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Genetic variability in salt tolerance of selected boreal woody seedlings

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Abstract

In order to select woody plant candidates suitable for revegetation of saline–alkaline soils, we tested selected woody plant species and seed lots: *Pinus contorta* (lodgepole pine), *Pinus banksiana* (jack pine), *Picea glauca* (white spruce), *Populus tremuloides* (trembling aspen), and *Alnus rubra*, syn. *Alnus oregona* (red alder). Pre-germinated seedlings were grown for 4 weeks in a greenhouse in a semi-hydroponic system containing 1/2 strength Hoagland solution with additional sodium concentrations (0, 25, 50, 75 mM) and composite tailings release water. A significant interaction between salt treatments and seed lots within plant species as well as between salt treatments and plant species was observed for weight and necrosis, indicating that the plant genotype responded differently to salt treatments. Of all examined woody plant species, jack pine (Syncrude seed source) exhibited the highest percent survival followed by white spruce (Syncrude seed source), red alder (seed lot No. 40457), and lodgepole pine (seed lot No. 7960007). Proportionately ranked means for dry biomass production showed lodgepole pine (seed lot No. 7960007) with the greatest biomass followed by aspen (seed lot No. Syncrude), red alder (seed lot No. Port Renfrew), jack pine (seed lot No. 8960049) and white spruce (Syncrude seed source). The best performing seed lots based on aggregation index which combines both the percent survival and dry weight averages were: red alder (seed lot Nos. 40457 and 45958), aspen (Syncrude seed source), jack pine (Syncrude seed source), lodgepole pine (seed lot No. 7960007). Based on variance components, most of the variation was explained by the treatment and seed lot effects. This reveals the importance of intra-specific variability and that selection should be based not only on inter-specific variation but also on the intra-specific variation for the development of salt-tolerant lines to be used in reclamation of saline habitats.

Keywords: Inter- and intra-specific variation; G × E interaction; Oil sands; Reclamation; Salt tolerance

1. Introduction

Salinity and water logging are a serious threat to food and fiber production worldwide (Khan and Ungar, 1995). The rapidly growing demand for food, fiber, and fuel in the face of rapidly declining productivity of agricultural land due to increased soil salinization makes it imperative that plant production under saline conditions be significantly increased. Salt affected soils make up 954.8 million hectares, or about 10% of the earth's surface (Szabolics, 1994). In the Canadian prairie, soils contain predominantly sulfate salts as well as some chloride salts (Curtin et al.,

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1993). The primary problems associated with salinity and sodicity are reductions in plant productivity due to water stress caused by the increased osmotic potential and changes in soil physical properties (dispersion with a resultant reduction in permeability) when salt or sodium concentrations in soils become too high. It is believed that salt tolerant plants may be of potential use in these degraded agricultural and forest areas to produce food, fiber and other commodities. The influence of physicochemical and biotic factors is important to the distribution and establishment of halophytes (Ungar, 1998). In natural forest ecosystems, soils rarely contain an excess of salt because of natural drainage and flushing by precipitation. As a result, salt tolerance has not been extensively studied in woody plant species compared to herbaceous species (Renault et al., 1998).

Anthropogenically created conditions, such as the bitumen extraction methods (hot water digestion and flotation) currently in use in the Athabasca oil sands deposit in northeastern Alberta (Canada), by two operating oil sands plants (Syncrude Canada Ltd. and Suncor Energy, 1998), alone are producing large volumes of fluid tailings containing elevated concentrations of sodium, sulfate, bicarbonate and chloride ions. Syncrude Canada Ltd. (1998) alone produced about 75 million m^3 of tailings in 1998 and the total disturbed areas requiring reclamation at closure is estimated at 22,000 ha of which tailings surface areas for dryland reclamation are 7500 ha (Li and Fung, 1998). Natural consolidation of the fluid tailings would require hundreds of years and are inappropriate for terrestrial reclamation. Therefore, emphasis is currently on composite tailings (CTs) reclamation in the oil sands industry in Alberta (Mikula et al., 1996a,b; TERRE, 1998). The composite tailings process consists of adding gypsum to fine tailings to produce non-segregating deposit (CT) and release water (CT water). These CT materials are characterized by high levels of salt concentrations (excess sodium sulfate, chloride, Ca, Mg) and high alkalinity, which are major causes of plant toxicity. Demands of today's society, as expressed in recent reclamation laws of many provinces in Canada, are not just to stabilize soil and vegetation on disturbed areas but to reconvert them into productive agricultural and forested lands comparable to pre-disturbed terrain providing an integrated range of end uses and values associated with forests.

Previous studies have examined the tolerance of selected woody and herbaceous boreal plants to salts associated with these composite tailings, fine tailings and the waters they contain (Renault et al., 1998, 1999, 2000). Knowing the salt tolerance levels of various potential reclamation plant species is an important factor to ensure the survival and growth of plants onto saline habitats. In these studies, however, little attention was given to the intra-specific variability of the plants tested. The purpose of our study was to examine the inter- and intra-specific variability of selected boreal woody species to various salt concentrations using a semi-hydroponic system (Hambling et al., 2001) and to determine their potential suitability for reclamation of saline habitats.

