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Water Potential of Radiata Pine Shoots Cultured *in vitro* under Different Relative Humidities

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The water potential of radiata pine shoots cultured for 21 days under different relative humidities (10, 30, 50, 70 and 90% outside the culture vessel during the photoperiod) was investigated for 10 hours at 85 and 75% relative humidities. Changes in shoot water potential with time were used to evaluate the effect of the relative humidity in the culture vessel on the degree of water stress experienced by the shoots after transplanting. A device was developed to control relative humidities in transparent boxes containing culture vessels. Relative humidity in the culture vessels ranged from 80-93% during the photoperiod and was higher than that outside the vessel for each treatment by about 5-70%. The shoots cultured under an outside relative humidity of 90% exhibited a rapid decrease in water potential after exposure to relative humidities of 85 and 75%, while the water potential of those cultured under an outside relative humidity of 10% gradually increased. The results indicate that reducing relative humidity in the culture vessel to an appropriate level may provide a way to produce shoots better able to withstand severe water stress after transplanting from *in vitro* conditions to *ex vitro* conditions.

Introduction

Relative humidity in the culture vessel is known to affect the water status or the events involved in the water relations of tissue-cultured shoots and plantlets, *e. g.* the rate of water loss[1,2], wilting after transplaning to *ex vitro* conditions[3], leaf surface structure[4], epicuticular wax formation[2,3,5,6] and water potential[6]. Grout & Aston[7] indicated the importance of reducing relative humidity in the culture vessel prior to transplanting to a low relative humidity environment, such as a greenhouse or directly to the field.

This study investigated the water potential of transplanted *Pinus radiata* D. Don (radiata pine) shoots following culture under different relative humidities, and evaluated the degree of water stress on the basis of the time course of their water potentials. The validity of utilizing water potential for evaluating the degree of water stress has been described by Kramer[8].

There have been several studies on water potentials of cultures[9-11], and there has been a report on the evaluation of plantlet water stress after transplanting to soil[12]. However, we found no reports evaluating the degree of water stress of tissue-cultured shoots or plantlets on the basis of water potential measurements. The novelty of this study lies in the use of water potential to evaluate the effect of relative humidity conditions in culture on the degree of water stress experienced by tissue-cultured shoots after transplanting to *ex vitro* conditions.

Materials and Methods

1. *Shoots*

Single shoots were excised from radiata pine shoot clumps of one clone (268. 323 Clone 7) which had been initiated and elongated using the method described by Aitken-Christie & Jones[13]. The shoot clumps had been grown on 100 ml of modified Lepoivre medium[14] with 30 g$\cdot l^{-1}$ sucrose and gelled with 2. 38 g$\cdot l^{-1}$ Gelrite plus 0. 5 g$\cdot l^{-1}$ Difco Bacto agar (gelled LP medium) in 580 ml glass jars with plastic petri-dish lids under a 16 h photoperiod, photosynthetic photon flux density of 80 μmol$\cdot$m$^{-2}\cdot$s^{-1} and temperature of 26$\pm$1°C/18$\pm$1°C (photo/dark period).

Six single shoots (fresh weight per shoot : 294 mg) were transplanted onto 125 ml of gelled LP medium in a 580 ml glass jar with a plastic screw lid. A piece of gas permeable membrane filter (CELGARD 4410, Celanese Plastics, U. S. A.) was inserted over a hole in the lid to give an effective area for gas exchange of 16 cm^2, pore size less than 0. 2 μm, under the same conditions as that described above. The dry weight per shoot and water potential estimated from destructive measurement of 7 other shoots of the same clone at same stage were 66 mg and $-$1. 3 MPa, respectively. Ten jars containing shoots were prepard. After transplanting, 2 jars were placed for 21 days in each of 5 transparent boxes with different relative humidities (boxes-A, see **Fig. 1**).

On day 21, 5 of the 6 shoots from a jar placed in each of the boxes-A were transplanted onto 50 ml of gelled LP medium in a petri-dish with no lid. These were placed in another transparent box (box-B, see **Fig. 1**). The remaining shoot in the jar was used for measuring water potential for a 0 h-value. Measurements of water potential for each of the other 5 shoots were made every 2 hours after exposure to humidity-controlled air in box-B.

