

Leaf Number Indicates Salt Tolerance of Young Seedling Families of European Aspen (*Populus tremula* L.) Growing in Different Soils

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Soil salinity limits plant productivity and quality. We evaluated the response of 12 aspen (*Populus tremula*) families to salt stress in two different soils irrigated for 4-weeks with 0, 80 or 160 mM saline solution. Easily measurable characteristics such as shoot height, leaf number, dry mass as well as the distribution of sodium (Na^+) ions were measured in 5-month-old aspen seedlings raised in controlled greenhouse conditions on two different soils. Growth among families varied significantly, and the interaction between family and soil type was significant. From 2–5 months, leaf number correlated with that of the first month and salinity tolerance. Sodium ions varied significantly within plants and among families; seedlings that accumulated higher Na^+ concentrations in root had more leaves and lower Na^+ in shoot. These results suggest that leaf number indicates salt tolerance in young seedlings. Seedling performance was also affected by soil type, especially the root/shoot ratio, suggesting an interaction between salt tolerance and growth medium. This study has identified significant intra-specific variation in salt tolerance of aspen in 160 mM saline and highlighted the potential to select and develop a method for efficient pre-screening of trees to be used in the reclamation of salt-affected land.

Keywords leaf number, *Populus tremula*, root/shoot ratio, salt tolerance

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1 Introduction

Soil salinity limits plant growth and productivity of arable land and is a major concern in boreal ecosystems (Howat 2000). The United Nations' Food and Agriculture Organization estimates that more than 800 million hectares throughout the world are salt affected (Munns and Tester 2008). Reclamation of saline soils requires adequate provision of low-electrolyte irrigation water as well as sufficient drainage to transport the leachate. In the absence of adequate drainage, all efforts to keep these lands under cultivation have been met with frustration. Trees can, however, process large amounts of water and present a natural and low-cost solution to salt removal from affected soil. Efforts are now underway to utilize trees for this purpose as well as create a renewable resource (Lambert and Turner 2000), but the success of such programs will depend on the selection of appropriate species and genotypes.

Salt tolerance is a complex trait governing the physiological response to osmotic and ionic stress (Munns and Tester 2008). While no macrophytes are true halophytes, there is considerable inter- and intra-specific variation in salinity tolerance (Flowers et al. 1986, Greenway and Munns 1980). Genetic diversity within a species thus provides a resource for screening and selection. Indeed, selective breeding of genotypes that tolerate higher soil salinities has proven successful in the improvement of crop and pasture plants (Munns and James 2003).

In order to understand salt tolerance and detect genotypic differences in relatively slow-growing trees, experiments require a long-term commitment of personnel, resources and time (Fortmeier and Schubert 1995). Ideally, one would be able to score salt tolerance in early life stages with a simple phenotypic metric. Short-term studies of variation in growth rhythm (Dietrichson 1969) and frost/drought hardiness (Nilsson and Eriksson 1986, Andersson 1992, Pulkkinen 1993) in wild and captive populations have led to the identification and selection of specific genotypes that are well-suited for different growing situations.

The individual plant response to salinity is also influenced by soil properties (Arzani 2008). Affected soils can vary greatly in terms of salinity and sodicity, but a low organic content has in

several cases been reported (Feng et al. 2007). Soil properties are a critical factor affecting plants response to environmental stress and are mainly responsible for the community of microbes that can play a role in salt tolerance (Smith and Read 1997). Given that salt-affected soils are variable, local conditions either promote or inhibit the growth of different salt-tolerant plant species.

The selection of tree species is of critical importance. *Populus* is a diverse genus with a large number of species that vary in salt tolerance (Chen et al. 2003, Sixto et al. 2005, Rae et al. 2007). Several species or their hybrids grow in low salinity soil, e.g., *P. alba* (Beritognolo et al. 2007), *P. tremula* × *alba* (Bolu and Polle 2004) and even in high salinity soil, such as *P. euphratica* (Ma et al. 1997). Salt-tolerant, *P. euphratica*, had a greater ability to control salt uptake and transport to the shoots, which contributes to its greater capacity for salt exclusion (Chen et al. 2003). Most physiological studies on salinity stress are carried out on one or few genotypes/clones per species, which do not represent, however, the natural variation of biological processes. In this study, we used European Aspen (*Populus tremula* L.), full-sib families that originate from the natural germplasm, as an experimental plant. European Aspen is native to cool-temperate regions of Europe and Asia and is a fast-growing pioneer species that may gain a competitive advantage under future climate models (Santamaria and Diez 2005).

In this study, our aims were to evaluate: 1) the suitability of several traits as screening indices of salt tolerance among young seedlings families, and 2) the effects of salinity on different young seedling families of *P. tremula* grown in different soil conditions.

2 Materials and Methods

2.1 Aspen Seedlings and Soil Types

The study was conducted at the Haapastensyrjä Tree Breeding Station of the Finnish Forest Research Institute in southern Finland (60°37'N, 24°26'E, 110 m a.s.l.). Using material collected from *P. tremula* occurring in a nearby natural stand, seeds were produced by artificial crossing

between four male and three female trees to yield 12 different families.

Seedlings were grown on two different soil types. The uppermost layer of soil (horizon A: 20–25 cm top soil) from an aspen-dominated hardwood forest close to the experimental site (Pulkkinen and Pirttilä 1999) was collected and the live plant material was removed by sieving (diameter, 2 cm). This soil was either used as it is (forest soil) or mixed with sand (sandy soil) prior to planting. Soil moisture, as field capacity, was determined after drying at 105 °C for 12 h and organic content was determined as loss-on-ignition after combustion at 550 °C for 4 h. Moisture and organic content were 28% and 15% for forest soil and 9% and 3% for sandy soil, respectively.