2. Materials and methods

2.1. Plant material

Seeds of lodgepole pine (*Pinus contorta* Dougl. ex Loud. Var. *latifolia* Engelm.), jack pine (*Pinus banksiana* Lamb.), white spruce [*Picea glauca* (Moench) Voss], trembling aspen (*Populus tremuloides* Michx.), and red alder (*Alnus rubra* Bong.), were obtained from various sources (Table 1). The seeds were surface sterilized in 2% (v/v) sodium hypochloride and rinsed six times with autoclaved water. They were then germinated in Metromix® (Grace Horticultural Products, W.R. Grace of Canada, Ajax, Ont.). When seedlings were 4–6 cm in height (about 2 weeks), they were transplanted into sand filled germination trays, in which each cell contained 50 cm^3 of sand autoclaved twice for 45 min each time and completely saturated with water. The seedlings were left for 5 days without irrigation of treatment solutions to minimize transplantation shock.

2.2. Experiment set-up in greenhouse

A semi-hydroponic system (Hambling et al., 2001) was used. The sub-irrigation system is activated by a timer set to supply power to water pumps. When activated, the pumps flood large holding trays with treatment solution to approximately half the height of the germination trays three times a week for 15 min at a time. The solution is re-circulated allowing for

Table 1
Description of seed sources (origin, seed lot number or name, supplier and geographic locations)

Species	Origin	Seed lot	Supplier[a]	Latitude (°N)	Longitude (°W)	Elevation (m)
A. rubra	Englishman River, BC	45958	TSC	49°13′	124°26′	400
	Mount Prevost, BC	40473	id.	48°50′	123°47′	530
	Jordan River, BC	40457	id.	48°41′	124°02′	5
	Tasu Inlet, BC	33252	id.	53°40′	132°15′	37
	Skidgate, BC	30017	id.	53°21′	131°57′	50
	Hanna Valley, BC	39844	id.	53°15′	132°15′	100
	Exstew River, BC	43086	id.	54°25′	129°09′	80
	Buckley Bay, BC	46208	id.	49°33′	124°54′	100
	Kleindale, BC	46242	id.	49°27′	123°57′	50
	Bachelor Bay, BC	Bachelor Bay	id.	55°22′	126°55′	30
	Channel, BC	Channel	id.	53°08′	132°15′	20
	Galiano Is., BC	Galiano Is.	id.	48°57′	123°28′	50
	Kingcome Inlet, BC	Kingcome Inlet	id.	51°30′	126°08′	30
	Port Renfrew, BC	Port Renfrew	id.	48°36′	124°14′	20
P. banksiana	Nampa, AB	8060006	NTSC	56°07′	116°55′	610
	Calling Lake, AB	8960049	id.	55°46′	113°22′	600
	Fort Kent, AB	9060060	id.	54°38′	110°51′	640
	Fort MacKay, AB	9360060	id.	57°20′	111°23′	325
	Fort McMurray, AB	Syncrude	EAS	57°02′	111°35′	315
P. contorta	Grande Prairie Forest, AB	7665180	NTSC	55°10′	118°48′	650
	Woking, AB	7960007	id.	55°40′	119°28′	800
	DS 66-10-5-84, AB	8461731	id.	54°38′	115°22′	930
P. glauca	Fort McMurray, AB	Syncrude	EAS	57°02′	111°35′	315
P. tremuloides	Fort Nelson, BC	40960	TSC	58°36′	122°20′	480
	Fort Nelson North, BC	42307	id.	59°00′	123°12′	520
	Fort Nelson South, BC	42308	id.	58°48′	122°12′	480
	Fort McMurray, AB	Syncrude	EAS	57°02′	111°35′	315

[a] TSC: Tree Seed Centre, Surrey, BC Ministry of Forests; NTSC: National Tree Seed Centre, Canadian Forest Service, Fredrickton, NB; EAS: Environmental Affairs Department, Syncrude Canada Ltd., Ft. McMurray, Alta.

mixing of the treatment solution and adequate aeration. We applied 12 l of five different treatment solutions including a control of half strength Hoagland's solution (Hoagland and Arnon, 1938 in Hewitt, 1966), half strength Hoagland's solution prepared with CT release water, and three salinity treatments consisting of 25, 50 and 75 mM sodium chloride in half strength Hoagland's solution to five boreal woody species in a nested and two-factor factorial design (Montgomery, 1991). Seed lots are nested within species (Table 1). The CT release water was obtained from the small-scale (u-shaped cell) composite tailings trial at the Syncrude Canada Ltd. site near Ft. McMurray, Alta. The CT water chemistry consists of: pH, 8.26; electrical conductivity, 6170 μS/cm; Ca, 55.2 mg/l; Mg, 33.2 mg/l; Na, 1580 mg/l; K, 34.1 mg/l; Fe < 0.04 mg/l; Mn < 0.003 mg/l; SO_4, 2020 mg/l; Cl, 691 mg/l; hardness, 275 mg/l; T Dis. Solids, 3950 mg/l; NO_2 and NO_3-N < 0.05 mg/l. The experiment was set-up in a greenhouse with a daytime temperature of 21 °C and an 18 °C nighttime, natural light was supplemented with sodium halide lights with intensity of 450 $\mu mol\ m^{-2}\ s^{-1}$ and a 15 h photoperiod. The relative humidity was approximately 35%. The position of each treatment set-up was changed once a week to allow all units to be exposed to homogenous environmental conditions.

