtain a water potential of -0.7 MPa. Callus osmotic potential was measured periodically over an eight week period (Figure 1). An eight week duration was used because this was the length of time previously used to evaluate the water stress performance of calli of several loblolly pine families (Newton et al 1985).

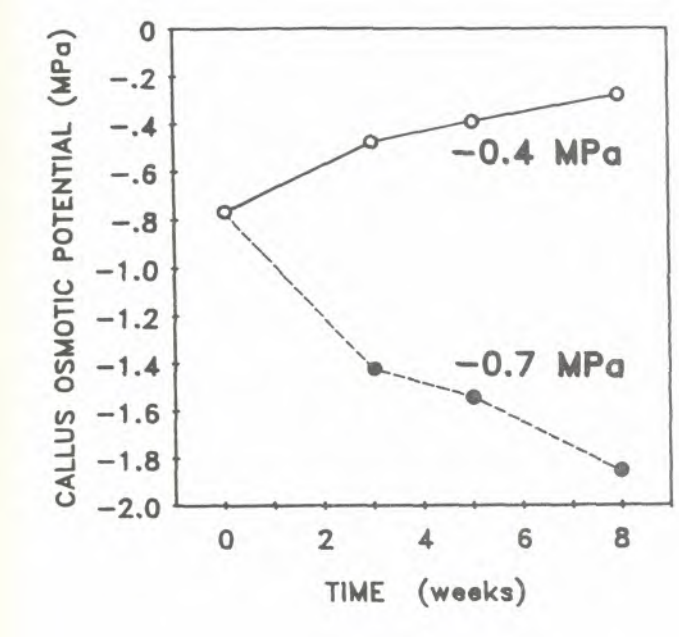

Figure 1. Osmotic potential of nonstressed (-0.4 MPa) and stressed (-0.7 MPa) loblolly pine callus.

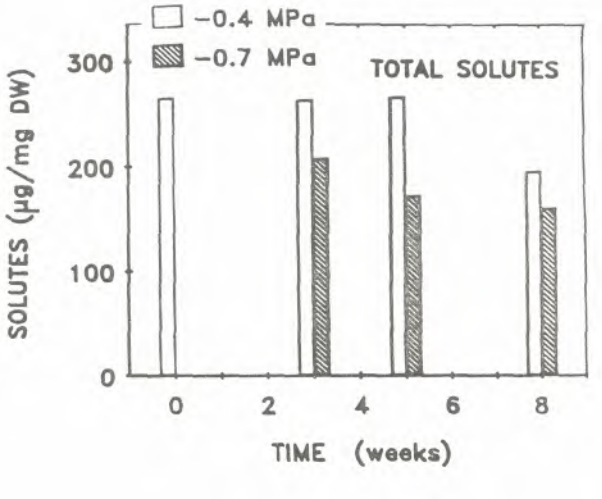

Figure 2. Total solutes (potassium, sugars, amino acids and organic acids) in nonstressed (-0.4 MPa) and stressed (-0.7 MPa) loblolly pine callus.

At the time of transfer, callus osmotic potential was -0.75 MPa (Figure 1). On control media (with a water potential of -0.4 MPa), callus osmotic potential increased to -0.3 MPa by the eighth week. Oppositely, callus transferred to a water stress media (with a water potential of -0.7 MPa), showed a reduction in osmotic potential from -0.75 MPa at week zero to -1.8 MPa after eight weeks (Figure 1).

Callus solute content was then measured to determine its relationship to the callus osmotic potential. In order to determine if there was accumulation, the data were expressed on both a dry weight and a per-callus basis. Common solutes known to accumulate in other plant systems were measured; these were: potassium, sugars, organic acids and amino acids. The total of these four solute fractions was observed to decrease in water-stressed callus (Figure 2 and 3). Therefore, it was apparent that the observed reduction in osmotic potential was not a result of solute accumulation, but was instead due to dehydration in the water stressed callus. This conclusion was verified by analysis of callus growth data.

After eight weeks of growth, fresh weight of callus growing on control media (-0.4 MPa) was over 600 mg while fresh weight on stress media (-0.7 MPa) was less than 100 mg (Figure 4). The initial inoculum callus at the beginning was about 40 mg; therefore, the stressed callus only doubled its fresh weight over the eight week period, whereas the nonstressed callus increased its fresh weight by 15X (Figure 4). Dry weight of the stressed callus was 18 mg and was 30 mg for nonstressed callus (data not shown). This indicated that the fresh

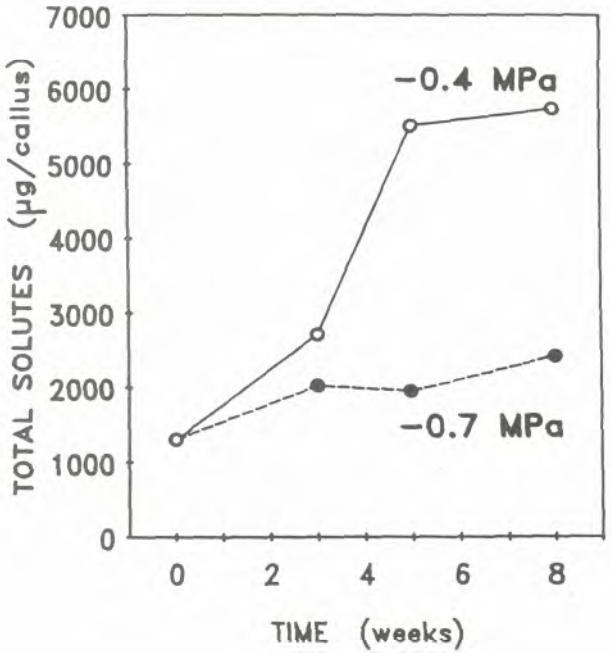

Figure 3. Total solutes (potassium, sugars, amino acids and organic acids) per callus in nonstressed (-0.4 MPa) and stressed (-0.7 MPa) loblolly pine callus.