2. *Humidity control device*

The relative humidities in the jars (culture vessels) were regulated by controlling the atmosphere in boxes-A. This was achieved for the 4 boxes in group A by connecting the box through an inlet pipe to either distilled water or saturated salt solutions in large Erlenmeyer flasks, as well as by altering the inlet pipe length (**Fig. 1**). For the remaining box-A, the base air of 10% relative humidity was supplied through an empty flask (**Fig. 1**). The rate of air flow at the pipe inlet of each box was approximately 3 $l\cdot$min^{-1}, and the inside volume of the box was about 18 l. Relative humidities in boxes-A were respectively controlled to be approximately 90% (box-A_{90}), 70% (box-A_{70}), 50% (box-A_{50}), 30% (box-A_{30}) and 10% (box-A_{10}) during the photoperiod.

Treatment names were given to the humidity treatments corresponding to each of relative humidities in boxes-A as follow : RH90 for 90%, RH70 for 70%, RH50 for 50%, RH30 for 30%, and RH10 for 10%. Relative humidity in box-B was controlled at approximately 85% for the first 4 hours and 75% for the following 6 hours.

Relative humidities were measured with a macromolecule humidity sensor (THT-A232, Shinyei, Japan). The sensor was calibrated and corrected using a divided flow type humidity generator (SRH-3MC, Shinyei, Japan). Readings were taken after the humidity stabilized.

3. *Water potential measurement*

Water potentials of shoots and media were measured using a thermocouple psychrometer (SC-10A, Decagon, U. S. A.) and a nanovolt-thermometer (NT-3, Decagon, U. S. A.). Samples were left in the psychrometer sample chambers for 1 h to equilibrate before measurement. Preliminary experiments showed that this was sufficient time for equilibration with radiata pine shoots and LP media. The psychrometer was calibrated against NaCl solutions at 25°C. Water potential was calculated according to the calibration line obtained. Shoot samples were cut into about 10 mm