2.3. Measurements and data analysis

The experimental design was a nested design with crossed factors according the following mathematical model $Y_{ijkl} = \mu + \mathrm{Sp}_i + \mathrm{Tr}_j + \mathrm{Pr(Sp)}_{k(i)} + (\mathrm{Sp\,Tr})_{ij} + \mathrm{Tr\,Pr(Sp)}_{jk(i)} + \varepsilon_{(ijk)l}$, where Y_{ijkl} is a score for the given experimental unit in treatment combination $\mathrm{Tr\,Pr(Sp)}_{jk(i)}$, μ the overall population mean, Sp_j the effect of the ith species, Tr_j the effect of the jth solution, $\mathrm{Pr(Sp)}_{k(i)}$ the effect of the kth seed lot within the jth species, $(\mathrm{Sp\,Tr})_{ij}$ the joint effect of species and solution treatment, $\mathrm{Tr\,Pr(Sp)}_{jk(i)}$ the interaction between treatment and seed lots within species, and $\varepsilon_{(ijk)l}$ the experimental error. Species and treatments were fixed factors, and seed lots were random factors. After 1 month of growth, survival, dry matter (biomass), and necrosis symptoms were recorded. The following scale was used for necrosis symptoms $(0 = \text{none}, 1 = \text{light}, 2 = \text{moderate}, 3 = \text{severe}, 4 = \text{dead})$. Normality and homoscedasticity of data were tested using the SAS Univariate procedure, and the Brown–Forsythe test by SAS for Windows (SAS release 8.0, SAS, 1999). Normality and homoscedasticity were not achieved even after transformations. Therefore, the analysis of variance was conducted using both the RANK and GLM procedures and variance components were calculated with the VARCOMP procedure model = type1 (SAS, 1999). Spearman correlations among growth traits and percent survival were computed from seed lot means over all solution treatments using the SAS CORR procedure. Proportionately ranked means for dry biomass production were obtained using the Bonferroni's correction for multiple pairwise comparisons with a maximum experimentwise error rate of 5%. Stepwise multiple regression analysis was used to relate biomass production of seed lots with their geographic origins (latitude, longitude, and elevation). Latitude, longitude, and elevation of seed lot origin, and their squares and cross products, were used as independent variables. The probability used for a variable to enter the equation was 0.10.

For practical ranking of seed lots for each species according to both their survival rate and dry biomass, we constructed a convex aggregation index (Khasa et al., 1995a). The formula constructed for this aggregation index was as follows:

$$\mathrm{AI} = \delta \frac{v_{c1} - (v_{c1\,max} + v_{c1\,min})/2}{(v_{c1\,max} - v_{c1\,min})/2} + (1-\delta)\frac{v_{c2} - (v_{c2\,max} + v_{c2\,min})/2}{(v_{c2\,max} - v_{c2\,min})/2}$$

where the v_{c1} criterion represents the average value of survival rate and the v_{c2} criterion represents the average dry biomass, and δ the weighting coefficient.

3. Results

The rank analysis of variance showed a significant interaction between salt treatments and seed lots within species as well as between salt treatments and species for the two measured traits (weight and necrosis, Table 2). The plant species, seed lots within species and salt treatments main effects were also significant (Table 2). The significant interaction plant genotype × salt treatment indicates a change in relative performance rank among genotypes from one salt treatment to the other. Based on variance components, the treatment and seed lot effects

Table 2
Anova on ranks and variance component for dependent variables weight and necrosis[a]

Source of variation	d.f.	Necrosis		Weight	
		F-value (Pr > F)	Variance component (%)	F-value (Pr > F)	Variance component (%)
Treatment	4	1135.78 (<0.0001)	57	157.54 (<0.0001)	18
Species	4	191.54 (<0.0001)	8	157.21 (<0.0001)	15
Treatment × species	16	15.89 (<0.0001)	3	5.00 (<0.0001)	1
Seed lot (species)	23	38.30 (<0.0001)	10	39.69 (<0.0001)	25
Treatment × seed lot (species)	88	5.42 (<0.0001)	6	3.11 (<0.0001)	7
Error	1331		15		34
Total	1466		100		100

[a] For "variance component" we used the Type 1 estimates method.

