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Responses of jack pine (*Pinus banksiana*) seedlings to root zone pH and calcium

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ABSTRACT

Plants growing in calcareous soils are exposed to high pH and elevated calcium levels which may reduce growth and produce high mortality in sensitive plants. In this study, we examined the hypotheses that in the high pH-sensitive jack pine (*Pinus banksiana*) seedlings, elevated root zone pH would reduce the ability of seedlings to control Ca uptake and the increase in Ca tissue concentrations would further aggravate the detrimental effects of high pH. We conducted two separate experiments to (i) investigate growth and physiological responses of jack pine (*P. banksiana*) seedlings to root zone pH ranging from 6 to 9, and (ii) examine the effects of 0.25 mM, 1 mM, 5 mM, and 10 mM Ca on physiological responses and nutrient uptake in jack pine seedlings exposed to the root zone pH of 6.5, 7.5, and 8.5 in nutrient solution culture. High root zone pH and high Ca concentrations significantly reduced growth, net photosynthesis, transpiration rates, needle chlorophyll concentrations, and root cortex cell lengths in seedlings. High root zone pH also decreased needle Ca and B concentrations, but the examined concentrations of Ca in nutrient solution had little effect on the needle composition of the examined essential elements including Fe, P, K, Cu, B, Mn, and Zn. We concluded that poor growth of conifer trees reported for calcareous soils is likely due to impaired root growth and the effects on gas exchange, likely caused by the reduced water uptake at high pH and elevated Ca levels.

1. Introduction

It is estimated that about 25–30% of the world land surface is calcareous (Wallace and Lunt, 1960). Calcareous soils are characterized by ample Ca supply and high soil pH, usually in the range of 7.5–8.5 (Marschner, 2012). Besides natural calcareous soils, human activities can also produce alkaline soils with high Ca concentrations (Renault et al., 2000). In the reclaimed areas following open-pit oil sands mining in northeastern Alberta, Canada, the pH of reclaimed soil is commonly higher than 8.0 (Howat, 2000). Since gypsum is also added to accelerate tailings consolidation process (Ramos-Padrón et al., 2010), the soil Ca content in some reclamation sites can exceed 400 mg kg^{-1} compared with less than 6 mg kg^{-1} typically found in the surrounding mixedwood forests (Visser, 2005).

High soil pH affects many different processes in plants in a complex manner. A common problem associated with high soil pH is reduced availability of certain essential elements including Fe, Mn, P, Zn, B, and Cu (Berger, 1962; Yang et al., 1994; Valentine et al., 2006; Marschner, 2012; Zhang et al., 2013). High soil pH can also reduce root water flux (Tang et al., 1993a; Kamaluddin and Zwiazek, 2004; Siemens and Zwiazek, 2011). The reductions in nutrient and water uptake by high pH may lead to stomatal closure (Tang et al., 1993a; Kamaluddin and Zwiazek, 2004), decreased shoot water potential (Tang et al., 1993a), and reduced leaf concentrations of photosynthetic pigments (Zhang et al., 2013) leading to growth reductions (Bertoni et al., 1992; Tang et al., 1992).

The plant root intracellular pH is affected by pH of the root growth medium (Felle and Hanstein, 2002). According to the acid growth theory, the root cell elongation is induced by acidification of apoplastic pH (Rayle and Cleland, 1992). The elongation of root cortex cells in *Lupinus angustifolius* L. was reported to be inhibited at pH 7.5 (Tang et al., 1993c). Calcium accumulation in the cell wall is thought to enhance cell wall rigidity by cross-linking pectin chains (Hepler, 2005). Therefore, Ca and protons appear to have opposing roles in the processes leading to cell elongation and the consequence of high Ca concentrations may be the inhibition of cell elongation (Tang et al., 1993b; Hepler, 2005).

