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Root histology, and nutrient uptake and translocation in tissue culture plantlets and seedlings of *Thuja occidentalis* L.

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Summary. Histological studies of the vascular connection in the root-shoot junction of tissue culture-derived plantlets and seedlings of *Thuja occidentalis* L. (Eastern white cedar) showed that in both plant types the xylem was well developed, smooth and not disrupted. Uptake and translocation of ^{32}P and ^{86}Rb were also similar for plantlets and seedlings. However, approximately 50% more ^{86}Rb could be desorbed from the root Donnan free space of plantlets than from seedlings. Uptake and translocation to the shoot was higher for ^{86}Rb than for ^{32}P, but no differences were found between the plant types. Tracer translocation rates to the shoot seem to be positively correlated with shoot fresh weight.

Key words: *Thuja occidentalis* – ^{32}P and ^{86}Rb uptake and translocation – Root histology – Tissue culture plantlets

Introduction

Plantlets of several conifers can now be produced by the rooting of shoots obtained through adventitious budding on seed and seedling explants in culture (David 1982; Dunstan and Thorpe 1986). However, very little information is available on the structural features and the physiological behaviour of these tissue culture-derived plantlets. To the best of our knowledge, no data on mineral nutrient uptake and translocation in conifer plantlets have been published. The efficiency of uptake and translocation of minerals would be dependent to a large extent on the nature of the root-shoot junction of the in vitro produced plantlets. In some plantlets, roots are initiated within the callus formed at the base of the shoot, while in others no callus is formed and roots are formed at base of the shoot, which may or may not become swollen prior to root initiation (David 1982; Rancilac 1979; Patel et al. 1987). Although the histology of this region has not been extensively studied in conifer plantlets, one study involving both black spruce and white spruce revealed that the root-shoot junction was rather complex and not as smooth and continuous as that normally found in conifer seedlings (Patel et al. 1987).

We have recently completed a micropropagation protocol for plantlet production of Eastern white cedar, starting with excised embryos and following the multi-staged organogenic route (Harry et al. 1987). We have used this system to compare the anatomy of the root-shoot junction region with that of greenhouse-grown seedlings. We have also investigated anion ($^{32}PO_4^{2-}$) and cation ($^{86}Rb^+$) uptake and translocation in both these seedlings and the tissue culture-derived plantlets. Our findings are reported here.

Materials and methods

Plant material. The tissue culture generated plantlets of *Thuja occidentalis* used in these experiments were produced according to procedures outlined by Harry et al. (1987). Four-month-old shoots (derived from excised embryos in vitro) were first hardened at 20° C for 3 weeks, and then rooted in a mist bed under non-sterile greenhouse conditions. Rooted shoots were potted separately and grown in the greenhouse. Seedlings (from open-pollinated seed) were also generated in the greenhouse. Seeds were first washed under running tap water for 2 days, and sown in commercial peat moss. After 2 months seedlings were separated and placed in individual pots.

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Approximately 3 months before the experiments began, vigorous specimens of both plantlets and seedlings were transferred to Spencer-Lemaire Rootrainers (Spencer-Lemaire Industries, Edmonton, Canada) containing peat and sand in a ratio of 2:1. At this stage, the plantlets were 5 months old (from rooting), and the seedlings were 2 months old. The Rootrainers were 20 cm deep and had grooved sides which allowed the roots to grow downwards rather than in spirals; they also allowed individual plants to be removed without causing any root damage. Containers were then placed in a controlled environment growth chamber (Conviron, Winnipeg, Canada), where humidity was maintained at approximately 50%–60%. Light intensity was kept at 35–40 $\mu E \cdot m^{-2} \cdot s^{-1}$ (PAR) for a photo-period of 16 h, by wide spectrum Sylvania Gro-Lux lights. Day temperature was maintained at 23° C and night temperature at 18° C. Both watering and feeding was done through an automatic misting system, which operated for 2 min every day. the water supplied through this system contained 20-20-20 NPK fertilizer (Peters, W. R. Grace & Co., Edmonton). With a water supply of 2.1 l m^{-2} per day, every plant was fed 0.45 mg of N, P_2O_5 and K_2O daily, which amounts to approximately 300 kg/ha/year. Also, after 2 months, they were foliar fed with an iron chelate containing 9% iron (Plant Products, Bramalea, Canada).