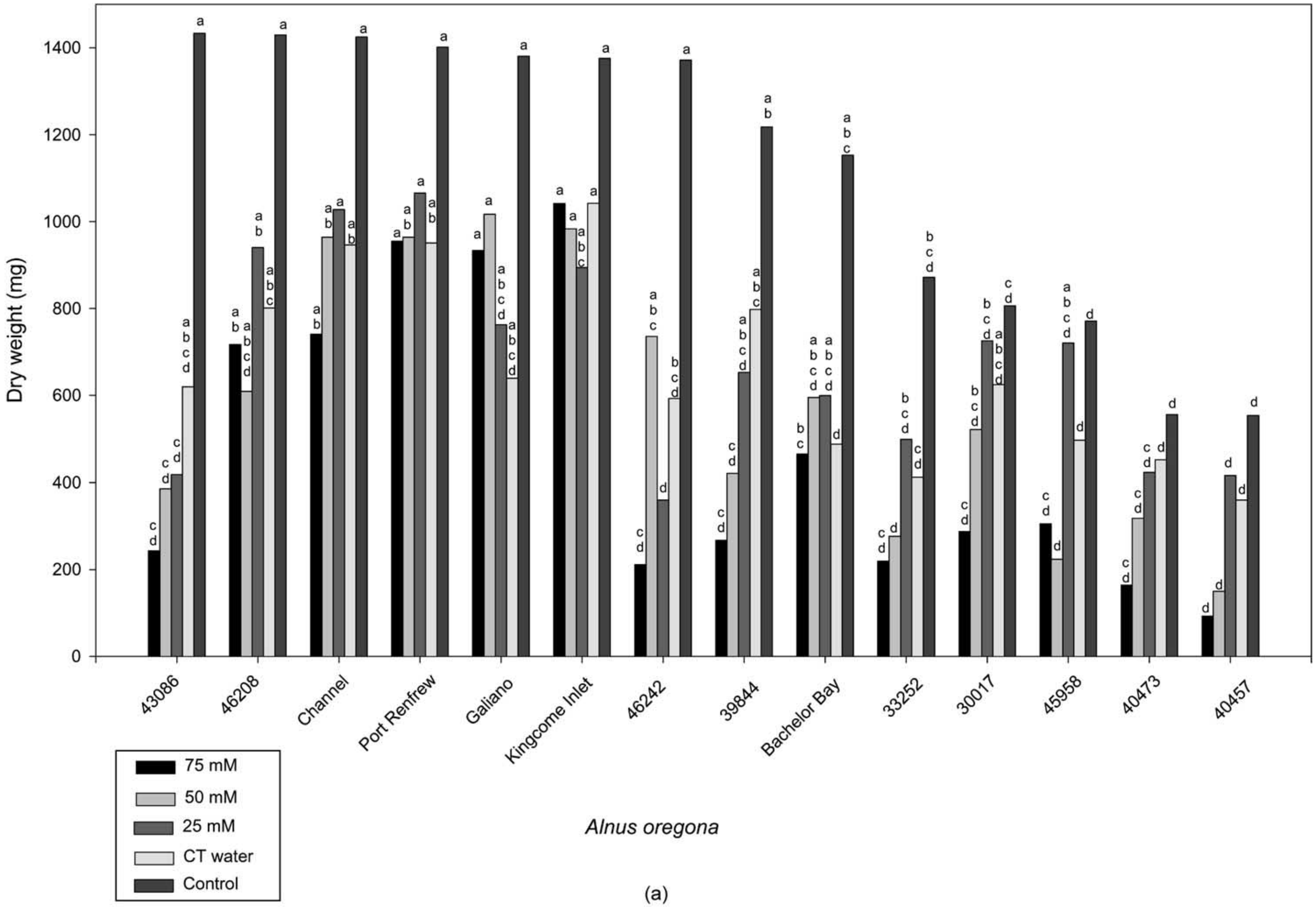

Fig. 1. Proportionally ranked means for dry matter (g) of different seed lots of *A. rubra* syn. *A. oregona* (a), *P. tremuloides* (b), *P. banksiana* (c), and *P. contorta* (d) at five salt treatments. Means followed by the same letter within each treatment are not significantly different at 5% significance level, using Bonferroni (Dunn) *t*-tests.

