Growth, water relations and solute accumulation in osmotically stressed seedlings of the tropical tree *Colophospermum mopane*

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**Summary**  Root and hypocotyl elongation, water status and solute accumulation were studied in osmotically stressed seedlings of the tropical tree, *Colophospermum mopane* (Kirk ex Benth.) Kirk ex J. Léonard, which grows in hot arid areas of southern and central Africa. Seeds were imbibed for 24 h and then subjected to a polyethylene-glycol-generated osmotic stress of −0.03 (control), −0.2, −0.8, −1.6 or −2.0 MPa for 60 h. Seedlings subjected to moderate water stress (−0.2 MPa) had higher root growth rates (2.41 ± 0.24 mm h−1), greater final root lengths (111 ± 3.8 mm) and longer cells immediately behind the root elongation zone than control seedlings (1.70 ± 0.15 mm h−1 and 93 ± 3.9 mm, respectively). Root lengths of seedlings in the −0.8 and −1.6 MPa treatments were similar to those of control seedlings, whereas the −2.0 MPa seedlings had significantly shorter roots. Both root and hypocotyl tissues exhibited considerable osmotic adjustment to the external water potential treatments. Seedlings in the −0.03, −0.2, and −0.8 MPa treatments had similar cell turgor pressures (0.69 ± 0.10, 0.68 ± 0.07 and 0.57 ± 0.04 MPa, respectively), whereas the −2.0 MPa treatment lowered cell turgor pressure to 0.17 ± 0.04 MPa. Root vacuolar osmotic pressures were generally similar to sap osmotic pressures, indicating that the increased root elongation obtained in moderately water-stressed seedlings was not caused by increased turgor pressure difference. Neutral-fraction solute concentrations, including the osmoticum pinitol, increased approximately two-fold in root sap in response to a low external water potential. In hypocotyl sap of seedlings in the −2.0 MPa treatment, pinitol more than doubled, sucrose increased from about 2 to 75 mol m−3 but glucose and fructose remained unchanged and, as a result, total sugars increased only slightly. The benefits of rapid early root elongation and osmoticum accumulation under conditions of water stress are discussed in relation to seedling establishment.

**Keywords:** osmotic stress, pinitol, root elongation, turgor pressure, solute accumulation, Zimbabwe.

**Introduction**

*Colophospermum mopane* (Kirk ex Benth.) Kirk ex J. Léonard is widespread in the low-altitude, high-temperature regions of southern Africa (Coates Palgrave 1983) and is able to establish and flourish in habitats characterized by arid or sodic soils (Bennett 1985). It is a preferred fuelwood species (Reh et al. 1989) and is also used as fodder (Coates Palgrave 1983). *Colophospermum mopane* is capable of germinating and establishing root growth at water potentials lower than those tolerated by species from similar regions, e.g., 80% germination at −0.51 MPa compared to less than 30% in *Acacia tortilis* (Forsk.) Hayne. (Choinski and Tuohy 1991). Under moderate water stress (−0.2 MPa) radicle elongation was greater after 3 days than that of control seeds grown at −0.03 MPa (Choinski and Tuohy 1991).

Generally, root extension growth is reduced at the onset of osmotic stress (Hsiao 1973, Pritchard et al. 1987, Pritchard et al. 1991, Spollen and Sharp 1991); however, in some studies it has been observed that root elongation growth is enhanced under conditions of moderate water stress (Sharp and Davies 1979, Meyer and Boyer 1981, N’guen and Lamant 1989, Triboulot et al. 1995). It is not clear how this drought-induced increase in root elongation is achieved. Although cell and organ expansion rate may be controlled by several factors, including water transport (Boyer 1985) and cell wall properties (Hsiao and Jing 1987, Passioura and Fry 1992, Pritchard et al. 1993), turgor pressure is the main driving force (Lockhart 1965). Thus, we compared effects of osmotic stress on root and hypocotyl growth of *C. mopane* seedlings with effects of osmotic stress on cell turgor to determine the role of turgor pressure in root elongation of seedlings under conditions of water stress.