At the time of transfer to Rootrainers, the average height of the plantlets was 2.8 cm and the seedlings, 1.7 cm. Most of the plantlets had mature scale-like leaves, and all had one or more side branches; the seedlings had no side branches or mature leaves. Several adventitious shoots usually appear in tissue culture plantlets, but for these experiments, only one was allowed to develop. After 3 months the average height and number of side branches were 4.5 cm and 4.8, and 3.4 cm and 1.9 for plantlets and seedlings, respectively.

For experimentation, the root systems of the seedlings and plantlets were washed free of the rooting substrate in tap water followed by a rinse in running distilled water. Single plants were cultured hydroponically for 48 h in glass tubes, each containing 50 ml of continuously aerated nutrient solution of the following composition: 2 mM KNO_3, 1 mM $Ca(NO_3)_2$, 1 mM $MgSO_4$, 1 mM NaH_2PO_4, 0.5 mM Na_2HPO_4, 0.2 mM Fe-ethylenediaminetetraacetate (EDTA), 0.38 μM $CuCl_2$, 0.7 μM Na_2MoO_4, 0.02 μM $ZnSO_4$, 3.7 μM H_3BO_3, 3.25 μM $MnSO_4$ (Jensen and Erlandson 1986). The hydroponic culture was carried out with the roots kept in the dark, while the shoots were exposed to the same light and day length regime as described above. After 24 h the volume of the nutrient solution was readjusted with distilled water. Root and shoot dry weight (DW)/fresh weight (FW) ratios were determined by using five plantlets out of each experiment after this 48 h period of hydroponic culture. Plants were first blotted dry; shoots and roots were then separated and their fresh weights recorded. The plant material was then dried to constant weight at approximately 85° C and the dry weights were recorded.

Clearing procedures. The hypocotyl regions of seedlings and tissue culture-derived plantlets were cleared using a 2% sodium hydroxide solution at 45° C for 2–3 days. The clearing procedure was terminated when the tissues appeared translucent. The tissues were then gently washed in several changes of distilled water, stained in a mixture of safranin, basic fuchsin, and crystal violet (0.5 g, 0.5 g, 0.2 g in 100 ml of 50% ethanol) for 24 h and dehydrated using an ethanol series and cleared in xylene.

Light microscopy. The hypocotyl regions of seedlings and tissue culture derived plantlets were fixed in 2% formaldehyde and 2% glutaraldehyde buffered with 0.05 M phosphate buffer. The tissues were then dehydrated using methyl cellosolve followed by absolute ethanol and embedded in LKB Historesin according to the method of Yeung and Law (1987). Two μm sections were stained using the periodic acid-Schiff's reaction and counter-stained with toluidine blue 0 (Yeung 1984).

Uptake and translocation studies. After 48 h of pre-culture under hydroponic conditions, as described above, each plant used for the ^{32}P uptake studies was given 50 ml fresh nutrient solution, to which 185 kBq ^{32}P-orthophosphate was added. The final specific radioactivity was 4.9 MBq/μmol. For ^{86}Rb uptake studies the nutrient solution given after 48 h of pre-culture (50 ml per tube and plant) contained 1 mM $RbNO_3$ instead of 2 mM KNO_3 (Jensen and Erlandson 1986), to which 185 kBq $^{86}RbCl$ was added giving a final specific radioactivity of 3.7 MBq/mmol.

