Evaluation of Argentine and Peruvian *Prosopis* germplasm for growth at seawater salinities

Marcelo Velarde[a], Peter Felker[a,b,c,d,*], Claudia Degano[c]

[a] *Universidad Catolica de Santiago del Estero, Argentina*
[b] *Secretaria de Produccion y Medio Ambiente Provincia de Santiago del Estero, Argentina*
[c] *Facultad de Agronomia y Agroindustrias, Universidad Nacional de Santiago del Estero, Argentina*
[d] *INSIBIO, Universidad Nacional de Tucuman, Tucuman, Argentina*

Abstract

The objectives of this study were (a) to identify individual plants of *Prosopis alba* and *P. pallida* that could grow at near seawater salinities to use directly as clonal propagules and to establish seed orchard and (b) to analyse the population structure to aid in future germplasm collections for salinity tolerance in *Prosopis*. This hydroponic greenhouse study compared the growth and survival of 27 *Prosopis* families (seed from single mother trees) as a function of salinity from 10 to 45 dS m^{-1}. Nine *P. alba* families from one saline area (provenance) near Santiago del Estero, Argentina were compared to 14 *P. pallida* families from six provenances in Peru and to one family each of *P. alba/flexuosa* from Chile, *P. juliflora* from Senegal, *P. flexuosa* from Argentina and *P. ruscifolia* from Argentina. The mean of the *P. pallida* families had a significantly greater survival (61.1% vs. 41.7%) and percentage of seedlings that grew (37.4% vs. 23%) at seawater salinities of 45 dS m^{-1} than *P. alba*. Families from *P. pallida* had the greatest mean growth at the highest salinity but *P. alba* families were ranked third and sixth from the top in growth. There were little differences in maximum height growth of the individual seedlings from these two species. For *P. alba*, the percentage of seedlings which survived at the highest salinity level had a lower correlation ($r^2 = 0.46$) with the number of seedlings which grew at that salinity level than for *P. pallida* ($r^2 = 0.70$). The correlation between mean family growth and maximum individual growth was lower for *P. alba* ($r^2 = 0.24$) than for *P. pallida* ($r^2 = 0.49$) suggesting that within *P. alba*, with a sufficiently high number of seedlings, it would be possible to find individuals with rapid growth rates in most families. Approximately 50 of the most rapidly growing seedlings at the highest salinity level have been repotted to be multiplied by rooting of cuttings and to serve as seed orchards for salt tolerance. Given the success in identifying rapidly growing *P. alba* individuals from only one provenance in Argentina, more extensive germplasm collections and testing are warranted to locate

*Corresponding author. Contact Peter Felker via e-mail for current address and telephone number.
E-mail address: peter_felker@hotmail.com (P. Felker).

individuals with superior growth rates. Due to the previously demonstrated erect form, high biomass productivity and sweet pods in these *P. pallida* families in Haiti, Cape Verde, India and Peru, extensive recollections and field testing of *P. pallida* are warranted.

1. Introduction

The negative impact of salinity on growth of plants in irrigated and non-irrigated areas of the world's arid regions continues to be a major problem. The classic works of Richards (1954) and Ayers and Westcott (1985) provides an excellent overview of the salt tolerance of plants and points out that while most of the temperate annual agricultural plants, soybeans, tomatoes suffer yield reductions from salinities with conductivities as low as 2–$3\,dS\,m^{-1}$, some agriculturally important plants, generally from arid zones (dates, barley), can tolerate salinities of 12–$18\,dS\,m^{-1}$ with only moderate reductions in yield. Within the legume family, most of the commercially important annuals, such as soybeans, beans, and cowpeas are salt sensitive showing yield decreases at salinities as low as $2\,dS\,m^{-1}$. Alfalfa is the most salt tolerant of the currently commercial legume species showing yield reductions of 50% at salinities of $9.6\,dS\,m^{-1}$ (Ayers and Westcott, 1985).

Efforts to identify plants that are tolerant to salinities equal to seawater were pioneered by Epstein for commercial crops such as wheat and barley (Epstein et al., 1979). Later other workers found that many trees and shrubs indigenous to coastal marine environments e.g. Spartina, Distichlis (Smart and Barko, 1980) and arid lands, Atriplex (Somers, 1979), *Suaeda* spp. (Flowers et al., 1986) and Eucalyptus (Van der Moezel et al., 1988; Sun and Dickinson, 1993) were highly tolerant of salinities close to seawater that would kill common agricultural plants. Among the terrestrial nitrogen-fixing plants the Casuarinas (El-Lakany and Luard, 1982; Ng, 1987), the Acacias (Craig et al., 1990) and the *Prosopis* (Felker et al., 1981; Rhodes and Felker, 1988; Ahmad et al., 1994; Baker et al., 1995) have shown the capability to grow in salinities of about $40\,dS\,m^{-1}$ which is nearly 20 times greater than salinities that can be tolerated by annual temperate legumes. More recent molecular work has made important progress in identifying some of the mechanisms for salt tolerance using model systems (Bohnert, 1999; Hasegawa et al., 2000). Among the highly salt-tolerant terrestrial plants and legumes in particular, *Prosopis* is unique in being able to provide nutritious pods suitable for human food and very dimensionally stable lumber useful for the furniture industry (Felker et al., 2000).