2.2 Growth Conditions and Saline Treatments

Fertilized seeds were sown on nursery peat (Kekkilä, Finland) and grown for 3 weeks, by which time they were 4–6 cm in height. Seedlings were then transplanted into containers (Takopot TA913: 40 × 60 × 12 cm, capacity 580 cm³, SCA Packaging, Finland) filled with either of the two soil types. Thirty-six to 48 seedlings from each family were applied in this experiment (960 total) in four randomized blocks replicated in different locations of the greenhouse. The greenhouse temperature profile and photoperiod matched the summer average for the site (11–13 °C night, 17–19 °C day).

A 4-week saline irrigation treatment began when seedlings were four months old. Following Munns and James (2003), we considered salinity levels of 50–100 mM NaCl to be moderately saline and >200 mM as highly saline, and used two test salines (80 mM and 160 mM) and a control (0 mM). Control seedlings were watered twice a week with tap water and once a week with nursery fertilizer solution (N:P:K 19:4:20; Nursery Seedling-*Superex* fertilizer, Kekkilä Corp., Finland). Test seedlings were watered once a week with tap water, once with nursery fertilizer solution, and once with saline water solution. Soil salinity was monitored by measuring electrical conductivity (ECe) of soil filtered water with an Aalsmeer-Holland EC-93 meter. Soil ECe

irrigated only with tap water increased slightly during the experiment (mean ± SE, 0.25 ± 0.02 dS/m, n = 10). Soil ECe after irrigation with 80 mM saline increased to 6.98 ± 0.15 dS/m and was considered moderately saline. Soil ECe after irrigation with 160 mM saline increased to 11.04 ± 0.48 dS/m and was considered highly saline. Each treatment was applied to 3–4 seedlings per block and considered one replicate, which involved a total of four blocks in the same arrangement as before salinity treatment.

2.3 Growth, Salt Tolerance and the Observation of Leaves

After the first month in the greenhouse, shoot height and leaf number were recorded monthly for all seedlings. These data were collected four times before saline irrigation began and once after it was completed. The response to saline treatment, as salinity tolerance, was calculated as the family-based mean dry mass of the whole plant. Salt tolerance was defined as % total biomass of salt-treated seedlings in relation to controls. Leaf necrosis was evaluated as % total number of leaf necrosis seedlings in relation to all tested seedlings. A seedling having more than three necrotic leaves is defined as a leaf necrosis seedling. The leaf necrosis was recorded one week after each salt irrigation.

Four weeks after the first salt treatment, test seedlings were grown for a week without saline irrigation in order to rinse the external salt. Following this period, one seedling per treatment from each block was randomly selected for growth examination. Roots were carefully removed from the soil and gently washed under tap water. Subsequently, leaves and stems were separated from shoot part. Finally, root, leaf and stem were dried at 70 °C for 72 h. The dry mass of three plant organs was measured. We calculated root/shoot ratio, as root dry mass/shoot (leaf + stem) dry mass, which is defined as growth indicator.

2.4 Na⁺ Determination of Different Plant Tissues

Four of 12 families (12–16 seedlings per family) were randomly selected to measure leaf, stem and root

Na⁺ concentrations after the plants were harvested. Leaf, stem and root were separately dried and ground before being subjected to nutrient element analysis. Dry weight concentrations of Na⁺ in each organ of plant were separately measured using dry ash and extraction with hydrochloric acid. The filtered solutions were analyzed by an inductively coupled plasma atomic emission spectrometer (ICP-AES, Thermol Jarrell Ash IRIS Advantage) according to the methods of SFS-EN ISO 11885.

2.5 Data Analysis

The main results were presented as average values and their standard errors. Data were analyzed in two steps: before and after saline treatment. We used arcsine transformation for saline tolerance values. The normality and the homogeneity of the variance of the residuals were examined using scatterplots and Q-Q plots. Analysis of variance (ANOVA) was applied to the experimental data collected from a randomized-block design. Differences were considered statistically significant at $p < 0.05$. Family, soil, salt treatment and block were fixed factors and no significant effect was found in block. In addition, shoot height and leaf number before saline treatment were used as covariates in the ANOVA of the post-treatment data. A post-hoc comparison was performed with the Tukey test. The effects of soil on shoot height, leaf number, salinity tolerance and root/shoot ratio were analyzed using an independent samples t-test. Fisher's protected least significant difference was used to analyze the variation of leaf numbers, root/shoot ratio, Na⁺ concentrations in root and shoot (leaf + stem) in 160 mM saline among four randomly selected families. The effects of family, salt and soil type on leaf necrosis ratio were tested by nonparametric test – Kruskal

Wallis test and Mann-Whitney test. The Pearson correlation was employed to evaluate the relationship of leaf number between 1st month and later each month over which data were collected under zero saline condition, and the relationship of leaf number between prior to saline treatment and after 4-week saline treatment including both saline levels. Entered linear regression technique was used for evaluating the relationship between leaf number of all tested families under 160 mM saline and their salt tolerance. All statistical analyses were computed with SPSS software (version 15.0; SPSS Inc., Chicago, Illinois).

3 Results

3.1 The Effect of Saline Treatment on 5-Month-Old Aspen Seedlings

Under zero saline condition, families grew differently in the two soil types after a four-month growth period. Shoot height was on average 41.74 ± 0.91 cm (29.33 ± 3.24 cm to 49.54 ± 1.22 cm) in forest soil and on average 41.07 ± 0.66 cm (34.46 ± 2.13 cm to 48.33 ± 2.21 cm) in sandy soil. Some families grew significantly better in the forest soil but others showed the opposite trend (Fig. 1a). Family and the interaction between family and soil explained significantly the variation of seedling heights before salinity treatment (Table 1). The explanation ratio of used ANOVA model was 0.29.