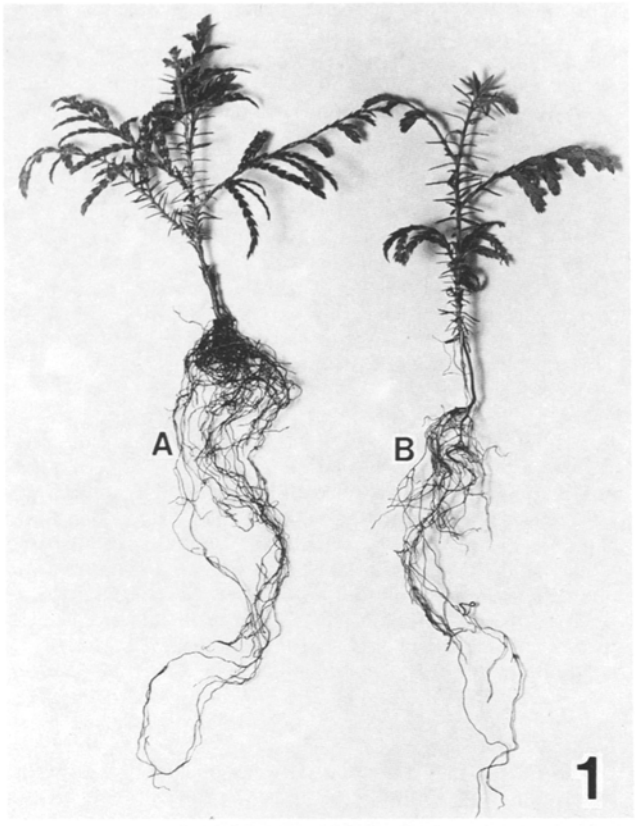
After 90 or 180 min, plants were harvested, the root systems blotted dry, and given a short rinse (1.0–1.5 s) in excess volume (3.5 l) of washing solution in order to remove any tracer adhering to the root surface and blotted dry again. For this rinsing step a solution containing 0.5 mM $CaSO_4$ plus 1 mM KH_2PO_4 was used in the ^{32}P studies (Cumming et al. 1986), while distilled H_2O was used in ^{86}Rb studies in order to avoid any ^{86}Rb exchange from the Donnan free space (DFS) by Ca^{2+} or K^+. The root systems of ^{32}P-labelled plantlets were then immersed in 100 ml ice cold 0.5 mM $CaSO_4$ plus 1 mM KH_2PO_4 solution for 10 min in order to eliminate tracer from the water free space (WFS) and blotted dry. The root system of the ^{86}Rb-labelled plantlets was desorbed in a two-step procedure. Firstly, the WFS was washed free of tracer for 10 min in 100 ml ice cold distilled H_2O and blotted dry; this was followed by the desorption of the DFS for 10 min in 100 ml of ice cold 10 mM $CaSO_4$ solution. After the desorption, roots and shoots were separated, weighed and wet ashed in a mixture of nitric and perchloric acids (2:1 v/v, 3 ml per 100 mg FW) at 120° C for 30 min. After cooling 1 ml of the resulting solution was pipetted into 10 ml distilled water in plastic liquid scintillation spectrometer (LSS) vials and the Cherenkov radiation counted in a Packard 411 "Minaxi" LSS. Counting efficiency was 33% for ^{32}P and 35% for ^{86}Rb, and 1 ml aliquots of the different desorption solutions were counted in organic scintillant (Scint A, Packard) against pre-established quench correction curves. Three single plants of either seed or tissue culture origin were used per experiment and uptake period, and all experiments repeated once.

Results

Table 1 shows percentage dry weight for roots and shoots of seedlings and tissue culture plantlets. There were no significant differences between the two plant types; however, shoot material contains almost twice as much dry matter as do the roots. Figure 1 shows a typical tissue culture

Table 1. Percentage of dry weight in roots and shoots of *Thuja occidentalis* seedlings and tissue culture plantlets

	% Dry weight	
	Root	Shoot
Seedlings	16.7 ± 1.3	31.3 ± 3.0
Plantlets	17.8 ± 1.7	29.7 ± 0.6


A
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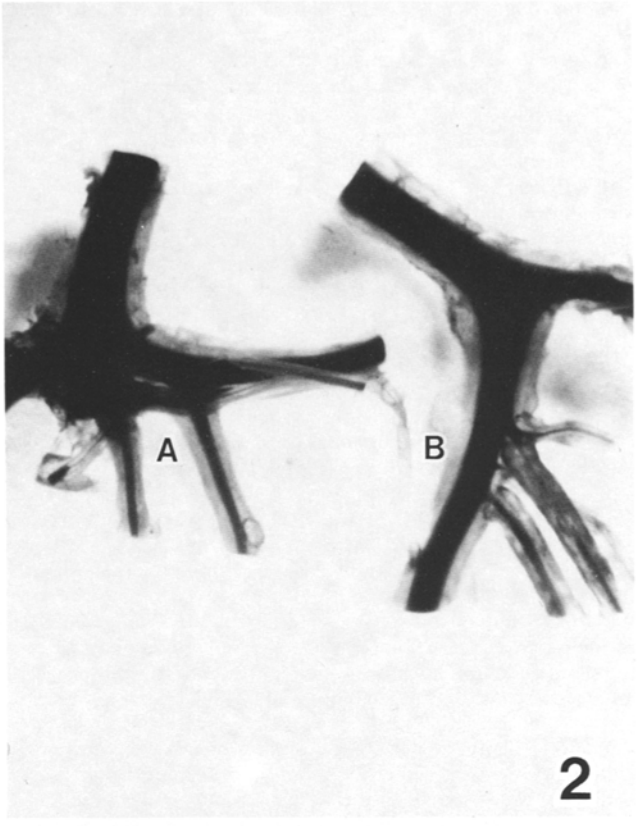
A
B
2