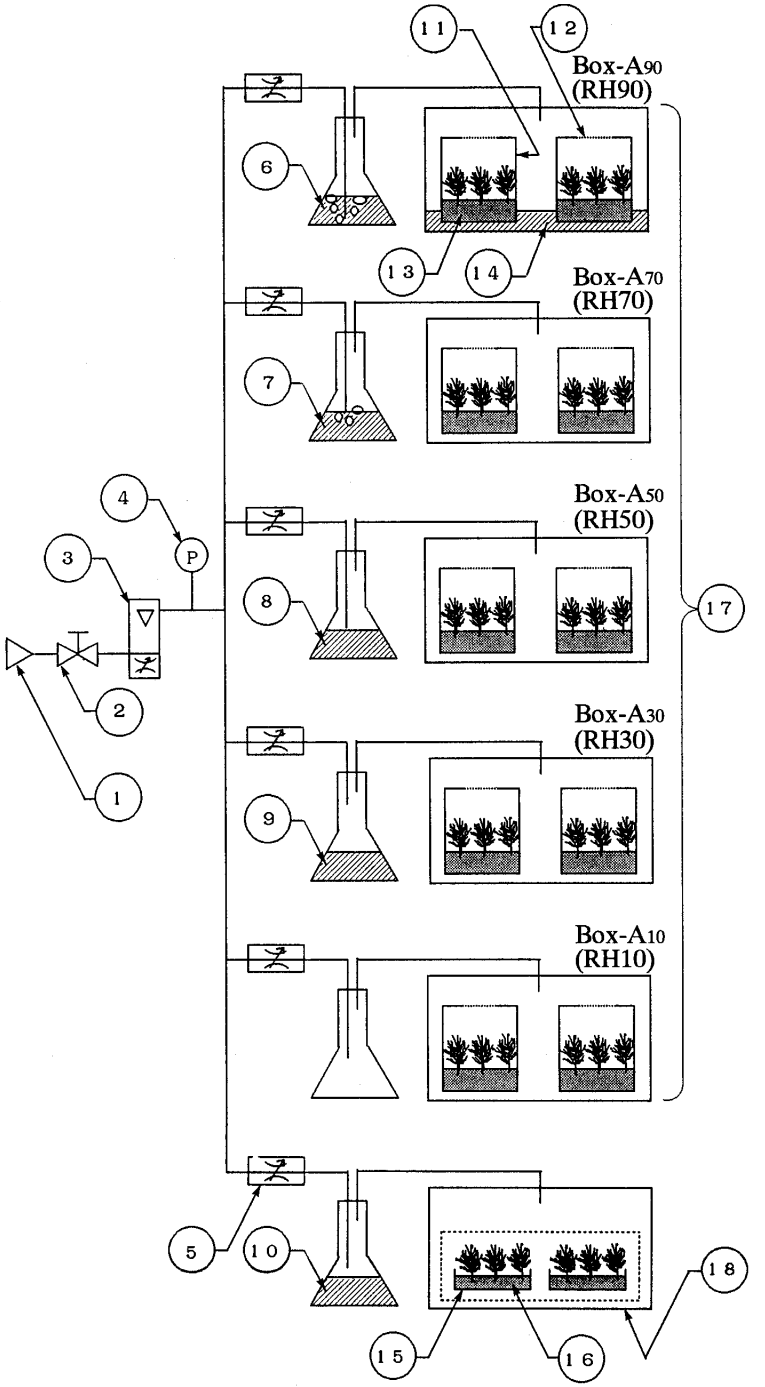

Fig. 1 Schematic diagram of a device for controlling relative humidity in transparent boxes (boxes-A and box-B).
Five shoots cultured in one jar in each of boxes-A were transplanted onto gelled LP medium in a petri-dish ; then placed in box-B on day 21.
①Air compressor, ②Valve, ③Flow meter with a discharge regulator, ④Pressure gauge, ⑤Discharge regulator, ⑥Distilled water, ⑦Distilled water, ⑧NaCl saturated solution, ⑨$Ca(NO_3)_2 \cdot 4H_2O$ saturated solution, ⑩Distilled water, ⑪Culture vessel (Jar with screw lid), ⑫Membrane filter, ⑬Gelled medium, ⑭Distilled water, ⑮Petri-dish, ⑯Gelled LP medium, ⑰Transparent box (boxes-A), ⑱Transparent box (box-B)

pieces of needles and stems after trimming off any brown-colored shoot needles. Medium samples were taken from around the shoot bases. Water potential measurements were made on days 0 and 21 once for each sample.

Results and Discussion

1. *Relative humidities in culture vessels*

The relative humidities measured in culture vessels (jars) during the photoperiod were 93, 91, 88, 85 and 80% for treatments of RH90, RH70, RH50, RH30 and RH10, respectively, and the values in the jars increased by 2-4% during the dark period (**Table 1**). The humidity conditions in the jar of RH90 can be regarded as approximately standard culture humidity conditions. The difference in relative humidity between the inside and outside of the jar increased markedly as relative humidity outside the jar decreased, although the jar had a considerably higher number of air exchanges[15], approximately 20 h^{-1}. It is likely that the marked increment of relative humidity in the jars, observed in relatively lower humidity treatments, was due to the higher rates of evaporation from the medium and transpiration from the shoots. The sum of the evaporation and transpiration rates in the jar from RH10 was estimated, using the values in **Table 1**, to be about 40 times higher than that of RH90.

2. *Water potentials and weights of media*

Water potentials and weights of all the media from the different relative humidity treatments on day 21 were lower than those of the fresh media (−0.38 MPa and 129 g), with decreasing relative humidity levels giving greater differences (**Table 2**). The large depression in both water potential and weight of media can be attributed to higher evaporation rates from the medium and water absorption rates of shoots from the medium caused by low humidities in the jar.

