GROWTH CHANGES IN LOBLOLLY PINE (PINUS TAEDA L.) CELL CULTURES IN RESPONSE TO DROUGHT STRESS

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Abstract.-- Two in vitro systems; callus proliferation from (1) cotyledons and (2) pre-existing callus, were used to evaluate growth responses to low tissue water potential among two loblolly pine sources including 6 families. Both systems showed significant differences in growth response to low tissue water potential between sources from Louisiana and Texas. Both systems also showed significant differences between a fast-growing family from Louisiana (A-1-14) and a slow growing family from Texas (GR1-8). Louisiana sources sustained more growth under in vitro drought stress than did Texas sources. The minimum tissue water potential below which callus growth was halted was -1.0 MPa. These preliminary results suggest that in vitro drought stress techniques may have applicability for predicting growth potential in the field under stressed and nonstressed conditions and investigating drought tolerance mechanisms which appear to be distinct and separate from drought avoidance mechanisms. Additional research is in progress to confirm these results.

Additional keywords: Tissue culture, polyethylene glycol, callus, water potential, drought tolerance.

Several investigators have shown that there are differences in loblolly pine (Pinus taeda L.) source responses to drought. Most of these studies have been concerned with drought avoidance whereby plants tolerate drought by maintaining a high tissue water potential. Whole plant studies have shown that increased root growth (Youngman 1965, van Buijtenen et al. 1976, Bilan et al. 1978, Cannell et al. 1978), reduction in epidermal conductance (Thames 1963, Knauf and Bilan 1974, van Buijtenen et al. 1976, Bilan 1977) and reduction in evaporative surfaces (Wells and Wakeley 1966, Wright and Bull 1968, Woessner 1972a,b; Venator 1976) are mechanisms by which high tissue water potentials are maintained in loblolly pine resulting in drought avoidance. Only a few studies have investigated the mechanisms whereby drought is tolerated at low tissue water potential. These whole plant

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studies have shown that solutes accumulated (Hodges and Lofio 1969) and that growth potential was maintained through osmotic adjustment (Hennessey and Dougherty 1984) as low tissue water potentials were experienced. Other workers have shown differential tolerance to low tissue water potential among loblolly pine families (Kaloyereas 1958, van Buijtenen et al. 1976, Newton and van Buijtenen 1984) when whole plants were subjected to desiccation.

A recently developed technique for investigating drought tolerance at low tissue water potential is in vitro tissue culture. Tissue culture has several advantages over whole plants: (1) drought avoidance mechanisms such as increased rooting, decreased stomatal conductance and leaf drop can be dismissed, (2) cellular mechanisms of drought tolerance at low water potential can be investigated, and (3) it could be used to screen a large number of different sources of germplasm for drought tolerance in a short period of time and in a small space. Therefore, if the suitability of tissue culture as a selection tool for drought tolerance could he demonstrated, it could aid existing tree improvement programs, particularly those in the western edge of the loblolly pine region.

The first objective of this investigation was to evaluate two tissue culture systems for their suitability in differentiating between loblolly pine sources in their growth response to low tissue water potential. To accomplish this objective, seed sources from Louisiana and Texas were compared. Based on previous, percent-survival data in response to drought, the Louisiana sources have been designated as drought-susceptible and the Texas sources as drought-hardy (Newton and van Buijtenen 1984). Drought-hardy and drought-susceptible families have been selected for avoidance and maintenance of high tissue water potential rather than tolerance of low tissue water potential. We wanted to evaluate them for drought tolerance at low water potential by exploiting the unique system of cell culture.

The second objective was to determine the tolerance limit of callus growth in loblolly pine. That is, we wanted to determine minimum water potentials whereby growth could be sustained when callus was subjected to drought stress. This information would be important for comparison with other callus systems, particularly crop plants and other woody species. Furthermore, drought responses of cell cultures have not been previously reported for loblolly pine or for any other woody plant species. The data presented here show that in vitro cultures can be used for determining differences between loblolly pine source tolerance to low water potential and that cell growth is not sustained at water potentials lower than -1.0 MPa.

MATERIALS AND METHODS

Six half-sib loblolly pine families from known sources were used. Three of the families (BA3R13-41, BA3L11-1, GR1-8) were taken from the western edge of the loblolly pine range in Texas (TX) and three families