Our initial work evaluating *Prosopis* for salt tolerance (Felker et al., 1981) found that a *P. tamarugo* and a *P. pallida* accession from Hawaii could grow at salinities equal to seawater. Later work (Rhodes and Felker, 1988) that examined a broader germplasm base found that *P. juliflora* from Senegal had the greatest combination of salt tolerance and rapid growth and that within *P. alba*, which is the most important commercial *Prosopis* species in Argentina, there appeared to be sufficient

biodiversity to select individual plants that could grow in salinities equal to seawater. Due to the close affinity of *P. juliflora* to *P. pallida* which is highly valuable for sweet pods, rapid and erect growth (Wojtusik et al., 1993; Harris et al., 1996; Harsh et al., 1996), a more detailed look at the salt tolerance of the Peruvian *P. pallida* seemed warranted. As the weedy *P. ruscifolia* (vinal) with its 15 cm long thorns, rapidly colonizes frequently flooded and/or saline areas (Morello et al., 1971), it was important to examine this species for its salt tolerance. Given the ever increasing harvest of *P. alba* for the Argentine furniture industry (more than 100,000 ton year^{-1} in the Province of the Chaco), the recent approval of subsidies to establish *P. alba* plantations (3000 ha approved for the Province of Santiago del Estero in 2001) and the continued salinization of Argentina's arid lands, we placed a priority on identifying individual trees within *P. alba* with increased salt tolerance.

2. Materials and methods

The origin of the germplasm is listed in Table 1. Except as noted, the seeds came from individual mother trees. The *P. alba* seeds (O1–O9) were provided by H. Ochoa who collected them from trees of good form and sweet pods from a saline area described in Table 1. The *P. juliflora* seeds were collected from abundant *P. juliflora* trees near the CNRA research station in Senegal and had low germination as they were provided in April 1978. This *P. juliflora* accession was also tested in the salinity trial of Rhodes and Felker 1988. The Peruvian accessions 417–545 were collected by Sagastegui of the Universidad Nacional de Trujillo and provided in May of 1979. The low germination of some of these accessions is probably attributable to the age of the seeds. As can be seen in the map in Fig. 1, the 14 Peruvian accessions came from six different regions (provenances). Accession 1117 was provided by Luis Zelado of CORFO from the stands at Pampa del Tamarugal of northern Chile that is approximately 50 km east of Iquique and thus this accession was a combination of various trees. While the seeds from accession 1117 came from trees of the section algarobia (and not from *P. tamarugo*), the species cannot be resolved with precision as the progeny were intermediate between *P. alba*, *P. flexuosa*, *P. nigra* and *P. chilensis*. Accession CAT1 was provided by Rafael Santa Cruz of the Universidad de Catamarca, Argentina from *P. flexuosa* stands in that area. The *P. ruscifolia* (vinal) was provided by M.R. Freyre, Universidad Nacional de Litoral, Province of Santa Fe, Argentina. UDEP accessions 1 and 2 were provided by Luis Alban from a saline area near Sechura, Peru.

The plants were essentially evaluated in a hydroponic system in which the salinity could be carefully controlled. The salinity/nutrient solution was pumped from a 600 tank, according to a time clock, into a 2.2 m by 2.2 m by 0.25 m box which was fitted with a siphon. The box contained 21 plastic trays, each with 128 cavities arranged in rows of 8 by 16 (maximum of 2688 seedlings for the box). The individual cavity dimension was 3.0 cm in diameter and 5 cm deep. The trays were filled with a 50% sand/vermiculite mixture. The time clock was set to pump sufficient solution to cover the tray with about 1 cm of solution. A siphon was also adjusted to this height so

Table 1
Description of *Prosopis* germplasm used for evaluation for growth in high-salinity conditions