3. *Appearance of shoots*

On day 21 the shoots from RH10 and RH30 exhibited a dark green color with brown-colored needle tips. The shoots from RH70 and RH90 were green and healthy. The humidity conditions of RH50 seemed to be more appropriate for radiata pine shoots from visual assessment, since the shoots were a darker green than shoots from RH70 and RH90 and had less browning on the needle tips than shoots from RH10 and RH30.

Table 1. Relative humidity inside and outside the culture vessels containing radiata pine shoots.

Treatment (box)	Inside		Outside	
	Photoperiod (26.0°C)	Dark period (18.5°C)	Photoperiod (25.5°C)	Dark period (18.5°C)
RH90 (box-A_{90})	93	97	89	98
RH70 (box-A_{70})	91	94	70	88
RH50 (box-A_{50})	88	92	49	66
RH30 (box-A_{30})	85	89	31	43
RH10 (box-A_{10})	80	82	12	14

The values in parentheses indicate temperatures inside and outside the culture vessels. Measurements were made on day 19. Relative humidity outside the vessels, namely in boxes-A, during the photoperiod was controlled so as to be constant for 21 days.

Table 2. Water potentials and weights of the media on day 21.

Treatment	Water potential [MPa]	Weight [g]
RH90	−0.64	104.7
RH70	−0.70	90.2
RH50	−0.78	77.5
RH30	−1.04	66.5
RH10	−1.31	45.3

Water potentials and weights of the media on day 0 were approximately −0.38 MPa and 129 g, respectively.

4. *Time course of water potential*

On day 21, shoot water potentials of the higher humidity treatments tended to be higher than those of lower humidity treatments (see the values at 0 hours in **Fig. 2**). These results are consistent with previous findings concerning the effect of relative humidity on the water potential of carnation shoots[6].

During the first 4 hours in box-B, shoot water potential of RH90 decreased rapidly. Shoot water potentials of RH70 and RH50 did not show marked decreases in spite of the relative humidities inside the jars in which they had been cultured being lower than the humidity of 85% in box-B for the first 4 hours (**Table 1**). This may be because the water potential (−0.38 MPa) of fresh media in petri-dishes on which the shoots were transplanted was higher than water potentials of media in the jars on day 21 (**Table 2**), or because the drop in humidity of 3-6% when transplanted from boxes

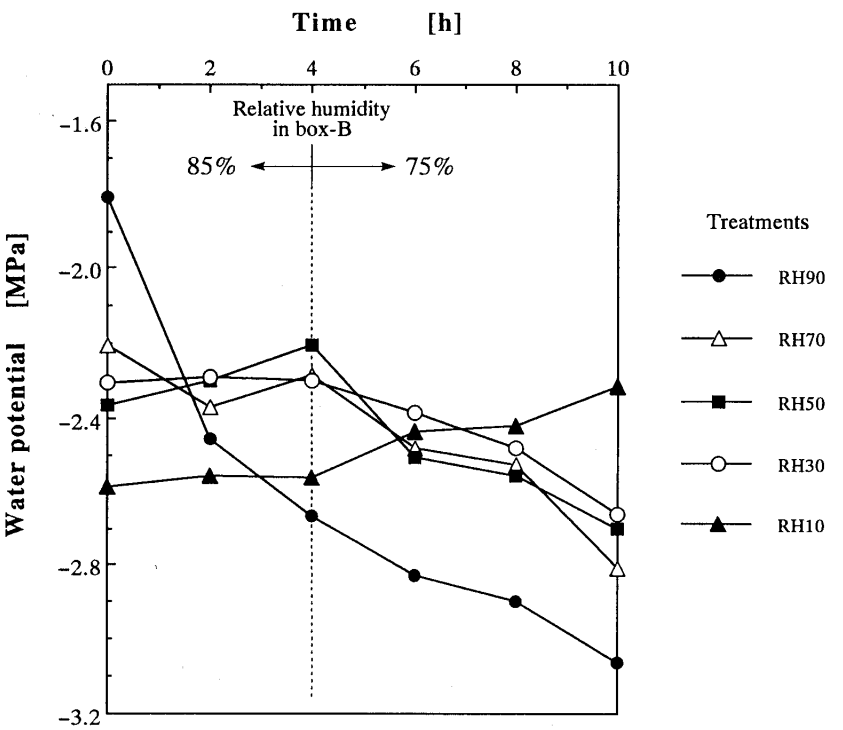

Fig. 2 Time course of water potential of radiata pine shoots cultured under different humidity conditions (see **Table 1**), immediately after transplanting on gelled LP media in a transparent box (box-B) in which the relative humidity was controlled to be approximately 85% for the first 4 hours and approximately 75% for the following 6 hours.