In addition to affecting cell wall properties, Ca plays a major role in maintaining membrane integrity and selectivity (Grattan and Grieve, 1998). Treatments of plants with Ca can significantly

Abbreviations: ANOVA, analysis of variance; chl, chlorophyll; Pn, net photosynthesis rate; E, transpiration rate; [Ca^{2+}], calcium ion concentration.

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ameliorate the negative effects of abiotic stresses including salinity and aluminum toxicity (Kinraide and Parker, 1987; Cabanero et al., 2004), by reducing plasma membrane permeability and preventing influx of toxic ions into the cytosol (Cramer, 2002). Ca^{2+} is a versatile signaling ion linking environmental and developmental stimuli and physiological responses of plants (Hepler, 2005; Bickerton and Pittman, 2012). The cytosolic $[Ca^{2+}]$ is approximately 0.1 μM while in the apoplast and storage compartments, such as vacuole and endoplasmic reticulum, $[Ca^{2+}]$ ranges from 0.1 to 10 mM (Hepler, 2005). Indeed, cytosolic $[Ca^{2+}]$ elevation is a ubiquitous feature of the signaling network when plants are exposed to almost all biotic and abiotic stresses (Bose et al., 2011). Calcium also affects water uptake as Ca^{2+} is involved in the opening and closing of aquaporin (Cabanero et al., 2006) and regulates guard cell turgor and stomatal aperture (Webb et al., 1996). However, despite the importance of Ca in plant functioning, the effects of Ca on the responses of plants to high pH have not been thoroughly studied.

Jack pine (*Pinus banksiana* Lamb.) is a native tree species of Canadian boreal forest, and is an early successional species found in sandy and nutrient-poor sites (Cayford et al., 1967). It is among the conifer tree species showing stunted growth in calcareous soils in southeastern British Columbia and western Alberta, Canada (Kishchuk, 2000) and one of the main tree species considered for the revegetation of the oil sands areas affected by high pH and Ca (Renault et al., 2000; Apostol and Zwiazek, 2004; Calvo Polanco et al., 2008). In the present study, we carried out two controlled-environment experiments to examine the growth and physiological responses of jack pine seedlings to neutral and high root zone pH in the presence of different Ca concentrations. We hypothesized that Ca would aggravate the effects of high pH through inhibited root growth and the resulting impairment of root function.

2. Materials and methods

2.1. Plant material and growth conditions

One-year-old container-grown (415D styroblocks™, Beaver Plastics, Acheson, AB, Canada) jack pine (*P. banksiana* Lamb.) dormant seedlings were obtained from the Boreal Horticultural Services Ltd., Bonnyville, AB, Canada. Seedling roots were gently washed to remove the potting medium and placed in 25% modified Hoagland's solution (Epstein, 1972) for two weeks in a controlled-environment growth chamber before applying pH and Ca treatments. Environmental conditions in the growth chamber were 22/18 °C (day/night) temperature, 65 ± 10% relative humidity, and 16-h photoperiod with 300 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) at the top of the seedlings provided by the full spectrum fluorescent bulbs (Philips high output, F96T8/TL835/HO, Markham, ON, Canada).