Maintenance of turgor pressure under conditions of external water stress requires an accumulation of solutes within cells, and hence an increase in internal osmotic pressures. An increase in vacuolar osmotic pressure can be achieved by accumulating a wide variety of solutes, whereas osmotic
adjustment in the cytoplasm is generally achieved by an accumulation of organic compounds, often sugars and amino acids. Plants also accumulate osmotically active low molecular weight compounds such as sugar alcohols (including pinitol), proline and glycine betaine (Hellebust 1976, Gorham et al. 1981, Wyn Jones and Gorham 1983) in response to abiotic stress. There have been few studies on pinitol in roots and none on tropical tree roots, but the compound has been found to increase in *Pinus pinea* L. roots in response to drought (N’guyen and Lamant 1988) and in soybean roots in response to high temperature stress (Guo and Oosterhuis 1995). To obtain a more detailed understanding of the role of osmotic accumulation during seedling establishment under conditions of water stress, we measured the concentrations of pinitol and other neutral-fraction sugars in roots and hypocotyls of unstressed and osmotically stressed *C. mopane* seedlings and calculated their contribution to osmotic pressure.

**Materials and methods**

**Plant material and collection site**

*Colophospermum mopane* seeds were collected from the Sapi Game Reserve in the Zambezi Valley, Zimbabwe (altitude 550 m, mean annual rainfall 750 mm, and mean annual maximum and minimum temperatures of 34.1 and 19.6 °C, respectively), and dispatched to Wales.

**Germination and growth of seedlings**

Fruit walls were removed and the seeds surface-sterilized for 3 min in 0.35% sodium hypochlorite containing a drop of detergent and then rinsed five times with distilled water. Surface-sterilized seeds were imbibed for 24 h in vermiculite mixed with 0.1 mol m\(^{-3}\) CaCl\(_2\) solution to give a water potential of −0.03 MPa (i.e., 3.72 ml CaCl\(_2\) solution per g dry vermiculite) (Sharp et al. 1988) at 30 °C. The imbibed seeds were then subjected to various external water potential treatments for 60 h. Polyethylene glycol (PEG) was made up in 0.1 mol m\(^{-3}\) CaCl\(_2\) solution to give water potentials of −0.03, −0.21, −0.8 and −2.1 MPa at 30 °C and mixed with vermiculite in the same ratio as used for seed imbibition. The PEG-vermiculite system placed the seeds in contact with PEG at the designated water potential, while allowing good aeration. Water potentials of the PEG-vermiculite mixtures were measured with a thermocouple psychrometer (Model 85, J.R.D. Merrill, Inc., Logan, UT). For the pressure probe and cell length measurements, germinated seeds were placed in the PEG-vermiculite mix in tall beakers (sealed with clingfilm to minimize evaporation), covered with aluminium foil and incubated at 30 °C for 60 h. For the growth studies, germinated seeds were grown in PEG-vermiculite mix in angled Perspex (Plexiglas) chambers (Sharp et al. 1988) so that root elongation could be determined *in situ*. Root elongation data are from a minimum of three roots per treatment from each of three separate experiments.

**Cell length determination**

Roots were fixed in 3% glutaraldehyde in 50 mol m\(^{-3}\) cacodylate buffer for 24 h under vacuum, dehydrated in 25% steps of ethanol over 3 days, infiltrated with 50/50 (v/v) ethanol/His-toresin (Reichert-Jung Ltd) overnight, left in pure resin for 2 days then embedded in fresh resin containing hardener. Longitudinal sections were made of three roots from each treatment and stained with toluidine blue. Cell lengths were measured with the aid of a microscope using an eyepiece graticule; 20 cells from each side of the central vascular strand at a point where cells had ceased to elongate (approximately 9 mm behind the root tip) were measured from four sections of root per treatment.