-A to box-B did not cause a significant increase in transpiration. Only minor changes in water potential were observed on the shoots of RH30 and RH10.

When the relative humidity in box-B was changed from 85% to 75% 6 hours after transplanting, the water potential of all shoots except for those of RH10 decreased. The increase in water potential of shoots from RH10 may be attributed to high water absorption ability and greater ability to control transpiration and water potential.

The most rapid decrease in water potential during exposure to both relative humidities (85% and 75%) was observed with the shoots from RH90, whereas the shoot water potential of RH10 increased gradually during the time period. There was no large difference in water potential between shoots of RH70, RH50 and RH30. These data clearly show that relative humidity during culture has effects on the change in water potential of tissue-cultured shoots when they are transplanted into different relative humidities. Reducing relative humidity in the culture vessel to a certain level may provide a way to improve the percentage survival of shoots/plantlets after transplanting to the greenhouse or field. Reduced humidity treatments prior to transplanting to soil were found to improve the survival of carnation plantlets[6] and to enable chrysanthemum plantlets to avoid severe wilting[3]. A follow-up survival and rooting *ex vitro* study with radiata pine shoots remains to be conducted.

5. *Water stress and importance of humidity control*

Kramer[8] stated that water potential seemed to be the best single measure of plant water status, and that measurements of water potential seemed to have maximum utility for estimating the degree of water stress. Radiata pine shoots cultured under relatively higher humidity conditions were water-stressed more rapidly after exposure to air at 85% and 75% relative humidities, and were more severely water-stressed 10 hours after exposure than those cultured under relatively lower humidity conditions. Therefore, shoots cultured under relatively higher humidity conditions suffer more readily from severe water stress resulting in wilting and a slowing down of growth rate, while those cultured under relatively lower humidity conditions suffer less. Appropriate control of relative humidity in the culture vessel would produce tissue-cultured shoots and plantlets more able to withstand severe water stress after transplanting to soil from culture. On the other hand, moderate water stress caused by reduced relative humidity in the culture vessel to a certain level may be beneficial to shoots and plantlets if applied before transplanting to soil.

Appropriate control of humidity conditions in the culture vessel will contribute to the improvement of the quality and production efficiency of tissue-cultured shoots and plantlets. The development of a simple and inexpensive method for controlling relative humidity in the culture vessel seems to be worthy of further study. Membrane filters[16] or paper filters[17] can be utilized in lids of the culture vessels so as to allow the vessels to release excess water vapor to the outside air and allow CO_2 enrichment. Bottom cooling of the culture vessel also reduces relative humidity in the vessel[18].

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《和文要約》

異なる相対湿度下で培養されたラディアータマツシュートの水ポテンシャル

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異なる相対湿度下で21日間培養したラディアータマツシュートを，相対湿度85%の培養器外雰囲気中に4時間，続いて75%の雰囲気中に6時間置いたときの，それらのシュートの水ポテンシャルの経時変化を測定した．また，その水ポテンシャルの経時変化から，それらのシュートを培養器外へ移植した後に生じる水ストレスの程度に及ぼす培養期間中の相対湿度の影響を評価した．明期の培養器外相対湿度90%下で培養されたシュートの水ポテンシャルは，上記培養器外雰囲気中に置かれた後急激に減少したのに対して，培養器外相対湿度10%下で培養されたシュートのそれは，時間の経過とともに上昇した．培養期間中の培養器内相対湿度をある程度低下させて培養することにより，培養器外へ移植した後に生じる強度の水ストレスに抗し得るシュートを生産できることが示された．