We investigated the effects of root zone pH and the effects of Ca on seedling responses to pH in two experiments. In Experiment 1, we examined the responses of jack pine seedlings to six root zone pH levels of 6, 7, 7.5, 8, 8.5, and 9. In Experiment 2, we selected three pH levels: 6.5, 7.5, and 8.5, and four Ca levels: 0.25, 1, 5, and 10 mM Ca, to study their combined effects on jack pine seedlings. These Ca treatment concentrations corresponded to 0.25, 1, 5, and 10*x* Ca concentration present in 25% modified Hoagland's solution. The quantity of $Ca(NO_3)_2{\cdot}4H_2O$ added in the nutrient solution was calculated from the formula of modified Hoagland's solution (Epstein, 1972). In both experiments, the plants were treated for 8 weeks. The hydroponic set-up for each treatment consisted of a 120 L opaque pail connected to three (Experiment 1) or two (Experiment 2) 30 L LDPE tubs through the PVC tubing. In each pail, a pump (Model 9.5 950GPH, Danner MFG Inc., New York, NY, USA) circulated 120 L of 25% Hoagland's solution between the pail and the tubs (Zhang et al., 2013). Each tub was covered with a styrofoam lid containing 3.8 cm holes through which seedlings were placed in nutrient solution and secured with foam plugs. Six (Experiment 1) or nine (Experiment 2) jack pine seedlings were placed in each tub. A pH controller (PHCN-70, Omega Engineering Inc., Laval, QC, Canada) connected with a pH electrode (Orion 9106 BNWP, Thermo Scientific, Rochester, NY) was used to continuously control the solution pH. An electronic valve (Model 8260G071 120/60 ASCO Valve, Inc., Florham Park, NJ, USA) was controlled by the pH controller and connected to a 5% (w/w) KOH solution container. A plastic ball valve (Model R-01377-84, Cole-Parmer Canada Inc., Montreal, QC, Canada) was connected to the electronic valve to release 5% (w/v) KOH solution slowly to the nutrient solution to maintain the desired pH levels (Zhang et al., 2013). During the experiments, the variation of solution pH was maintained within ±0.1 range. The nutrient solution was replaced every two weeks. The concentrations of dissolved O_2 were measured with the oxygen electrode (YSI 5000, YSI Inc., Yellow Springs, OH, USA) and were no less than 6 mg L^{-1} throughout the experiment.

2.2. Elemental analysis of nutrient solution

To quantify the solubility of selected essential elements at different pH and Ca levels in Experiment 2, 1 L of 25% modified Hoagland's solution without Ca was prepared in triplicates ($n = 3$), then $Ca(NO_3)_2{\cdot}4H_2O$ was added to adjust Ca concentrations to 0.25, 1, 5, and 10 mM. The solution pH was then adjusted to 6.5, 7.5, and 8.5 with 5% KOH (w/w) or 1% H_2SO_4 (v/v). For each treatment, three 20 mL nutrient solution samples were filtered with 0.45 μm PVDF syringe-driven filter unit (EMD Millipore Corporation, Billerica, MA, USA). The concentrations of B, P, K, Ca, Fe, Mn, Cu, and Zn in filtered solution were measured with the inductively coupled plasma mass spectrometry (ICP-MS) (Zarcinas et al., 1987) in the Radiogenic Isotope Facility of the University of Alberta.

2.3. Dry weights and needle chlorophyll concentrations

Shoot and root dry weights were determined for all seedlings from each treatment ($n = 18$). Roots and stems were dried in an oven at 70 °C for 72 h. The needles were divided into old and young needle classes. Needles that were fully elongated before the onset of treatments were regarded as old leaves, while the needles which started elongating after the onset of treatments were regarded as young needles. The needles were separated from stems and immediately placed in an ultra-low temperature freezer at −80 °C, and freeze-dried for 72 h before storage. To determine shoot dry weights, the dry weights of all needles and stems from each plant were added.

Chlorophyll-a (chl-a) and chlorophyll-b (chl-b) concentrations of old and young needles were determined in six randomly selected seedlings per treatment ($n = 6$). The freeze-dried leaves were ground with a Thomas Wiley Mini-Mill (Thomas Scientific, NJ, USA). Chlorophyll was extracted from pulverized leaf samples (10 mg dry weight) with 8 mL dimethyl sulfoxide (DMSO) at 65 °C for 22 h. Chlorophyll concentrations were measured in DMSO extracts with a spectrophotometer (Ultrospec, Pharmacia LKB, Uppsala, Sweden), at 648 nm for chlorophyll-a and 665 nm for chlorophyll-b. Total chlorophyll concentration was calculated using the Arnon's equation (Sestak et al., 1971).

2.4. Net photosynthesis (NP) and transpiration (E)

After 8 weeks of treatments, six jack pine seedlings ($n = 6$) were randomly taken from each treatment for the measurements of NP and E. For the measurements, about 5-cm distal parts of needles