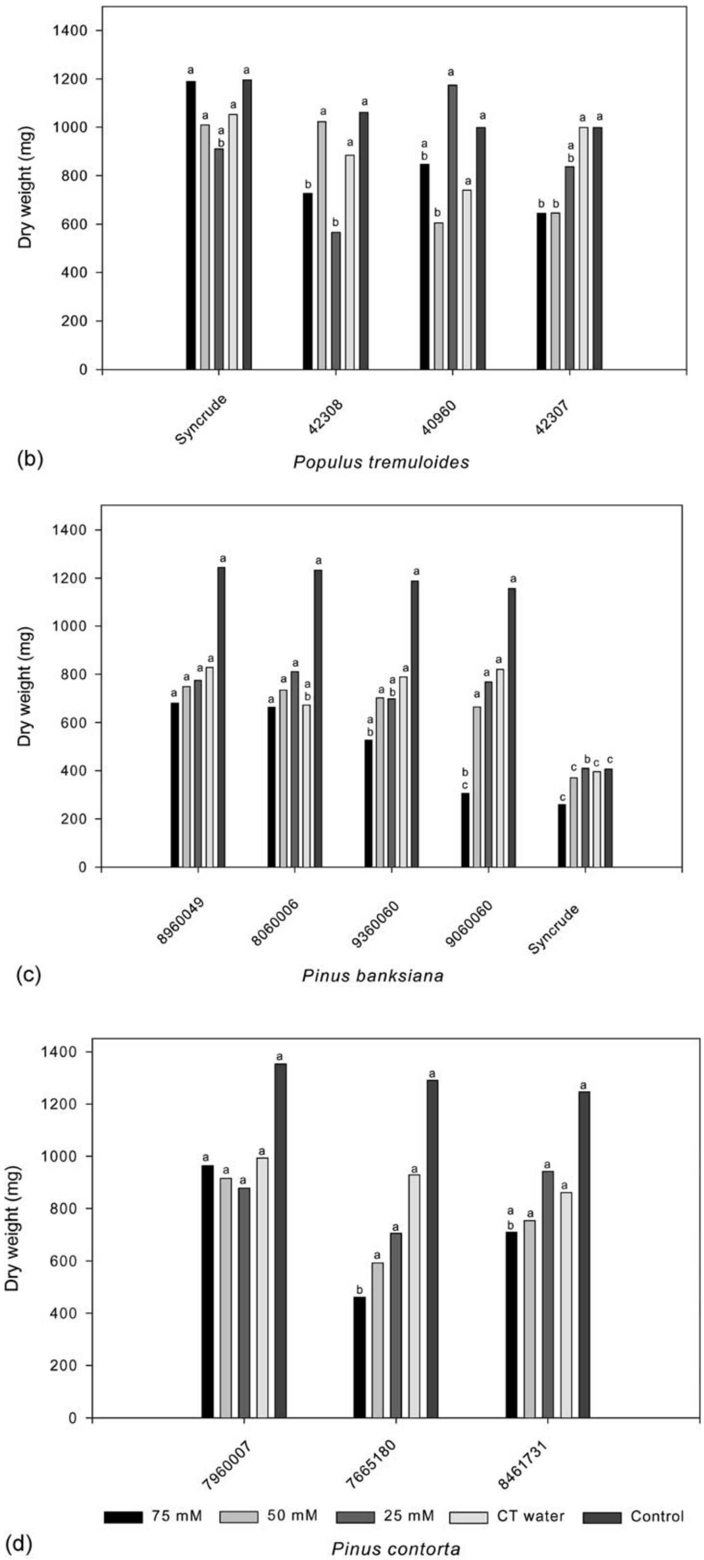

Fig. 1. (*Continued*).

Table 3
Percent survival of the different seed lots and species under different salt treatments

Species	Seed lot	Treatment[a]					Average survival rate over each seed lot
		0	25	50	75	CT	
A. rubra	Bachelor	83	0	0	0	0	17
	46242	75	0	0	0	0	15
	Kingcome	100	0	0	0	0	20
	Galiano	100	0	0	0	0	20
	39844	92	8	0	0	0	20
	43086	100	0	0	0	0	20
	46208	100	0	0	0	0	20
	Channel	100	0	0	0	0	20
	Port Renfrew	100	0	0	0	0	20
	30017	100	42	0	0	17	32
	45958	100	58	0	0	58	43
	40473	100	33	0	0	83	43
	40457	100	58	0	0	75	47
	33252	100	50	0	0	50	40
Average		96.4	17.8	0	0	20.2	26.9
S.E.		2.1	6.5	0	0	8.4	2.9
P. tremuloides	40960	83	0	0	0	0	17
	42307	83	0	0	0	0	17
	42308	100	0	0	0	0	20
	Syncrude	100	0	0	0	0	20
Average		91.5	0	0	0	0	18.5
S.E.		4.9	0	0	0	0	0.9
P. banksiana	9360060	100	8	0	0	0	20
	8060006	100	0	0	0	33	27
	Syncrude	100	92	100	67	100	92
	8960049	100	17	0	0	50	33
	9060060	100	0	0	0	27	25
Average		100	23.4	20	13.4	42	39.4
S.E.		0	17.4	20	13.4	16.6	13.3
P. contorta	7665180	100	14	0	0	73	37
	7960007	100	67	0	0	75	48
	8461731	100	50	0	0	42	38
Average		100	43.7	0	0	63.3	41.0
S.E.		0	15.6	0	0	10.7	3.5
P. glauca	Syncrude	100	92	92	50	17	70

[a] See Section 2 for definition of treatments.

accounted for a substantial amount of the variation for the two measured traits. Spearman rank correlations were significant among necrosis, weight and percent survival for red alder; between necrosis and weight for aspen and white spruce; between necrosis and weight, and survival and weight for lodgepole pine (results not shown). Our results and discussion will focus more on weight and survival traits.

For red alder, the seed lots Kingcome Inlet, Galiano, Port Renfrew, Channel and 46208 produced the greatest amount of dry weight even at the highest salt concentration (75 mM NaCl) but were all dead at

Table 4
Multiple regression of dry weight with geographic variables of seed lot origins (summary of stepwise selection by species[a])

Species	Variables in model	Model R-square	F-value ($\Pr > F$)	Parameter estimate	Type 11 SS
A. rubra	Intercept			1548.73516	6651335
	Elevation × elevation	0.7350	57.28 (<0.0001)	−0.00164	11462585
	Latitude × longitude	0.8700	10.63 (0.0012)	−0.12081	1775989
P. tremuloides	Intercept			8234.50870	2902044
	Latitude	0.0660	16.82 (<0.0001)	−125.59115	2299416
P. banksiana	Intercept			−41072.00000	1618413
	Latitude × elevation	0.1283	41.01 (<0.0001)	−1.82075	1140651
	Latitude × latitude	0.1547	8.78 (0.0033)	15.31770	2802439
	Elevation	0.2447	33.34 (<0.0001)	108.91621	1267921
	Longitude	0.2809	14.06 (<0.0001)	−89.43321	1230039
P. contorta	Intercept			−6476.5862	565866
	Latitude × latitude	0.0215	3.44 (0.0654)	4.10406	592016
	Longitude × longitude	0.0408	10.63 (0.0784)	−0.36581	367579

[a] All variables left in models are significant at the 10% level using backward elimination procedure.