The mean number of leaves was 20.48 ± 0.36 (17.3 ± 1.4 to 24.5 ± 0.9) in forest soil and 18.22 ± 0.34 (14.7 ± 1.1 to 20.3 ± 1.9) in sandy soil (Fig. 1b). Seedlings from three families had significantly more leaves in forest soil than in sandy soil. Family and soil type explained a significant

Table 1. ANOVA analyses of shoot height and leaf number of 12 families of *Populus tremula* under zero or negligible salt conditions.

Source of variation	df	Shoot height		Leaf number	
		F-value	P	F-value	P
Family	11	6.767	<0.05	4.304	<0.05
Soil	1	0.439	ns	22.588	<0.05
Family * Soil	11	3.017	<0.05	1.246	ns

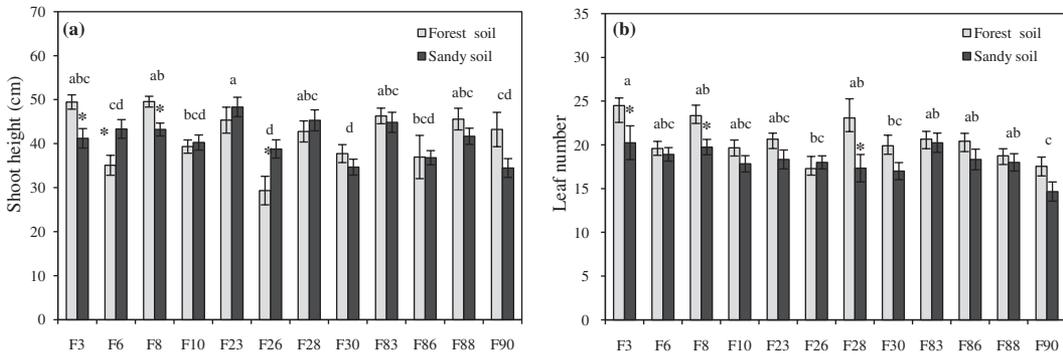


Fig. 1. Shoot height (cm) (a) and leaf number (b) of 12 families of *Populus tremula* before saline treatment grown for four months on two types of soil. Bars are means and error bars are standard errors (SE). Bars with different letters are significantly different among families (n=72–96, Tukey test, p<0.05). Asterisks above bars indicate significant differences between soil types (n=36–48, t-test, p<0.05).

Table 2. Leaf number correlations during five months (LN1–5) experimental period. For seedlings grown in saline conditions, the correlation is between pre-treatment (LN4) and treatment (LN5) value. Data are means of seedlings of 12 families of *Populus tremula*.

Condition		LN1	LN2	LN3	LN4	LN5
Non-saline*	LN1		0.61	0.067	0.194	0.225
			0.000 ^a	0.261	0.001 ^a	0.028 ^b
	LN2	0.61		0.235	0.227	0.171
		0.000 ^a		0.000 ^a	0.000 ^a	0.096 ^c
	LN3	0.067	0.235		-0.032	0.189
		0.261	0.000 ^a		0.594	0.065 ^c
	LN4	0.194	0.227	-0.032		0.072
		0.001 ^a	0.000 ^a	0.594		0.487
	LN5	0.225	0.171	0.189	0.072	
		0.028 ^b	0.096 ^c	0.065 ^c	0.487	
Saline	LN5				0.217	
					0.003 ^a	

* For non-saline seedlings, LN1–LN4, n=285–287; LN5, n=96;

For saline seedlings, LN4–LN5, n=189–191

** Correlation is significant at the (a) 0.01 level, (b) 0.05 level, (c) 0.1 level

portion of the variation in leaf number (Table 1: ANOVA explanation ratio 0.24). The leaf number of seedlings in the 1st month was significantly correlated with the leaf number in the 2nd month, the 4th month and even the 5th month under zero saline condition (Table 2).

After 4 weeks of saline treatment, in forest soil, the mean value of shoot height was 66.1 ± 2.7 cm (45.3 ± 15.1 cm to 80 ± 3.8 cm) under zero saline, 61.1 ± 2.7 cm (41.6 ± 6.3 cm to 78 ± 2 cm) under 80 mM saline and 60.5 ± 2.3 cm (45.5 ± 4 cm to

76.3 ± 5.7 cm) under 160 mM saline (Fig. 2a). In sandy soil, the mean value of shoot height was 61.3 ± 2.6 cm (34.4 ± 3.9 cm to 79.1 ± 3.2 cm) under zero saline, 61.5 ± 2.0 cm (44 ± 6 cm to 79.5 ± 7.3 cm) under 80 mM saline and 53.9 ± 1.6 cm (43.9 ± 8.1 cm to 60.8 ± 3.3 cm) under 160 mM saline (Fig. 2b). The significant differences of shoot height were observed among tested 12 families in both soil types. Salt strongly affected shoot height of seedlings, and shoot height was significantly decreased by saline treatment

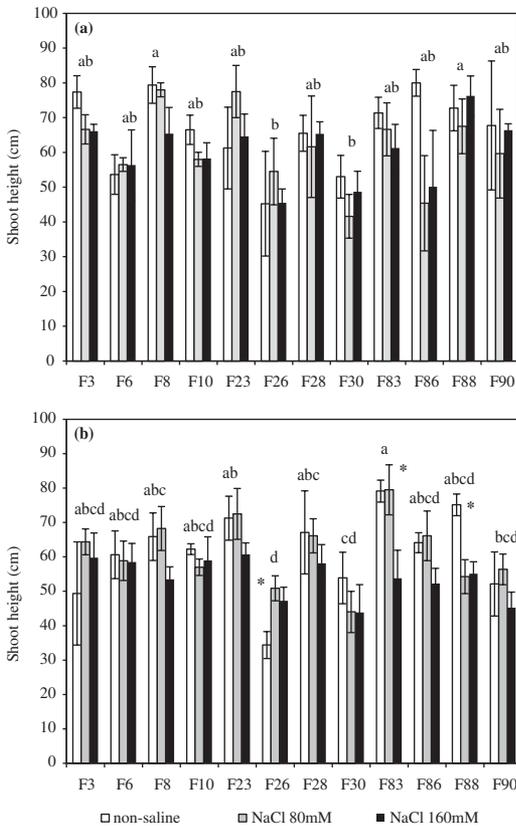


Fig. 2. Shoot height (cm) of 12 families of *Populus tremula* after a 4-week saline treatment grown on forest soil (a) and sandy soil (b). Bars are means and error bars are standard errors (SE). Bars with different letters are significantly different among families (n=72–96, Tukey test, p<0.05). Asterisks above bars indicate significant differences among saline treatments. (n=12–16, Tukey test, p<0.05).