**Bulk sap osmotic pressures**

Root tip (first 10 mm) and hypocotyl (total) samples of seedlings that had been osmotically stressed for 60 h were placed in Eppendorf tubes, frozen in liquid N\(_2\), and stored at −18 °C until required. Sap was collected as described by Gorham et al. (1984). A minimum of three samples of both root and hypocotyl tissue per treatment were prepared and osmotic pressures of 10-µl sub-samples were measured with a vapor pressure osmometer (Model 5100B, Wescor, Inc., Logan, UT).

**Pressure probe measurements**

Roots of intact seedlings were exposed to a solution of PEG with a water potential identical to that of the PEG-vermiculite mix in which the seedlings had been grown for 60 h, in a Plexiglas holder as described previously (Pritchard et al. 1987). Turgor pressures were measured with a cell pressure probe (Hüsken et al. 1978, Pritchard et al. 1987) along and across the growing zone on 1–5 cells per root and 3–4 roots per treatment. Vacular osmotic pressures were measured cryscopically on sap collected from vacuoles using the pressure probe (Malone et al. 1989). Three measurements per root comprising 2–3 epidermal and cortical cell vacuoles mixed were made.

**Bulk solute concentrations**

Seedlings were separated into root tip (first 10 mm) and hypocotyl and replicate samples of between 200 to 400 mg of tissue from each treatment from two separate experiments were placed in Eppendorf tubes. Each tube containing the tissue sample was weighed, frozen in liquid N\(_2\), returned to room temperature and sap expressed as described for the osmotic pressure measurements. Expressed sap was immediately re-frozen in liquid N\(_2\) to minimize invertase activity. Just before analysis, sap was thawed, a 40-µl aliquot was diluted with 120 µl of deionized water, centrifuged through 0.45 µm mesh micro centrifuge filters (Whatman, UK) and analyzed by HPLC on a Sarasep sodium-form carbohydrate column at 80 °C with 25 mol m\(^{-3}\) Na\(_2\)SO\(_4\) as eluent with a Marathon auto sampler and Shodex refractive index detector.
Results

Root length, growth rate and cell length

Roots of seedlings that had been moderately stressed (−0.2 MPa) for 60 h were significantly (P < 0.05) longer than those of control seedlings (−0.03 MPa) (111 ± 3.8 versus 93 ± 3.9 mm, respectively). Root growth rates of seedlings in the −0.2 MPa treatment were also greater than those of control seedlings (2.41 ± 0.24 versus 1.70 ± 0.15 mm h⁻¹) (Table 1). Root lengths of seedlings in the −0.8 and −1.6 MPa treatments were similar to those of control seedlings (Table 1). Root cell lengths in the zone of maximum elongation were greater in moderately stressed roots than in control roots (Figure 1A). There were no significant differences in root cell lengths between the other treatments.

In contrast to roots, hypocotyl extension was lower in seedlings in all of the water stress treatments than in control seedlings. Even the moderate water stress (0.2 MPa) treatment caused an approximately 30% reduction in hypocotyl extension (Table 1).

Osmotic and turgor pressures

Tissue sap osmotic pressures increased in both roots and hypocotyls in response to decreasing external water potential (Figure 1B), indicating that osmotic adjustment occurred in both tissues. A differential in osmotic pressure was maintained between the root and hypocotyl tissues, with the sap osmotic pressure of the root being lower than that of the hypocotyl in all treatments (1.26 ± 0.07 MPa in root tissue versus 1.43 ± 0.08 MPa in hypocotyl tissue).

Turgor pressures of cells in the root growing zone were similar in seedlings in the −0.03, −0.2 and −0.8 MPa treatments, ranging from 0.71 ± 0.13 MPa at −0.03 MPa to 0.57 ± 0.04 at −0.8; however, the 2.0 MPa treatment caused root turgor pressure to drop to 0.17 ± 0.08 (Figure 1D). None of the treatments had a significant effect on hypocotyl turgor pressure (Figure 1D).