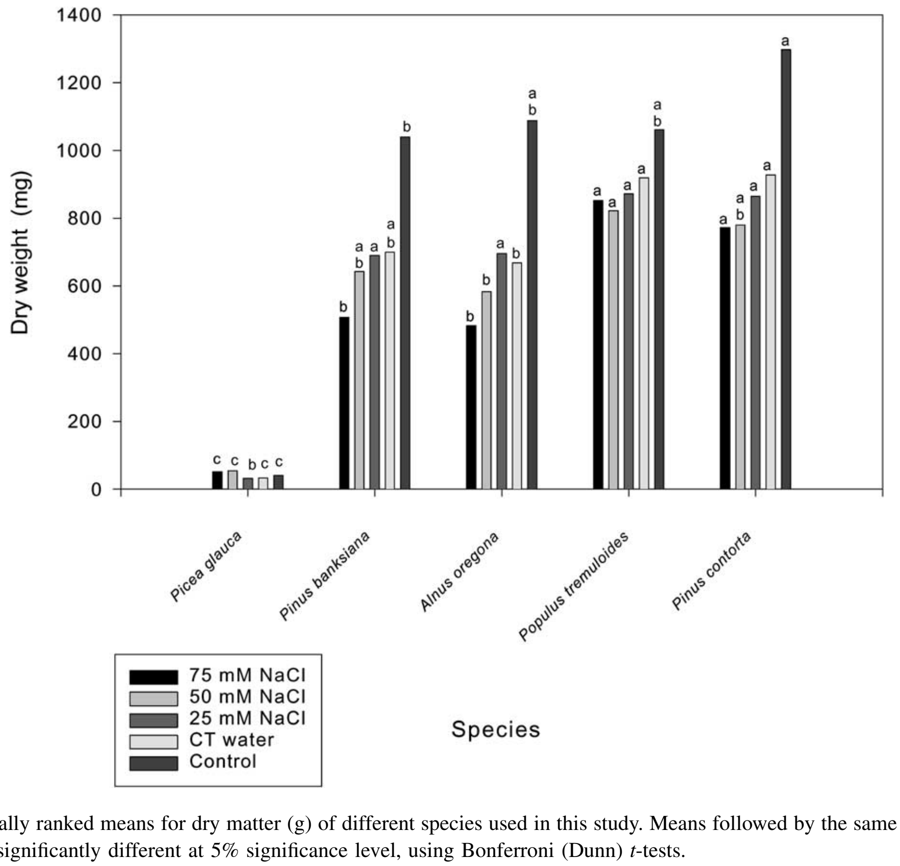

Fig. 2. Proportionally ranked means for dry matter (g) of different species used in this study. Means followed by the same letter within each treatment are not significantly different at 5% significance level, using Bonferroni (Dunn) t-tests.

Table 5
Ranking of performance of species seed lots based on aggregation index (AI)

Species/seed lot	Rankings based on AI weighting coefficients (0.1, 0.2, 0.3, 0.4, 0.5)				
	$\delta = 0.1$	$\delta = 0.2$	$\delta = 0.3$	$\delta = 0.4$	$\delta = 0.5$
A. rubra					
Bachelor	13	13	13	13	13
46242	14	14	14	14	14
Kingcome	6	6	6	5	1
Galiano	9	9	9	9	6
39844	11	11	11	11	11
43086	12	12	12	12	12
46208	10	10	10	10	9
Channel	8	8	8	7	4
Port renfrew	7	7	7	6	2
30017	5	5	5	8	10
45958	2	2	2	1	3
40473	3	3	3	3	8
40457	1	1	1	2	5
33252	4	4	4	4	7
P. tremuloides					
40960	3	3	3	3	3
42307	4	4	4	4	4
42308	2	2	2	2	2
Syncrude	1	1	1	1	1
P. banksiana					
9360060	5	5	5	5	4
8060006	3	3	3	3	2
8960049	2	2	2	2	1
9060060	4	4	4	4	5
Syncrude	1	1	1	1	3
P. contorta					
7665180	3	3	3	3	3
7960007	1	1	1	1	1
8461731	2	2	2	2	2
P. glauca					
Syncrude	ND[a]	ND	ND	ND	ND

[a] Not determined.

time of harvest (Fig. 1a, Table 3). These seed lots are "early fast-growing" and produced more dry biomass than any others before death. The seed lots 40457, 40473, 45958 and 33252 had at least 40% average survival over all treatments (Table 3). For aspen, the Syncrude seed lot produced the greatest amount of dry weight at the highest salt concentration but all four seed lots were dead at the harvest time even at the lowest concentrations (25 mM NaCl) (Fig. 1b, Table 3). For jack pine, the seed lots 8960049, 8060006 and 9360060 produced the greatest amount of dry weight at all salt treatments and the Syncrude seed lot was the poorest (Fig. 1c). In contrast, the Syncrude seed lot had the greatest survival rate (92%) over all treatments, followed by the seed lots 8960049, 8060006, 9060060 and 9360060 (Table 3). For lodgepole pine, the seed lots 7960007 and 8461731 produced the greatest amount of dry weight at the highest salt treatment while the seed lots 7960007 and 7665180 had the highest percent survival (Fig. 1d, Table 3). The one white spruce seed lot from Syncrude tested in this study produced the least dry weight among the five tested species but was second best in survival after jack pine (Fig. 2, Table 3). Based on our study, lodgepole pine showed the greatest biomass followed by aspen, red alder, jack pine and white spruce (Fig. 2). Jack pine exhibited the highest percent survival followed by white spruce, red alder, and lodgepole pine (Table 3). Stepwise multiple regression analysis used to relate biomass production of seed lots with their geographic origins showed moderate amount of geographic variation with a coefficient of determination of 8.7, 6.6, 28.1 and 4% for red alder, aspen, jack pine, and lodgepole pine, respectively (Table 4).