especially in families 83 and 88 on sandy soil (Fig. 2b). Family, soil type and saline concentration all explained significant portions of the variation in shoot height (p<0.05). Shoot height before and after salt treatment was significantly correlated. However, the interactions were not significant. The explanation ratio of the ANOVA model employed was 0.70 (Table 3).

Leaf number was significantly affected by family and salinity (Table 3). In forest soil, the mean number of leaves was 23±4 under zero saline, 20±2 under 80 mM saline and 16±3

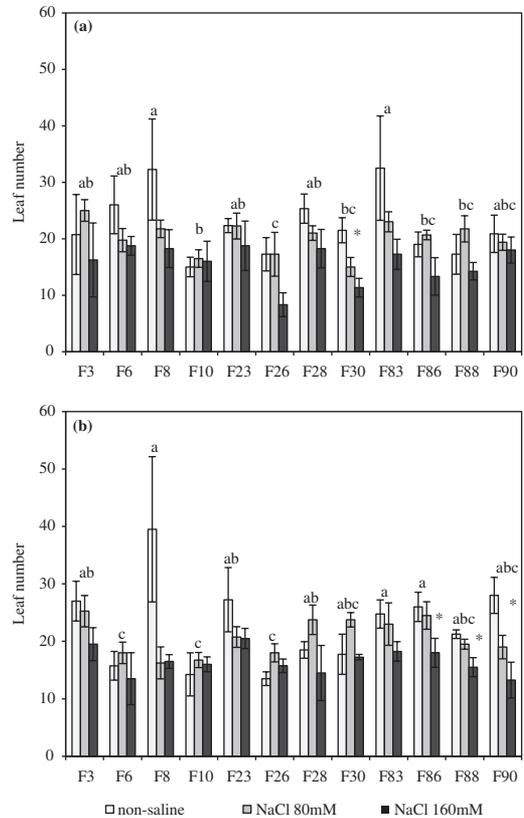


Fig. 3. Leaf number of 12 families of *Populus tremula* after a 4-week saline treatment grown on forest soil (a) and sandy soil (b). Bars are means and error bars are standard errors (SE). Bars with different letters are significantly different among families (n=72–96, Tukey test, p<0.05). Asterisks above bars indicate significant differences among saline treatments. (n=12–16, Tukey test, p<0.05).

under 160 mM saline (Fig. 3a). In sandy soil, the mean number of leaves was 23±4 under zero saline, 21±2 under 80 mM saline and 17±2 under 160 mM saline (Fig. 3b). Leaf number of seedlings was not significantly affected by soil type. Only one family had significantly fewer leaves in forest soil but there were several families with reduced leaf number in sandy soil with increasing salinity. Although there was some overlap, the results suggest that most families had fewer leaves under higher salinity. The explanation ratio of the ANOVA model used was 0.42. No interaction

Table 3. ANOVA analyses of shoot height, leaf number, root/shoot ratio and salinity tolerance across 12 families of *Populus tremula*, two types of soil and three levels of salt treatments.

Source of variation	df	Shoot height (SH)		Leaf number (LN)		Root/shoot ratio		Salt tolerance	
		F-value	P	F-value	P	F-value	P	F-value	P
Family	11	2.288	<0.05	3.151	<0.05	3.444	<0.05	1.574	ns
Soil	1	5.147	<0.05	1.114	ns	45.544	<0.05	3.320	<0.05
Salt	2	13.554	<0.05	20.511	<0.05	1.280	ns	17.686	<0.05
Family * Soil	11	1.705	ns	1.139	ns	0.861	ns	1.298	ns
Family * Salt	22	1.144	ns	1.518	ns	0.703	ns	0.330	ns
Soil * Salt	2	2.075	ns	0.020	ns	0.590	ns	0.565	ns
Family * Soil * Salt	22	0.892	ns	0.987	ns	1.029	ns	1.756	<0.05
Covariation (SH before salt treat)		219.092	<0.05						
Covariation (LN before salt treat)				2.179	ns				

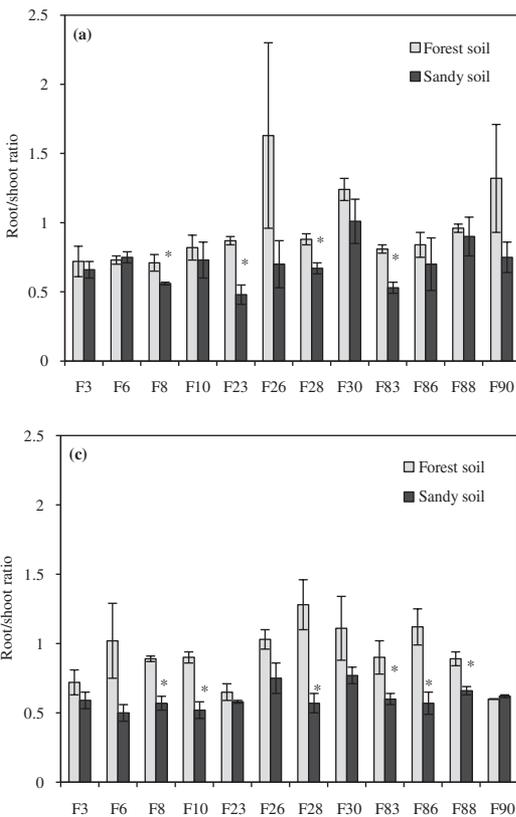


Fig. 4. Root/shoot ratio of 12 families of *Populus tremula* after a 4-week saline treatment grown on two types of soil, control (zero saline) (a), 80 mM saline (b), and 160mM saline (c). Bars are means and error bars are standard errors (SE). Bars with different letters are significantly different among families (n=8, Tukey test, p<0.05). Asterisks above bars indicate significant differences between soil types (n=4, t-test, p<0.05).

between or among effects was found with respect to shoot height or leaf number. A positive correlation was still observed for leaf number before and after saline treatment (Table 2).