Accession number	Species	Origin
O1	*P. alba*	Saline area between Loreto and Rio Saladillo, Prov Santiago del Estero, Argentina
O2	*P. alba*	Saline area between Loreto and Rio Saladillo, Prov Santiago del Estero, Argentina
O3	*P. alba*	Saline area between Loreto and Rio Saladillo, Prov Santiago del Estero, Argentina
O4	*P. alba*	Saline area between Loreto and Rio Saladillo, Prov Santiago del Estero, Argentina
O5	*P. alba*	Saline area between Loreto and Rio Saladillo, Prov Santiago del Estero, Argentina
O6	*P. alba*	Saline area between Loreto and Rio Saladillo, Prov Santiago del Estero, Argentina
O7	*P. alba*	Saline area between Loreto and Rio Saladillo, Prov Santiago del Estero, Argentina
O8	*P. alba*	Saline area between Loreto and Rio Saladillo, Prov Santiago del Estero, Argentina
O9	*P. alba*	Saline area between Loreto and Rio Saladillo, Prov Santiago del Estero, Argentina
44	*P. juliflora*	Centre National Recherche Forestier, Ross-Bethio, Senegal, West Africa
417	*P. pallida*	El Nino (Viru) (Proc), Trujillo (Prov), La Libertad (Dpto), Peru
422	*P. pallida*	Algarrobal (Proc), Contumaza (Prov), Cajamarca (Dpto), Peru
423	*P. pallida*	Algarrobal (Proc), Contumaza (Prov), Cajamarca (Dpto), Peru
424	*P. pallida*	Algarrobal (Proc), Contumaza (Prov), Cajamarca (Dpto), Peru
425	*P. pallida*	Chao (Viru) (Proc), Trujillo (Prov), La Libertad (Dpto), Peru
439	*P. pallida*	Huancaco (Viru) (Proc), Trujillo (Prov), La Libertad (Dpto), Peru
537	*P. pallida*	Sullana (Proc), Sullana (Prov), Piura (Depto), Peru
541	*P. pallida*	La Union (Proc), Piura (Depto), Peru
543	*P. pallida*	La Union (Proc), Piura (Depto), Peru
545	*P. pallida*	Sullana (Proc), Piura (Depto), Peru
553	*P. pallida*	Piura (Proc), Piura (Depto), Peru
556	*P. pallida*	Piura (Proc), Piura (Depto), Peru
1117	*P. alba/nigra*	Pampa del Tamarugal, Chile
CAT1	*P. flexuosa*	Catamarca, Argentina
VINAL	*P. ruscifolia*	Santa Fe, Argentina
UDEP 1	*P. pallida*	Sechura, Piura (Depto), Peru
UDEP 2	*P. pallida*	Sechura, Piura (Depto), Peru

that the solution would begin to drain when this height was reached. About 12 min was required to fill the box and also for the siphon to drain the tank. The trays were irrigated from 1 to 3 times a day depending on the temperature. The overall system was constructed for less than $500 using a common greenhouse irrigation timer ($40) a 24 V relay ($25) that controlled a $\frac{1}{3}$ hp pump ($40), a 600 l tank ($75), $\frac{3}{4}$ in PVC plastic pipe for the pressure side and 1.5 cm internal diameter flexible hose for the siphon. The major costs were the materials for the wooden box and the plastic trays.

Fig. 1. Location of the *P. pallida* collection sites in Peru.

The experimental design contained a replicated and a non-replicated portion. The replicated portion contained 25 accessions of *Prosopis* in a randomized complete block design with four replicates. Each replicate consisted of a row of 16 seedlings. The non-replicated portion contained nine accessions of *P. alba* (also included in the replicated portion) with 128 plants each and was designed to screen larger numbers of Argentina's economically important priority species *P. alba*.

In spite of the randomized complete block experimental design with 16 plants per replicate, a non-conventional data analysis system was used with essentially no statistics. The justification for this data analyses is that our goal was not to compare

the mean performance of the seed sources but rather to identify the 1% of the population (and thus > 2 standard deviations from the mean) that had the greatest growth at high salinity levels. Thus, the imposition of treatments that lead to mortality, or cessation of growth, in greater than 90% of the plants lead to a database that was not amenable to standard statistical analyses. Thus, while the replicates were isolated in different blocks to minimize location effects, the survivals and height growth were based on the means of all the 64 possible plants per accession and not on the means of the four replicates. However, a correlation matrix was used to examine the means of variables measured (percent survival, percentage seedlings with growth) at the highest salinity level.

The seeds were nicked on the blunt end with a utility knife and then sown in a 50% sand/50% vermiculite mixture in the plastic trays. The seedlings were germinated in a nutrient solution without salinity since Argentine *Prosopis* plantations will be established with well-established seedlings and not direct seeding. The nutrient solution was a commercial mixture of Peters soluble 20–20–20 mix containing macro- and micronutrients and used at the following concentration (mg l^{-1}); N— 200, P$_2$O$_5$—200, K$_2$O—200, Mg—5, B—0.2, Cu—0.5, Fe—1, Mn—0.5, Mo—0.005, Ca—0.0 and Zn—0.5. Since this mixture was low in calcium and magnesium an additional 490 mgl^{-1} of MgSO$_4 \cdot$7H$_2$O and 345 mgl^{-1} of CaSO$_4 \cdot$2H$_2$O was added to the tank. As this was not completely soluble, on alternating weeks a mixture of either 500 mg l^{-1} magnesium sulfate or calcium sulfate was sprayed on the plants until dripping occurred. Iron sequestrene 138 was also added at a concentration of 100 mg l^{-1}.