By using the aggregation index (Khasa et al., 1995a), which takes into account two criteria (survival and dry weight), red alder (seed lot Nos. 40457 and 45958), aspen (Syncrude seed source), jack pine (Syncrude seed source), lodgepole pine (seed lot No. 7960007), seemed best amenable for revegetation of saline–alkaline sites such as the CT materials (see Table 5 for the following ranks). Varying relative weights given to the two criteria (Table 5) affected, the seed lot rankings little.

4. Discussion

Identifying suitable plant seed lots and families within-seed lots for CT reclamation is one of the important environmental issues being addressed by the oil sands industry in Alberta. Therefore, a rapid screening method is needed for assessing several plant species and genotypes for tolerance to toxic compounds such as those present in CT materials (Hambling et al., 2001). The CT materials are characterized by high levels of salt concentrations and alkalinity, and naphthenic acids, which are major causes of plant toxicity (Renault et al., 1999). This study has identified potential seed lots, which may be used for CT revegetation.

4.1. Dry matter production and survival

We have identified seed lots of woody plants that may produce more dry matter and have a good survival rate under saline conditions. Both of these measures of tolerance may be useful when selecting seed lots and families within-seed lots to grow at a given level of soil salinity. High salinity increased necrosis and decreased dry biomass of seedlings. These results are in agreement with other studies (Khan and Ungar, 1995; Renault et al., 1998, 2000; Rogers et al., 1996, 1997). Indeed, an excess of sodium salts in plant tissues can lead to enzyme inactivation, inhibition of nucleic acid and protein synthesis, membrane lesions that result in leakage of solutes from the cells and cause injury to tissues (Banuls et al., 1996; Renault et al., 1998, 2000). A study of different salts of Na^+ and K^+ on the growth of *Atriplex prostrata* (Chenopodiaceae) showed that all plant growth parameters decreased with a lowering of the medium osmotic potential, and that K^+ salts were more inhibitory than Na^+ salts (Egan and Ungar, 1998). However, at low osmotic potential (−1.5 MPa) the rate of germination was more inhibited by Na^+ salts than by K^+ salts (Egan et al., 1997). In some plants such as sorghum, an accumulation of Na^+ leads to a loss of potassium from leaves (Khan et al., 1995). Sodium toxicity impairs the germination and growth of plants and makes them more susceptible to pest and environmental stresses. Sodium also has an antagonsitic effect on Ca^{2+} and Mg^{2+} uptake. The decrease in Mg^{2+} could play a role in the observed leaf chlorosis, as Mg^{2+} is one of the key elements that compose the chlorophyll molecule essential for plant photosynthesis.

Salt tolerance in plants involves changes in morphology and in physiological processes (Greenway and Munns, 1980; Khan and Ungar, 1995). Several mechanisms have been proposed to explain salt resistance in woody plants including plant exclusion of Na^+ or Cl^- (Allen et al., 1994) and ion compartmentalization in vacuoles to avoid toxic effects in the cytosol (Jacoby, 1994). Ungar (1991) divided halophyte plants into four groups based on their mechanism of ionic adjustment: (i) plants that restrict ion uptake, many of which belong to the Poaceae, (ii) sodium and chloride accumulators, most of which are in the Chenopodiaceae, (iii) sulfate accumulators, represented by the Brassicaceae, and (iv) plants that accumulate a high concentration of inorganic ions, but are relatively low in organic substances (e.g., members of the Plantaginaceae).

It should be pointed out that stress resistance can vary among plant species and seed lots of plant species. For instance, in woody plants, oak has been reported to be more tolerant to salt stress than birch, Dutch elm and Norway spruce (Dragsted and Kubin, 1990). Salt tolerance in *Eucalyptus camaldulensis* Dehn (Karschon and Zohar, 1975) and *Acer rubrum* stress (Dochinser and Townsend, 1979) significantly differed depending on the seed source. Based on our study, lodgepole pine showed the greatest biomass, followed by aspen, red alder, jack pine and white spruce. Both lodgepole pine and jack pine exhibited a good survival rate at around 40% followed by red alder and aspen.

The CT water treatment with its phytotoxic compounds (Renault et al., 1998, 1999) is one of the best representations of the challenging problems facing the terrestrial reclamation in the oil sands industry. Based on the CT treatment, the following seed lots had at least 50% survival rate and may be used in the revegetation of CT: jack pine (seed lots Syncrude and 8960049), red alder (seed lot Nos. 40473, 40457, 45958, 33252), and lodgepole pine (seed lot Nos. 7960007 and 7665180). Overall treatments, the aggregation index indicated that red alder (seed lot Nos. 40457 and 45958), aspen (Syncrude seed source), jack pine (Syncrude seed source), lodgepole pine (seed lot No. 7960007) were the best performers. It is difficult to draw an informed decision on white spruce since we tested only one seed lot. The genetic variability of these species, suggests that emphasis be placed on intra-population sampling for both white spruce (King et al., 1984; Cheliak et al., 1985; Innes and Ringius, 1990) and aspen (Cheliak and Dancik, 1982; Hyun et al., 1987). Thus, it is worth testing more seed lots and families within-seed lots of these species to find the best suitable ones for CT revegetation. As well, it would have been better to test more seed lots in the previous studies involving several plant species native to the boreal forest (Renault et al., 1998, 1999), before making decisions on species performance to high salinity. Indeed, some species might be rejected outright because of susceptibility of one seed lot to high salinity. As an example, jack pine showed the lowest survival rate in the CT treatment (Renault et al., 1999). This is contrary to our findings where jack pine exhibited the highest percent survival because more seed lots were screened and some of them did exceptionally well in CT treatment (e.g., Syncrude seed lot, 100% survival in CT treatment). Speckled alder [(*Alnus incana* ssp. *Rugosa* (Du Roi)], indigenous to Alberta, collected at the Syncrude site survived better to the salt treatments than the Pacific Northwest red alder but its germination rate was very low (data not shown). There is a need to understand the physiology and biochemistry of the speckled alder seeds in relation to germination. If the germination problems are overcome, seed lots/ families of this actinorhizal species could be inoculated with specific *Frankia* strains for CT revegetation.