3


4

Table 2. Fresh weight (FW) and tracer uptake per gram root FW of *T. occidentalis* seedlings and tissue culture plantlets

	Phosphate				*Rubidium*				
	mg FW		nmol ^{32}P per plant	% ^{32}P in shoot	mg FW		nmol ^{86}Rb per plant	% ^{86}Rb in shoot	nmol ^{86}Rb in DFS (root)
	Root	Shoot			Root	Shoot			
90 min uptake									
Seedlings	221 ± 56	195 ± 48	290.2 ± 36	9.9 ± 2.3	290 ± 51	729 ± 98	483.5 ± 56	17.5 ± 2.3	186.0 ± 31
Plantlets	320 ± 63	384 ± 115	268.3 ± 12	9.8 ± 1.0	367 ± 66	580 ± 151	467.5 ± 66	13.0 ± 3.9	337.2 ± 51
180 min uptake									
Seedlings	838 ± 49	243 ± 42	405.4 ± 51	9.8 ± 1.0	335 ± 35	637 ± 95	701.2 ± 108	18.9 ± 3.0	201.8 ± 44
Plantlets	292 ± 19	222 ± 18	324.2 ± 17	10.6 ± 2.3	467 ± 64	672 ± 227	770.5 ± 144	17.1 ± 4.7	297.8 ± 41

Data for ^{32}P studies represent the mean ± standard error (SE) of one experiment with three replicates. The data of the other experiment showed the same shoot to root ratio but were of different absolute values. Data for ^{86}Rb studies represent the mean ± SE of two experiments with three replicates each. DFS = Donnan free space

plantlet (A) and seedling (B) used for histological, as well as nutrient uptake and translocation studies carried out in these investigations. The plantlets usually had a somewhat larger root system, as is depicted in Fig. 1 A (compare also fresh weight data in Table 2). The light micrographs in Fig. 2 show cleared preparations of tissue culture plantlets (A) and seedlings (B). The central darkly stained core represents the tracheary elements of the xylem. The cleared specimens demonstrate that the vascular tissue of the shoot is directly connected with the roots and with no apparent disruptions in the tissue culture plantlets. Histological examinations of that same region indicate that the central core is composed of tracheary elements in both plant types with no apparent break of vascular tissues at the root-shoot junction (Figs. 3, 4). More rays can be found in the plantlets than in the seedlings.

Table 2 shows the average fresh weight of the roots and the shoots of the plants used in these experiments. As can be seen, it was not always possible to match shoot and root weights of seedlings and plantlets. Nevertheless, the weights were comparable within a certain range and the largest deviation between the two plant types is seen in the 90 min ^{32}P uptake studies. However, even here, it was kept within an average of about 220 mg vs 320 mg for the roots and 200 mg vs 380 mg for the shoots of seedlings and plantlets, respectively. Uptake of ^{32}P per plant and its translocation to the shoot (percentage of total) over a period of 90 and 180 min are given in Table 2. Total ^{32}P uptake was the same for seedlings and plantlets during the 90 min experiments, while 20% less was taken up by the latter during the 180 min period. Nevertheless, the percentage of ^{32}P translocated to the shoot was the same for the two plant types.

Also, there were no great differences in the ^{86}Rb uptake and translocation to the shoot between the seedlings and the plantlets (Table 2), although the ^{86}Rb uptake was 70% and 100% higher after 90 and 180 min of uptake, respectively, than those for ^{32}P in both plant types. The higher uptake of ^{86}Rb in comparison with ^{32}P was also reflected in a higher translocation of ^{86}Rb to the shoot (2.6 and 3.6 times higher than ^{32}P translocation for the 90 and 180 min uptake periods, respectively).