Variation in the root/shoot ratio was significantly explained by family and soil type, but not by salt treatment or their interaction (Table 3). Under control conditions (zero salinity), the root/shoot ratio was significantly higher in forest soil (1.19 ± 0.25) than in sandy soil (0.7 ± 0.03) (Fig. 4a). No significant difference was found

among the tested families (family effect, $p > 0.05$; soil effect, $p < 0.05$; interaction of family and soil, $p > 0.05$, the explanation ratio of the ANOVA model used was 0.38). When seedlings were irrigated with 80 mM saline, the root/shoot ratio decreased to an average of 0.87 ± 0.04 in forest soil and 0.66 ± 0.04 in sandy soil (Fig. 4b). Family and soil explained a significant portion of the variation in root/shoot ratio (family effect, $p < 0.05$; soil effect, $p < 0.05$; interaction of family and soil, $p > 0.05$, ANOVA model explanation ratio 0.54). Under 160 mM saline, the root/shoot ratio was an average of 0.93 ± 0.06 in forest soil and 0.61 ± 0.02 in sandy soil. Seedlings from half of the tested families had significantly higher root/shoot ratios in forest soil than in sandy soil (Fig. 4c). Soil type strongly explained the variation of root/shoot ratio (family effect, $p > 0.05$; soil effect, $p < 0.05$; interaction of family and soil, $p > 0.05$, ANOVA model explanation ratio 0.47). Overall, the root/shoot ratio was not significantly affected by salt, but family and soil type explained a significant portion of its variation. The ANOVA model explanation ratio was 0.40.

3.2 Salinity Tolerance

All seedlings were alive after four weeks of saline irrigation. The mean salt tolerance for seedlings irrigated with 80 mM saline was $104.98 \pm 4.90\%$ ($72.93 \pm 4.02\%$ to $155.32 \pm 36.16\%$) in forest soil and $89.02 \pm 4.03\%$ ($63.02 \pm 9.06\%$ to $127.5 \pm 17.9\%$) in sandy soil (Fig. 5a). There was no significant difference among families or for the interaction between family and soil type. Under 160 mM saline, salt tolerance decreased to $76.98 \pm 4.12\%$ ($47.61 \pm 10.43\%$ to $114.56 \pm 26.40\%$) in forest soil and $70.87 \pm 4.54\%$ ($43.81 \pm 9.61\%$ to $96.75 \pm 9.60\%$) in sandy soil (Fig. 5b). Soil type and salinity level explained a significant portion of the variation in salt tolerance. The interaction among family, soil and salt was significant (Table 3). The ANOVA model explanation ratio was 0.53. The significant difference of salt tolerance among tested families was found in 160 mM saline condition (Fig. 5b). Moreover, leaf number of families irrigated with 160 mM saline correlated positively with salt tolerance ($r = 0.47$, $p < 0.05$) (Fig. 6).

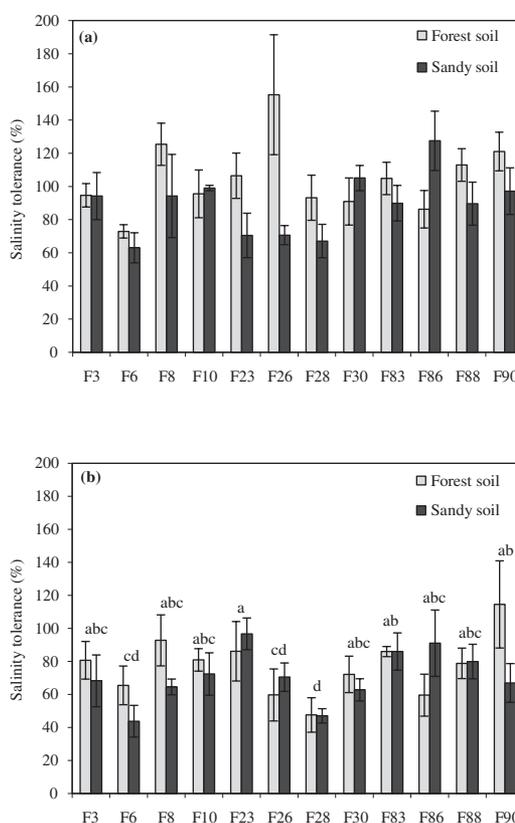


Fig. 5. Salt tolerance (%) of *Populus tremula* seedlings grown in 80 mM saline (a) and 160 mM saline (b). Bars are means and error bars are standard errors (SE). Bars with different letters are significantly different among families ($n = 8$, Tukey test, $p < 0.05$); no significant differences between soil types soil ($n = 4$, t-test, $p < 0.05$).

The symptom of leaf necrosis was clearly observed in the second week of 80 mM saline irrigation in most of tested families ranging from 0–62% (Fig. 7). Only the seedlings of family 10 grown in sandy soil did not have any leaf necrosis. Difference of leaf necrosis among tested families was significant at the beginning; however, it became non-significant later when salt irrigation lasted more than 3 weeks or saline level increased to 160 mM (Table 4). Only slight soil effect on leaf necrosis was observed in the fourth week under 80 mM saline (Table 4). Leaf necrosis was significantly influenced by saline level (data not shown).