(A-1-4, A-1-7, A-1-14) were obtained from Crown Zellerbach Corporation and originated from Louisiana (LA). Explant and media procedures were modified from Mott and Amerson (1982). Seeds were placed in sterile flasks containing 1% H_2O_2 at room temperature (27°C) to stimulate germination (Ching and Parker 1958). After 4-5 days, seeds were further surface sterilized in 15% clorox solution for five minutes followed by three rinses of sterile water. Embryos were then aseptically removed and the cotyledons were excised as explants. In one in vitro system, the cotyledons were inoculated onto the experimental liquid media and allowed to produce callus (cot->cal) and in the second system, excised cotyledons were first placed on a complete agar media, allowed to proliferate callus for four weeks and then callus was inoculated onto the experimental media (cal->cal). Both the agar and the experimental liquid media were amended with napthalene acetic acid (NAA) and benzylamino purine (BAP) (see RESULTS). The cotyledons or callus were inoculated on Heller supports in test tubes containing Gresshoff-Doy (GD) (1972) liquid media with polyethylene glycol (PEG, Mol. Wt. 8000, Sigma) added to provide varying water potential (04). The media water potential was determined with a microvoltmeter (Wescor HR 33T) and thermocouple psychrometer chamber (Wescor C52). The standard media without PEG had a water potential of -0.4 MPa. **Six** sets of PEG media were prepared with a water potential of -0.6, -1.0, -1.3, -1.8, -2.3 and -2.7 MPa. In the cot->cal system the initial cotyledon explant weights were between 1.1 and 1.5 mg, and there were no significant differences between families in regard to their initial weights. In the cal->cal system, 80 to 90 mg of callus tissue were placed into each tube. The inoculated tissues were subjected to drought stress for eight weeks in a controlled environment chamber with a temperature of 21°C and continuous light (fluorescent and incandescent) with an intensity of $pEm^{-2} sec^{-1}$. Each treatment was replicated ten times. Test tubes were placed in a randomized block design and the position of each rack of tubes was randomly arranged every 24 hours to ensure equal treatment. After eight weeks the resulting callus was frozen in liquid nitrogen, lyophilized, and the dry weights recorded.

All data were analyzed with Analysis of Variance and Duncan's Multiple Range Test.

RESULTS

It was important first to determine which combination of napthalene acetic acid (NAA) and benzyl-amino-purine (BAP) would provide the most callus growth. One mg/lNAA and 3mg/1BAP resulted in maximum growth of callus after 8 weeks on a GD-agar medium. This modified medium was used thereafter as the standard medium for all subsequent experiments.

In vitro fresh weight growth of callus from cotyledons (cot->cal) and from pre-existing callus (cal->cal) over an eight week period in normal media with no PEG added are shown in Table 1. The water potential was -0.4 MPa. In both systems, family A-1-14 increased in fresh weight more than the other five families and family GR-1-8 had the least growth. Family rankings based on mean fresh weight were also similar in both systems with

the exception of BA3R13-41 and A-1- 4 which were reversed.

TABLE 1. *In vitro* fresh weight growth of 6 loblolly pine families at a water potential of -0.4 MPa.

		Mean Fresh Weight (mg)	
Family	Source	Cot->Cal[1]	Cal->Cal[1]
A-1-1	LA	58.0 a[2]	263.8 a[2]
A-1-7	LA	54.9 a	237.8 ab
A-1-4	LA	43.3 ab	173.9 ab
BA3R13-41	TX	42.8 ab	184.8 ab
BA3L11-1	TX	29.5 ab	128.4 ab
GR-1-8	TX	18.1 b	108.9 b

[1] 10 replicates per family
[2] means sharing a common letter are not significantly different at an alpha level of 0.05

Cultures from each family were next tested for their capacity to grow when PEG was added to the media. Cot->cal and cal->cal systems were both used with water potentials ranging from -0.6 to -2.7 MPa and -0.6 to -2.3 MPa for the two systems, respectively. The overall mean cot->cal fresh weight at 6 different water potentials was the largest for family A-1-14 and was smallest for family GR1-8 (Table 2). The overall mean cal->cal fresh weight at 5 different water potentials was also larger for A-1-14 and smaller for GR1-8 (Table 2). Growth of GR1-8 was 60 and 40% less than growth of A-1-14 in the cot->cal and cal->cal systems, respectively. Fresh weight growth under stressed and nonstressed conditions was significantly different between these two families (Table 1,2). Analysis of Variance showed significant differences at the 6% level between sources in response to drought stress (Table 2). Louisiana sources sustained more growth under drought stress than did Texas sources.

TABLE 2. In vitro fresh weight growth of 6 loblolly pine families averaged over all water potentials.

		Mean Fresh Weight (mg)	
Family	Source	Cot->Ca11,4	Cal->Ca12,4
A-1-14	LA	11.7 a[3]	93.7 a
A-1-7	LA	10.9 a	84.3 ab
A-1-4	LA	9.4 ab	66.9 bc
BA3R13-41	TX	8.5 ab	67.3 bc
BA3L11-1	TX	8.2 ab	56.9 c
GR-1-8	TX	4.5 b	55.0 c

[1] 6 different water potentials with 10 replicates per family
[2] 5 different water potentials with 10 replicates per family
[3] means sharing a common letter are not significantly different at an alpha level of 0.05
[4] sources are significantly different at an alpha level of 0.06

It was most meaningful to determine the minimum tolerance levels for in vitro growth by loblolly pine since this has not been reported earlier. This was accomplished by pooling the family fresh weights for each water potential and comparing the overall means (Table 3). Mean fresh weight growth of the cot->cal system was 63% less than the control when subjected to drought stress at -0.6 MPa and 96% less than the control at -1.0 MPa. (Table 3). There was no growth at water potentials lower than -1.0 MPa. Fresh weight of the cal->cal system was decreased by 64% at -0.6 MPa and 74% at -1.0 MPa compared to the control. However, final fresh weight of the cal->cal tissue was smaller than the original inoculum fresh *weight at all stress treatments; only* the *callus at* -0.4 MPa *was larger than* the initial callus fresh weight (Table 3). At water potentials less than -1.0 MPa the fresh weight of both systems remained relatively constant. The minimum tolerance level for cot->cal growth was -1.0 MPa and was -0.6 MPa for cal->cal growth (Table 3).