The interaction genotype × environment (salt treatments in our case) was significant, meaning changes in relative performance rank among seedlings of species or seed lots within species from salt treatment to salt treatment. In other words, dry matter production of all species or seed lots within species did not decrease as salinity increased and there were significant differences in salt tolerance among seedlings of species or seed lots within species. Because genotype × environment interactions exist, it is necessary to identify the sources of interactions before making decisions about species or seed lots within species selection and testing methods (Kremer, 1986; Khasa et al., 1995b,c). The variance components showed that most of the variation was explained by the treatment and seed lot effects. Renault et al. (1998) also observed a large individual variability in stress (CT water treatment) response of conifer seedlings for needle tip necrosis and water potentials. This suggests that there is a high degree of individual resistance within the species and that genetic differences should be considered in the selection of salt tolerant trees. Our results and others (Rogers et al., 1996, 1997) suggest that, in order to fully evaluate potential species for saline conditions, it may be worthwhile exploiting the intra-specific variation for dry matter production and survival that exists within species and selecting for increased productivity and survival. Therefore, the use of aggregation indices such as those presented in this study or more refined selection indices appears essential for the selection of seed lots and families, but also for clonal selection within-seed lots or families (Khasa et al., 1995a).

4.2. Geographic patterns

For red alder, differentiation among seed lots demonstrated geographic patterns with those from low elevations and south (early fast-growing seed lots) exhibiting the greatest amount of dry matter ($R^2 = 6.6\%, p < 0.0001$, see Tables 1 and 4, Fig. 1). This negative elevational and latitudinal trends observed in the present study is in agreement with earlier studies by Xie et al. (1994) who found that seed lots from south, inland and low elevations tend to grow faster than those from north, coast and high elevations. Coastal seed lots may be more tolerant to salt because of sodium brought about by wet marine deposition (Khasa et al., 1995c). The study on genetic variability and performance of red alder in British Columbia showed that the genetic variability was substantial both among and within-seed lots for traits such as height, diameter, leaf abscission, and bud flushing (Xie et al., 1994). Dang et al. (1994) also found highly significant differences among seed lots in ecophysiological traits such as photosynthetic rate, mesophyll conductance, transpiration rate, stomatal conductance, stomatal sensitivity to water vapor pressure deficit, intercellular to ambient CO_2 concentration ratio, and middy xylem water potential, but no significant between-seed lot differences in water use efficiency and no significant differences between families within-seed lot in any of the ecophysiological variables. For aspen, the latitude was important, with a negative trend ($R^2 = 6.6\%, p < 0.0001$). In jack pine, the three geographic variables (longitude, latitude and elevation, $R^2 = 28.09\%, p < 0.0001$) were important. A study on height growth and survival of within- and between-seed lot crosses in jack pine has also shown these parameters were low at the most northern-most site as the latitude increased (Magnussen and Yeatman, 1988). In lodgepole pine, no significant trend was observed ($R^2 = 4.08\%$, $p = 0.0389$). However, a rich structure of allozymic variation associated with geography was revealed; both latitude and longitude being important, with northern populations exhibiting a greater extent of genetic differentiation (Yeh et al., 1985). In white spruce, no trend could be derived since only one seed lot was used. However, Li et al. (1997) showed that patterns of white spruce seed lot variation followed mainly a south–north cline and to a lesser extent a west–east cline for height.

5. Conclusion

The considerable variability of long-lived woody species (Hamrick et al., 1992) suggests that attractive gains might be obtained through breeding programs aimed at developing salt tolerant tree genotypes. A tree improvement program could be time-consuming (Zobel and Talbert, 1991), but an early selection strategy with molecular markers, associated with tolerance to high levels of salts might be adopted to accelerate the rate of genetic improvement (Cheliak and Rogers, 1990; Tuskan, 1992; Monforte et al., 1996; Kubisiak et al., 2000) and the development of salt-tolerant lines.

Selection of the most promising ectomycorrhizal fungi for use in the saline–alkaline habitats has already been accomplished (Kernaghan et al., 2001). Therefore, the best seed lots selected in this study or families to be selected later can be inoculated with specific salt-tolerant ectomycorrhizal fungi. This work forms the basis for further studies to evaluate the inoculation biotechnology of tree seedlings or cuttings with these salt tolerant ectomycorrhizal fungi under field conditions over several seasons in order to fully assess the performance under saline soil conditions. A two-step procedure involving the selection of the plant genotypes and of their microsymbionts is likely to optimize the plant establishment and productivity on high salt-containing tailings generated from oil sands operations.

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