Root vacuolar osmotic pressure increased with increasing stress (Figure 1C). Vacuolar osmotic pressures were similar to sap pressures except in the −2.0 MPa treatment where the vacuolar osmotic pressure was about 0.4 MPa higher than the sap pressure (2.5 ± 0.36 versus 2.1 ± 0.04 MPa). Osmotic pressures were essentially constant along the apical 8 mm of the root (data not shown), with values similar to those measured 4–6 mm from the tip (see Figure 1D).

Solute concentrations

Total sugar (sucrose, fructose and glucose) and pinitol concentrations (mol m⁻³) were consistently higher in hypocotyl sap than in root sap (Figures 2A and 2B), except in seedlings in the −2.0 MPa treatment where total sugar concentrations were similar in both organs (Figure 2A). In the root sap, concentrations of pinitol, total sugars, and the individual sugars, sucrose, fructose and glucose increased with increasing external stress. Both pinitol and total sugar concentrations were over three times higher in roots in the −2.0 than in the −0.03 MPa treatment (Figures 2A and 2B and Table 2).

The effects of the treatments on solute concentrations was less clear in the hypocotyl sap than in the root sap. Although the pinitol concentration of hypocotyl sap increased with increasing external stress, total sugar concentrations were similar in control and moderately stressed plants (about 230 mol m⁻³) and only slightly higher (about 300 mol m⁻³) in the −0.8 and −2.0 MPa treatments (Figure 2A). Hypocotyl glucose and fructose concentrations remained similar in all treatments, but sucrose concentrations were about 15 times higher in the −2.0 MPa treatment than in the other treatments (75 versus approximately 5 mol m⁻³) (Table 2). In contrast to the other solutes measured, sucrose concentrations were lower in hypocotyl sap than in root sap, except in the −2.0 MPa treatment (Table 2).

<table>
<thead>
<tr>
<th>External water potential (MPa)</th>
<th>Root length (mm)</th>
<th>Hypocotyl length (mm)</th>
<th>Root growth rate (mm h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−0.03</td>
<td>93 ± 3.9a</td>
<td>25 ± 2.4a</td>
<td>1.70 ± 0.15a</td>
</tr>
<tr>
<td>−0.2</td>
<td>111 ± 3.8b</td>
<td>17 ± 1.8b</td>
<td>2.41 ± 0.24b</td>
</tr>
<tr>
<td>−0.8</td>
<td>86 ± 6.4a</td>
<td>10 ± 0.8c</td>
<td>1.55 ± 0.12a</td>
</tr>
<tr>
<td>−1.6</td>
<td>91 ± 6.1a</td>
<td>8 ± 0.87c</td>
<td>ND</td>
</tr>
<tr>
<td>−2.0</td>
<td>57 ± 3.5c</td>
<td>8 ± 1.0c</td>
<td>0.38 ± 0.04c</td>
</tr>
</tbody>
</table>

Table 1. Effects of external water potential on elongation growth in hypocotyls and roots, and root growth rates in mopane seedlings. Lengths were measured to the nearest mm; values are given as mean ± SE. ND = not determined. Within each column, means without a common letter are significantly different at P ≤ 0.05.
Table 2. Concentrations (mol m$^{-3}$) of individual sugars in root and hypocotyl sap of mopane seedlings stressed for 60 h at various external water potentials.