TABLE 3. *In vitro* fresh weight growth of loblolly pine as influenced by decreasing water potential.

	Mean Fresh Weight (mg)	
ψw(MPa)	Cot->Cal[1]	Cal->Cal[1]
-0.4	41.6 a[2]	182.9 a[2]
-0.6	15.1 b	66.4 b
-1.0	1.6 c	47.9 bc
-1.3	1.3 c	45.9 bc
-1.8	1.0 c	43.1 c
-2.3	1.0 c	37.6 c
-2.7	0.8 c	

[1] n = 60 with 10 replicates per family
[2] means sharing a common letter are not significantly different at an alpha level of 0.05.

Dry weight decreased in the cot->cal system when subjected to drought stress, but cal->cal dry weight remained constant at all stress levels (Table 4). Dry weight of the cot->cal system was decreased by nearly 90% at -1.0 MPa compared to cultures at -0.4 MPa. Therefore, the decrease in fresh weight of cot->cal (Table 1) was due to both water loss and dry weight decrease (Table 3) whereas, the fresh weight decrease of cal->cal was primarily water loss (Table 1) with very little change in dry weight (Table 3).

DISCUSSION

Two in *vitro* culture systems were used to compare growth between loblolly pine families and sources. Both systems showed significant differences between fast-growing and slow-growing sources during drought stress. The family ranking of mean fresh weight growth under nonstressed conditions (Table 1) was the same as their ranking under stressed conditions (Table 2). Family A-1-14 from Louisiana consistently grew better under nonstressed and stressed conditions compared to the other 5 families and family G1-8 from Texas was a consistent, poor performer. These

TABLE 4. *In vitro* dry weight growth of loblolly pine as influenced by water potential.

	Dry Weight (mg)	
ψw(MPa)	Cot->Cal[1]	Cal->Cal[1]
-0.4	7.2 a[2]	20.2 a[2]
-0.6	4.4 b	22.3 a
-1.0	0.8 c	20.5 a
-1.3		21.8 a
-1.8		21.3 a
-2.3		20.7 a

[1] n = 60 with 10 replicates per family
[2] means sharing a common letter are not significantly different at an alpha level of 0.05

data indicated that fast growing families under nonstressed conditions also perform well under stressed conditions and that Louisiana sources grew significantly better than Texas sources. Although preliminary, these data also show that source tolerance responses to drought may be different from source avoidance responses.

Some interesting comparisons with field observations can be made here. Louisiana sources are known for their rapid growth under field conditions (Yeiser et al. 1981). Under drought stress, however they suffer excessive mortality (Zobel and Goddard 1955), although surviving trees may grow quite well for a period of time. Mortality of Louisiana source outplantings in drought prone areas decreases per acre yields compared to seed sources selected for drought resistance (van Buijtenen, unpublished). it will be most interesting to pursue the possibility that in *vitro* growth is indicative of growth rate observed under field conditions.

Callus proliferation from cotyledons appeared to be very limited if the water potential was less than -1.0 MPa. This is similar to the level of drought tolerance of other in *vitro* systems such as grain sorghum (Newton et al., submitted). At water potentials less than -1.0 MPa, the fresh weight of the cot->cal cultures (0.8 to 1.6 *mg)* were not significantly different from the weight (1 mg) of the initial, inoculated cotyledons

(Table 3). Control medium in these experiments was at a water potential of -0.4 MPa; higher water potentials were not tried. It would be most helpful to determine if growth is increased at these higher water potentials.

Even though callus fresh weight produced from callus was severely reduced by the slight stress of -0.6 MPa, the dry weight of the cal->cal system was not affected (Table 4), indicating that the tissue was becoming more dehydrated as it experienced more drought stress. For example, callus tissue at a water potential of -0.4 MPa contained 160 mg of water with a dry weight of 20.2 mg whereas tissue at -1.0 MPa had 27 mg of water with a dry wt of 20.5 mg (Table 3, 4).

In conclusion, Louisiana sources sustained more growth under in vitro drought stress than did Texas sources. The minimum tissue water potential below which callus growth ceased was -1.0 MPa. In vitro drought stress techniques may have applicability for predicting growth potential in the field under stressed and nonstressed conditions. Furthermore, the data indicate that this technique may be suitable for investigating drought tolerance mechanisms which appear to be distinct and separate from drought avoidance mechanisms. Additional research is in progress to confirm these preliminary results.

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