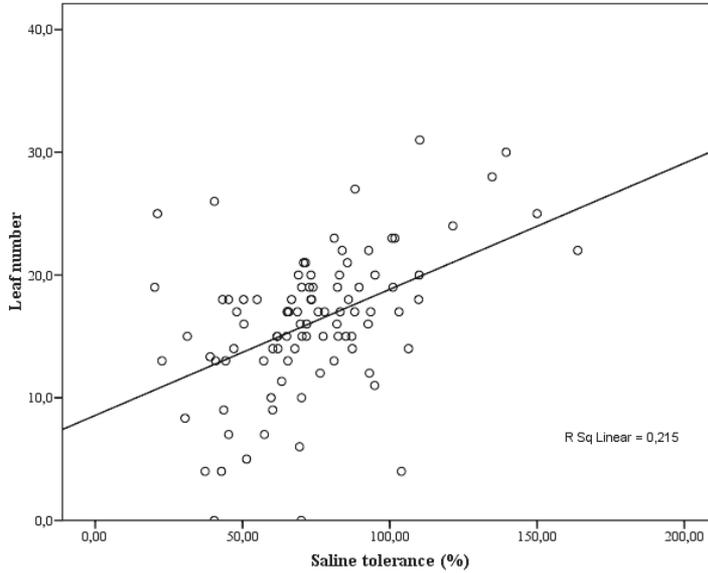


Fig. 6. Correlation between leaf number and saline tolerance (%) of *Populus tremula* seedlings grown under 160 mM saline for four weeks ($p=0.000$).

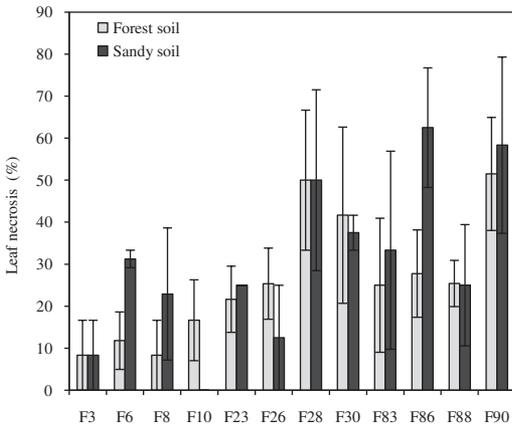


Fig. 7. Leaf necrosis (%) of *Populus tremula* seedlings grown in 80 mM saline for two weeks. Bars are means and error bars are standard errors (SE). The effect of family was tested by Kruskal-Wallis test ($p=0.007$). The effect of soil was tested by U-test ($p=0.655$).

Table 4. Nonparametric tests of the family and soil effects on leaf necrosis of 12 *Populus tremula* grown on two types of soil during 4-week saline treatment.

Source of variation	NaCl 80 mM			NaCl 160 mM		
	Week 2	Week 3	Week 4	Week 2	Week 3	Week 4
Family ^{a)}	0.007*	0.066	0.484	0.202	0.264	0.112
Soil ^{b)}	0.655	0.624	0.079	0.482	0.482	0.125

^{a)} Kruskal Wallis test (n=8)

^{b)} Mann-Whitney U test (n=48)

* Asymp Sig. (2-tailed) <0.05

3.3 Na⁺ Accumulation in Different Plant Organs

Under zero saline condition, some Na⁺ was detected from root organ from both types of soil, but only very limited amount of Na⁺ was found in leaf and stem organs. The differences of Na⁺ concentrations among three organs were significant (Fig. 8a, 8b). After 4-week 80 mM saline irrigation, the accumulation of Na⁺ in different organs was significantly different on forest soil, 64% of Na⁺ was distributed in roots and only 10%

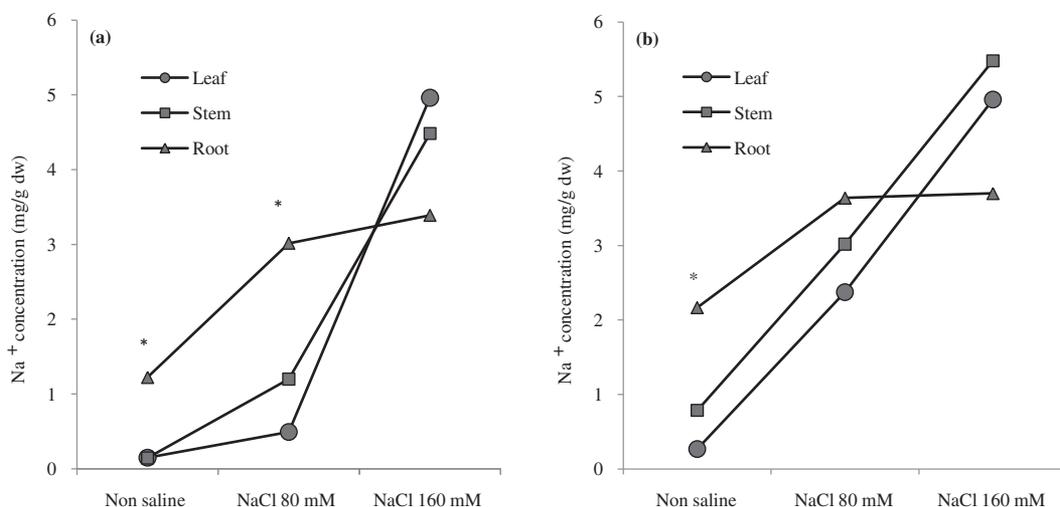


Fig. 8. Distributions of sodium (milligrams Na⁺ per gram dry mass) among leaf, stem and root organs after 4-week saline treatments in four families of *Populus tremula* growing on forest soil (a), and sandy soil (b). Asterisks above lines indicate significant differences among different organs (n=68–72, Tukey test, p<0.05).