<table>
<thead>
<tr>
<th>External water potential (MPa)</th>
<th>Sucrose (mol m$^{-3}$)</th>
<th>Glucose (mol m$^{-3}$)</th>
<th>Fructose (mol m$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root Hypocotyl</td>
<td>Root Hypocotyl</td>
<td>Root Hypocotyl</td>
</tr>
<tr>
<td>−0.03</td>
<td>13 ± 1.3 1.2 ± 0.33</td>
<td>21 ± 0.5 103 ± 8.1</td>
<td>43 ± 1.8 110 ± 9.9</td>
</tr>
<tr>
<td>−0.2</td>
<td>15 ± 0.2 4.9 ± 3.3</td>
<td>31 ± 0.5 102 ± 5.8</td>
<td>67 ± 2.6 129 ± 12</td>
</tr>
<tr>
<td>−0.8</td>
<td>29 ± 2.8 2.0 ± 0.2</td>
<td>65 ± 2.5 131 ± 10</td>
<td>106 ± 2.5 157 ± 14</td>
</tr>
<tr>
<td>−2.0</td>
<td>44 ± 3.4 75 ± 6.3</td>
<td>140 ± 12 96 ± 13</td>
<td>176 ± 8.4 138 ± 18</td>
</tr>
</tbody>
</table>

Figure 2. Effects of external water potentials on total sugar (A) and pinitol (B) concentrations (mol m$^{-3}$) in roots and hypocotyls of mopane seedlings. Roots = shaded bars; hypocotyls = open bars. Data are means ± SE.

The combined contribution of sugars and pinitol to osmotic pressure, calculated according to the van’t Hoff equation, was about 30% of the total root osmotic pressure at an external stress of −0.03 MPa and 41% at −2.0 MPa. The corresponding values for the hypocotyl were 70% and 45%, respectively. Although the concentration of pinitol increased with increasing stress, the overall contribution of pinitol to the pinitol + sugars component of root osmotic pressure did not increase significantly, being 30% in control roots and 33% in roots grown at −2.0 MPa. In the hypocotyl, the osmotic contribution of pinitol to the pinitol + sugars component of osmotic pressure doubled from 22% in control plants to 44% at −2.0 MPa.

Discussion

As in other plant species, primary root elongation of mopane seedlings was less sensitive to low external water potentials than hypocotyl extension (cf. Westgate and Boyer 1985, Sharp et al. 1988, Spollen et al. 1993). Water stress generally leads to a reduction in root growth (Pritchard et al. 1991, Spollen et al. 1993); however, in mopane, there was a significant stimulation of root elongation under conditions of moderate water stress (−0.2 MPa) (Table 1). Increased root mass, lateral branching and depth of rooting in response to long-term water deficits have also been observed in other plant species (Sharp and Davies 1979, Osonubi and Davies 1981, Reader et al. 1992) but during early radicle development in mopane (Choinski and Tuohy 1991) and Pinus pinaster (Triboulot et al. 1995). Enhanced radicle development in response to mild water stress is not typical of all tropical African trees. In two species of African Acacia, including A. tortilis a species common to more arid regions, PEG treatment inhibited radicle development (Choinski and Tuohy 1991). Although roots were longer than controls in the moderately stressed (−0.2 MPa) mopane seedlings 60 h after stress was imposed, seedlings in the −0.8 MPa treatment had the longest roots 14 days later (data not shown). Similarly, maize roots grown in vermiculite at −2.0 MPa were initially shorter than roots of control seedlings but after 44 days there was no difference in length (Sharp et al. 1988). There may also be differences in both the timing and the location of root growth responses to water stress. For example, water stress may cause an increase in elongation growth in younger cells, while causing a decrease in older cells, leading to either an overall increase or an overall decrease in root elongation.

In the root growing zone, turgor pressure and expansion decrease immediately after drought stress is imposed (Hsiao and Jing 1987, Frencsch and Hsiao 1994). Thereafter, depending on the severity of the stress, partial or total turgor recovery occurs. Following 24 h of exposure to 300 mol m$^{-3}$ mannitol, root turgor of wheat plants recovered to unstressed values in the region of accelerating root growth, but growth rates were reduced (Pritchard et al. 1991). In maize roots subjected to long-term exposure to −1.6 MPa water stress, growth rates were maintained in the region of accelerating growth, but turgor pressures were about 0.3 MPa lower than in unstressed controls (Spollen and Sharp 1991). Hence, in wheat, cell wall tightening must account for the reduction in growth, whereas in maize the cell walls must loosen in response to stress to maintain growth at reduced turgor. In mopane roots, growth rates increased under conditions of moderate stress but turgor pressures were not significantly different to those of unstressed controls. This suggests that increased root growth in response to moderate water stress results from increased cell wall loosening. Increased root growth rates at a turgor pressure similar to that of unstressed controls also occurs in moderately water stressed (−0.15 MPa) Pinus pinaster (Triboulot et al. 1995), but not in maize (Sharp et al. 1988).