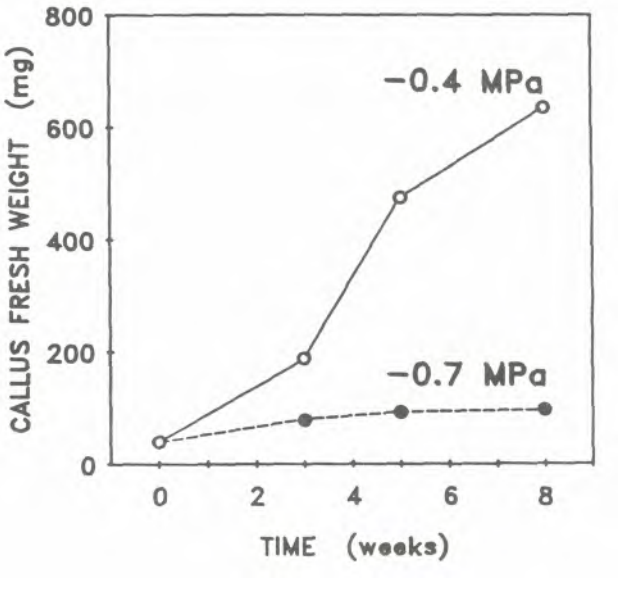

Figure 4. Fresh weight of nonstressed (-0.4 MPa) and stressed (-0.7 MPa) loblolly pine callus.

weight growth difference observed between the contral and stressed callus was due primarily to a reduced water content (fresh weight -- dry weight) in the stressed callus. After eight weeks, the water content of the stressed callus was 80 mg compared to the control callus with a water content of 600 mg.

The reduction in water content of the stressed callus (Figure 4) was also accompanied by a reduction in solute content (Figures 2 and 3) and osmotic potential (Figure 1). The reduced osmotic potential observed was a result of dehydration and not solute accumulation; therefore, there was no osmotic adjustment in the stressed callus. The reduction in total solutes per callus appeared to be a result of reduced callus growth, perhaps a reduction in cell number per callus and/or cell volume.

In contrast, the increase in osmotic potential of nonstressed callus after eight weeks (Figure 1) suggested that cell volumes and/or cell number increased as the fresh weight increased, thus diluting the solute concentration.

Turgor has been shown to be an important component of growth and its total and/or partial maintenance is accomplished by osmotic adjustment (Meyer and Boyer 1981). Since there was no osmotic adjustment observed in stressed callus and there was some maintenance of growth (Figure 4), it is reasonable to assume that at least partial turgor was maintained. In addition to osmotic adjustment, cells can also maintain turgor by reducing their volume (Cutler et al 1977).

The cell population of callus is heterogeneous, ranging from very elongated, rectangular cells to rounded, spherical ones. Both elongated and spherical cell volumes were measured in stressed and nonstressed callus after eight weeks of growth. Cell volumes were reduced 50 to 60% in the stressed callus compared to nonstressed callus (Table 1). This suggested that the callus responded to water stress by producing cells with reduced volumes which perhaps increased the solute concentration and resulted in a reduced osmotic potential. Although cell number/callus was not determined, it is quite likely that it too was reduced when callus was stressed. Therefore if the total solutes/callus (Figure 3) were associated with fewer cells and small cell volumes in response to water stress, the total solute concentration per cell would increase and this would explain the reduction in osmotic potential that was observed (Figure 1). In nonstressed callus, the solute increase (Figure 3) appears to be associated with large cell volumes (and perhaps more cells) and an increase in callus osmotic potential.

Table 1. Cell Volumes of Two Cell Types of Loblolly Pine Callus[1]

Cell Type	Water Potential		Percent Reduction
	-0.4MPa	-0.7MPa	
Spherical	$436 \pm 90 \mu^3$	$164 \pm 66 \mu^3$	62%
Long	$720 \pm 109 \mu^3$	$361 \pm 139 \mu^3$	50%

[1]Callus eight weeks old; n-270

Assessment of turgor maintenance can be accomplished by measuring both callus water potential and osmotic potential and estimating the turgor potential by a difference calculation of these two measurements. We are presently making these measurements in our laboratory in order to estimate turgor. We also are measuring callus turgor with a pressure probe which will be used to corroborate turgor estimates.

The results of this investigation should not be interpreted as to mean that osmotic adjustment does not occur in loblolly pine callus tissue in response to water stress. Under similar experimental conditions, osmotic adjustment was not apparent in stressed callus of a drought tolerant cultivar of sorghum either (Newton et al 1986). Osmotic adjustment is greatly dependent upon the rate of dehydration in the tissues when subjected to water stress (Jones et al 1981). When the water potential of fully expanded sorghum leaves decreased at a rate of over 1.0 MPa/day, no osmotic adjustment occurred, but when the stress rate was 0.1 MPa/day, osmotic adjustment was most evident (Jones and Rawson 1979). It is quite possible that callus mounted on Heller supports is more stressed than indicated by the media water potential in which the support is immersed. If so, rapid dehydration with a rapid decrease in water potential would prevent osmotic adjustment.

ACKNOWLEDGEMENT

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