Table 5. Performances of seedlings of *Populus tremula* after treated by 160 mM saline for four weeks.

Families	Soil type	Leaf number	Root/shoot ratio	Salt tolerance (%)	Na ⁺ (mg/g d.w.) in root	Na ⁺ (mg/g d.w.) in shoot
F8	Forest soil	18.3±3.3 a**	0.89±0.02* a	92.8 a	4.8±1.0 a	7.6±1.2 b
	Sandy soil	16.5±1.2	0.60±0.05	64.6	5.3±0.4	8.1±0.6
F26	Forest soil	8.3±2.1 b	1.03±0.07 a	59.7 a	3.4±0.5 ab	16.8±4.4 a
	Sandy soil	15.8±1.2	0.75±0.12	70.5	4.0±1.7	9.8±1.1
F83	Forest soil	17.3±2.7 a	0.90±0.07* a	86 a	3.1±0.5 ab	4.7±1.0 b
	Sandy soil	18.3±1.7	0.60±0.04	86.1	3.5±1.0	9.5±2.4
F86	Forest soil	13.3±3.3 ab	1.12±0.33 a	59.6 a	2.3±0.9 b	11.1±4.4 a
	Sandy soil	18.0±2.5	0.57±0.08	91.1	2.5±0.6	14.7±3.3
Effects	Family	p<0.1	ns	ns	p<0.05	p<0.05
	Soil	ns	p<0.05	ns	ns	ns
	Family×Soil	ns	ns	ns	ns	ns
Explanation ratio of used ANOVA-model		0.27	0.37	0.27	0.44	0.52

* Values are significantly different between soil types (n=4, t-test, p<0.05)

** Values are significantly different among four families with different letters (n=4–8, LSD, p<0.05)

accumulated in the leaves (Fig. 8a). However, the Na^+ concentration was sharply increased in above-ground organs on sandy soil, even under 80 mM saline (Fig. 8b). When the saline level increased to 160 mM, there was no significant difference of Na^+ accumulation among three organs on both types of soil. There were significant differences of Na^+ accumulation among tested families. Seedlings of families 8 and 83 contained significantly higher concentrations of Na^+ in roots, but had less Na^+ in shoot under 160 mM saline treatment (Table 5). In relation to the leaf number of the same seedlings, those containing significant less Na^+ in the shoot had significant more leaves (Table 5). The leaf number of the tested four families correlated positively with Na^+ concentration in root under zero saline condition ($r=0.61$, $p<0.01$) (data not shown).

4 Discussion

The test plant material responded in a variety of ways when subjected to saline irrigation in different types of soil. The results showed the potential of aspen for use in soil desalination programs. This study indicated three key findings: (1) *P. tremula* exhibited growth variation under different soil conditions, including soil salinity; (2) leaf number indicated salinity tolerance of young seedlings; (3) root/shoot ratio indicated the effect of soil type on seedling saline stress.

Growing seedlings under controlled conditions, such as in a greenhouse, provides the means to test environmental factors such as various soil stressors in a relatively small space. Such an experimental approach could identify relationships between early (juvenile) and late (adult) salt tolerance. Such relationships have been demonstrated in frost tolerance of trees in short-term greenhouse trials and more long-term studies in field situations (Andersson 1992).

This study showed that family, soil type and soil salinity all have a strong influence on seedling growth of *P. tremula* in the greenhouse. Prior to salt treatment, our experimental families varied considerably in shoot height and leaf number after four months of growth in either soil type. A significant interaction between family and soil

type meant that some families grew better in the more organic forest soil while others fared better in sandy soil. Seedlings growing in forest soil, however, typically had more leaves. Genetic differences among individuals have been found for many characters showing continuous variation within natural populations of forest tree species such as European conifers (Müller-Starck et al. 1992), *Acacia* and *Eucalyptus* (Davidson and Galloway 1993, Mahmood et al. 2003). Although aspen relies on asexual reproduction (Suvanto et al. 2005), the species maintains a high level of genetic variation within populations. Such variability may be caused by environmental or genetic effects. Genetic differences in growth among offspring from same seed parent are, however, difficult to measure because genetic variation typically is confounded with at least some degree of environmental variation in progeny tests where each genotype is represented only once. To address this question, a study is on-going using clone materials to test the saline tolerance of aspen, which offers the possibility of measuring within-family genetic effects. Regardless of the reason of the family effect, families of *P. tremula* exhibit considerable variation in salt tolerance, thus, a selective breeding program cultivating highly salt-tolerant genotypes could result in more effective planting programs on saline soils.

In this study, we used full-sib families that originated from the natural germplasm of *P. tremula*. Therefore, they are representative of natural genetic resources potentially useful in breeding programs. During the experimental period, irrigation with saline at 80–160 mM caused no fatalities in the test families. Additionally, we did not detect a significant difference in salt tolerance among families irrigated with 80 mM saline, but did detect the significant difference in 160 mM saline. Usually, plant responses occur in two distinct phases: osmotic and ionic. The osmotic phase begins immediately after salt treatment whereas ionic stress affects growth much later (Munns and Tester 2008). Many families showed leaf necrosis at 80 mM in second week of saline treatment. Thus, the additional salt clearly had a negative effect on plant vitality. The leaf necrosis is the clear symptom of salt accumulation in leaves (Munns and Tester 2008). Irrigation with 160 mM saline did not cause in reduction in toler-