At an external stress of −0.8 MPa, neither root growth rates nor turgor pressures were significantly different from those of unstressed controls. In other species, such severe water stress can lead to sharp reductions in root elongation rates, e.g.,
approximately 40% in soybean, 60% in squash (Spollen et al. 1993) and 50% in Pinus pinaster (Triboulot et al. 1995). In our study, the most severe external water potential treatment, −2.0 MPa, caused an approximately 60% reduction in root turgor pressure and an approximately 75% reduction in root growth rate. We do not have growth rates or turgor pressures for roots grown at −1.6 MPa, but overall root length at −1.6 MPa was similar to that at −0.8 MPa. Hence, we conclude that there was a threshold stress value between −1.6 and −2.0 MPa at which the change from turgor and growth maintenance to reduced turgor and growth occurred.

Although root growth was not reduced until the external water stress was greater than −1.6 MPa, hypocotyl elongation was reduced by about 33% even under conditions of moderate stress (−0.2 MPa); however, hypocotyl turgor pressures were the same in all treatments. Hsiao and Jing (1987) suggested that cell wall properties of shoots have the opposite response to those of roots. There is evidence that in moderately water-stressed plants, despite the maintenance of turgor pressures at control values, cell wall tightening in shoots is responsible for reduced growth (Tomas and Pritchard 1994).

The changes in osmotic pressure required to maintain turgor for extension growth are achieved by solute accumulation in the cells of the growing region (see Figures 1B and 1C). The compounds accumulated vary among species, but tend to be of low molecular weight. For example, there is an accumulation of proline in droughted wheat roots (Ober and Sharp 1994) and of pinitol in legumes, including chickpea (Laurie and Stewart 1990), pigeon pea (Keller and Ludlow 1993) and soybean (Guo and Oosterhuis 1995). Concentrations of pinitol vary from 10 to 30 mol m$^{-3}$ in stressed Mesembryanthemum crystallinum L. and Sesbania aculeata (L.) Merr. to 100 mol m$^{-3}$ in leaves of unstressed Robinia pseudacacia L. (Thonke 1990, cited in Popp and Smirnoff 1995). In mopane trees growing in an arid area of Zimbabwe, leaf and twig pinitol concentrations can be as high as 370 and 230 mol m$^{-3}$, respectively (Popp, unpublished data); however, pinitol concentrations in the seedlings we studied were lower, ranging from 50 (controls) to 125 mol m$^{-3}$ (at −2.0 MPa) in hypocotyls and 25 to 85 mol m$^{-3}$ in roots (Figure 2C). On a dry weight basis we found that pinitol occurred in root in mopane seedlings growing at external water potentials of −0.03, −0.2 and −0.8 MPa, but the gradient was not observed in seedlings in the −2.0 MPa treatment.

Other soluble carbohydrates, particularly sucrose, accumulate in response to water deficit, possibly because of a reduced shoot sink demand as a result of reduced growth (Popp and Smirnoff 1995). In mopane, sucrose concentrations were low in roots of seedlings in the −0.03, −0.2 and −0.8 MPa treatments but were higher in roots of seedlings growing at −2.0 MPa where growth was reduced.

We conclude that there is a relationship between osmotic adjustment and pinitol accumulation in roots of mopane seedlings subjected to drought. However, we were unable to determine whether osmotic adjustment and pinitol accumulation are related to root elongation or to increased drought resistance.

Acknowledgments

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References