Desorption of the DFS of the root system with 10 μM $CaCl_2$ revealed that 50%–80% more ^{86}Rb was bound to the DFS of the roots of the tissue culture plantlets than those of the seedlings

Fig. 1. Photograph showing the morphology of a tissue culture derived plantlet (**A**) and a seedling (**B**) of *Thuja occidentalis*. Note the abundance of roots in (**A**)

Fig. 2. Light micrograph showing cleared preparations of a tissue culture-derived plantlet (**A**) and a seedling (**B**) of *T. occidentalis*. The central darkly stained core represents the tracheary elements of the xylem. X 15

Fig. 3. Light micrograph showing a longitudinal section through the shoot-root junction of a tissue culture-derived plantlet of *T. occidentalis*. Vascular tissues of the adventitious root (*) are connected to the vascular tissues of the shoot with no apparent break at the junction. Rays (*arrows*) X 76

Fig. 4. Light micrograph showing a longitudinal section through the corresponding shoot-root region of a seedling of *T. occidentalis*. Rays (*arrows*) X 76

(Table 2). When the radioactivity data of the shoots were evaluated, it appeared that the shoot size itself had an influence on tracer translocation from root to shoot. Indeed, a positive correlation was found between the shoot fresh weight and the amount of tracer translocated to the shoot, when calculated as a function of the amount of tracer taken up per unit of root.

Discussion

In tissue culture plantlets the root system originates via adventitious roots and in seedlings it develops from the radicle. However, histological examinations of the root-shoot junctions of plantlets and seedlings of *T. occidentalis* did not reveal any major differences in the vascular connection. In both plant types they appeared to be well developed, smooth and not disrupted. However, a smooth connection of the xylem between adventitious roots and the base of the shoot is not always seen in plantlets obtained through tissue culture techniques, e.g. in plantlets of black spruce and white spruce (Patel et al. 1987).

Besides the structural examinations, the functional comparison between the two plant types (employing ^{32}P as a representative of anionic nutrient and ^{86}Rb as a cation and K^+ analogue) clearly showed that the nutrient uptake and translocation pattern for the two ions tested were about the same in the two plant types. However, a comparison of the ^{32}P and ^{86}Rb uptake data indicates that rubidium, at least at the concentrations employed, is taken up more rapidly than phosphorus. This was also observed by Barber (1979) in intact maize roots and might reflect the higher demand for potassium than for phosphorus (Mengel and Kirkby 1982). An interesting, but as yet unexplained, feature of the root system of tissue culture plantlets is that they seem to have a 50% larger DFS when compared with seedling root systems. A possible reason for this effect could be that the somewhat larger root systems of the tissue culture plantlets used in these studies have a higher proportion of parenchyma cells. However, more detailed studies will have to be carried out before this phenomenon can be satisfactorily explained.

Although the observed data for ^{32}P accumulation in *T. occidentalis* were about half of that reported for red spruce seedlings (Cumming et al. 1986), the ^{32}P translocation to the shoot was in the same order of magnitude for both species (~ 8 and $\sim 15\ nmol \cdot g^{-1}\ FW \cdot h^{-1}$ for red spruce and Eastern white cedar, respectively). Moreover, in the experiments reported here, the shoot apparently exerted a direct influence on nutrient translocation from root to shoot in both plant types. This finding is not unreasonable, and there is evidence in the literature that the shoot per se, as the sink for mineral nutrients, might participate in the regulation of nutrient translocation from root to shoot (Clarkson and Hanson 1980; Lüttge 1983; Pitman 1972; Graham and Bowling 1977). This effect is probably due to the higher overall transpiration rate of the larger shoots as opposed to the smaller ones (Lüttge 1983). However, other shoot-borne factors influencing nutrient translocation to the shoot, such as photosynthate availability to the root (Pitman 1972; Graham and Bowling 1977) or phytohormonal signals (Clarkson et al. 1978) cannot be ruled out.

In conclusion, it is apparent that the mineral nutrient uptake and translocation system in tissue culture plantlets of *T. occidentalis* were structurally and functionally similar to those of seedlings, although the origins of the root systems are different. Thus, in these aspects, the performance of the tissue culture-derived plantlets of *T. occidentalis* in the field should be normal.

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