ance. The results of this study suggest that some seedling families of *P. tremula*, irrespective of genotype, might be able to cope with a salinity of up to 80 mM for at least a month, which is consistent with several previous studies dealing with *P. tremula* (Jouve et al. 2004, Ehlting et al. 2007) and other *Populus* species (Bolu and Polle 2004, Beritognolo et al. 2007). Shoot height, leaf number and seedling dry mass (root/shoot ratio) were dependent on family, soil type and irrigation saline, except for leaf number which was not influenced by soil type. Among the tested variables, total dry mass and leaf number were both significantly correlated with salt tolerance. For at least the five months of growth, the number of the 1st month leaves correlated positively with later leaves number in our study and did not vary between soil types. In addition, leaf number prior to saline treatment correlated positively with post saline treatment. These results suggest that leaf number of early stage seedlings can be used as an indicator of salt tolerance among young seedling families of *P. tremula*. Leaf number is an easily measured and non-destructive external index that enables comparison among plants grown in different environments (Zalesny et al. 2007). Earlier studies have explored the relationship between leaf number, plant growth and stress tolerance (e.g., Kodani 1999, Pellegrino et al. 2005), but our study is among the first to find a clear correlation among these factors. More studies are needed but the basis for salt tolerant selections could come initially from leaf counts.

Salt-tolerant plants employ a variety of physiological mechanisms to minimize the influence of salt on growth. Indeed, the most salt tolerant species of *Populus* (*P. euphratica*) has the greatest ability to restrict Na^+ movement from roots to shoots (Maas 1993). Several studies (Chen et al. 2002, 2003, Sun et al. 2009) also reported root-born processes such as limited ion loading into the xylem, and restriction of salt transport from roots to leaves. Exclusion may be due to active excretion from the roots, or be associated with impermeable membranes (Lambers et al. 2008). A study dealing with salt-sensitive poplar (*Populus × canescens*) showed that Na^+ strongly increased in leaves after two weeks of salt treatment (75 mM NaCl), however, the maximum Na^+ concentration reached only about 75% of root and stem

values (Escalante-Pérez et al 2009). The ability to maintain low Na^+ concentration in leaves and growing shoots is crucial for plant growth under salinity conditions. In our case, the average Na^+ concentration in leaf was about 12–36% of total amount of Na^+ in root and stem under 80 mM saline, and 54–63% under 160 mM saline. It seems that *P. tremula* has a potential to exclude Na^+ from leaves. Here, our results clearly showed that those families with a greater ability to accumulate Na^+ in roots had significantly more leaves and lower Na^+ concentrations in shoot. This result also suggests that leaf number could be one of the useful indicators of salt tolerance in aspen.

Is the observation of having more leaves and uptaking less Na^+ related to saline tolerance of *P. tremula*? We suggest two possible explanations to these observations. Firstly, increased leaf number could lead dilution of the Na^+ toxicity in whole plant. The main site of Na^+ toxicity for most plants is the leaf blade, where Na^+ accumulation stems, rather than in the roots (Munns 2002). This was confirmed in case of family 8, which had significantly higher number of leaves and lower concentration of Na^+ in shoots among the tested four families (Table 5). Secondly, a variable number of leaves in seedlings of the same age means a variable capacity of leaf photosynthesis. Under salt stress, a higher number of leaves could help maintaining a functional photosynthetic apparatus, new leaves are formed while the older ones accumulate salt and undergo necrosis. In addition, the storage compartments in roots are first filled with Na^+ also could delay severe salt impact on photosynthesis. This hypothesis was proved by Escalante-Pérez et al's work (2009).

Plant responses to salinity stress can be strongly modified by soil and by the interaction between salt and soil. This study showed that soil type significantly affects shoot height, dry mass and root/shoot ratio. Of particular interest is that root/shoot ratios in many test families were significantly higher in forest soil than in sandy soil. Many studies have proved that nursery growth response of the roots provide the best indication of seedling performance after outplanting. Rytter et al. (2003) investigated the field performance of containerized seedlings of silver birch and Norway spruce and suggested that shoot length was of lesser importance to future growth than a high root/

shoot ratio, which is of known importance in the growth of other conifers. Furthermore, it has been reported that the production of new roots can rapidly alleviate the reduced growth of seedlings caused by transplant shock (Davis and Jacobs 2005) and improve nutrient uptake by supporting more ectomycorrhizal fungi (Vaario et al. 2009). The fact that aspen seedlings have more roots in forest soil than in sandy soil (corresponding to higher root/shoot ratio) could also be explained by the higher amount of organic matter in the former. The organic matter could provide more nutrients, improve soil aggregation, structure, water retention and increase the capacity for cation exchange (Donald 2003). Soil fertilization plays an important role in the amelioration of salt stress tolerance because it helps to compensate and correct nutritional imbalances in salt-stress plants (Gomez et al. 1996). In addition, soil also plays a key role in sustaining a more diverse microbial community that, in turn, enhances plant tolerance of salt stress. In this way, the observation of less Na^+ accumulation in leaf when grown on forest soil could be explained.

The main objective of this study was to evaluate the utility of several external and easily measured traits as early indicators of salt tolerance among aspen seedlings. We did not analyze the Na^+ concentration of different organs in all families but results from a random sample suggested an ability to restrict salt transport from roots to leaves. Furthermore, soil containing a higher amount of organic matter allows seedlings to develop a higher root/shoot ratio, which might enhance seedling salinity tolerance. In order to determine the generality of these findings, similar studies should be conducted with different tree species and different seedling clones.

In conclusion, we detected significant variation in salt tolerance among European aspen families in 160 mM saline and this variation was significantly correlated with leaf number in young seedlings. These results identify leaf number as an early indicator of salt tolerance and future growth, and are representative of natural genetic resources potentially useful in breeding programs. In concert with the strong role played by soil type, our findings encourage a comprehensive evaluation of European aspen for use in salt-affected land reclamation programs. Increasing

the organic matter and fertilizer in soil could help trees to improve root growth, promote a diverse microbial community and ECM establishment, and thereby enhance salinity tolerance. Extensive screening and a selective breeding program could help to develop families or clones for commercial application.

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