The salinity was increased by adding NaCl until the desired conductivity was achieved. The conductivity was measured, and corrected if necessary, 3 times per week. Just before the next salinity regime was imposed (about 4-week intervals), the heights of the live portions of all the seedlings were measured. In some cases at the high salinity, tip dieback occurred and in this case we measured the height of the live portion. Thus, in many cases at the high salinity, a negative growth occurred. The mortality and differences in height growth were used to assess resistance to salinity.

3. Results

Evidently, due to the long time in storage (> 20 years) in three germplasm collections (Univ. California, Riverside, Texas A&M University, Kingsville and Universidad Nacional de Santiago del Estero) the germination percentage of some of the Peruvian accessions was very low (Table 2). In contrast the germination of the freshly harvested *P. alba* ranged from 85% to 97%.

For virtually all of the seedlings, there was little loss in mortality from the 0 to 10 dS m^{-1} salinities as has been also reported by other workers (Rhodes and Felker, 1988) who found that salinities below 12 dS m^{-1} have little effect on the survival or growth of *Prosopis*. There was, however, an abrupt drop in survival going from the 10 to 25 dS m^{-1} salinity. Virtually, all of the *P. alba* species had an approximate 50%

Table 2
Percentage of *Prosopis* seedlings which survived at increasing salinity levels and the percentage which grew at 45 dS m^{-1}

Accession	Initial no.	Seedlings which survived (%)					Seedlings that grew at 45 dS m^{-1} (%)
		0 dS m^{-1}	10 dS m^{-1}	25 dS m^{-1}	35 dS m^{-1}	45 dS m^{-1}	
44 (*P. juliflora*)	14	100	93	64	64	64	29
1117 (*P. alba/nigra*)	12	100	92	50	50	50	25
Vinal (*P. ruscifolia*)	37	100	97	87	77	87	46
CAT (*P. flexuosa*)	64	100	98	38	31	38	14
O1 (*P. alba*)	58	100	100	48	45	38	21
O2 (*P. alba*)	60	100	100	52	47	43	27
O3 (*P. alba*)	61	100	98	59	48	46	23
O4 (*P. alba*)	62	100	100	63	52	42	21
O5 (*P. alba*)	62	100	97	35	32	29	11
O6 (*P. alba*)	62	100	95	53	53	47	27
O7 (*P. alba*)	54	100	100	50	44	43	33
O8 (*P. alba*)	50	100	96	66	66	60	26
O9 (*P. alba*)	60	100	100	43	35	27	17
Mean of *P. alba*		100.0	98.4	52.1	46.9	41.7	23
95% CI		0	1.31	6.35	6.55	6.4	4
Peru Prov. 1 422	16	100	94	88	88	81	56
423	23	100	91	65	65	65	48
424	25	100	92	84	84	84	52
Mean		100.0	92.3	79.0	79.0	76.7	52
95% CI		0	1.73	13.91	13.91	11.56	5
Peru Prov. 2 417	60	100	98	85	85	83	48
425	23	100	100	43	39	39	13
439	24	100	83	67	63	63	38
Mean		100	94	65	62	62	33
95% CI		0	11	24	26	25	20
Peru Prov. 3 UDEP1	53	100	98	91	87	85	34
UDEP2	3	100	33	33	33	33	0
Mean		100	66	62	60	59	17
95% CI		0	64	57	53	51	33
Peru Prov. 4 553	18	100	89	72	72	72	61
556	10	100	80	80	70	70	40
Mean		100	85	76	71	71	56
95% CI		0	9	8	2	2	32
Peru Prov. 5 541	12	100	67	67	67	67	58
543	7	100	86	14	14	14	0
Mean		100	77	41	41	41	29
95% CI		0	19	52	52	52	57
Peru Prov. 6 537	4	100	50	25	25	25	25
545	8	100	100	88	88	75	50

Table 2 (*continued*)

Accession	Initial no.	Seedlings which survived (%)					Seedlings that grew at $45\,dS\,m^{-1}$ (%)
		$0\,dS\,m^{-1}$	$10\,dS\,m^{-1}$	$25\,dS\,m^{-1}$	$35\,dS\,m^{-1}$	$45\,dS\,m^{-1}$	
Mean		100	75	57	57	50	38
95% CI		0	49	62	62	49	24
		All Peruvian accessions			Mean	61.1	37.4
					95% CI	13.3	10.8

Seeds were sown in four reps of 16 each for a total of 64 seeds total, but as some of the seeds were more than 20 years old (44, 1117 and the accessions 417–556) many low germination rates were obtained.

drop in survival while the Peruvian accessions were less sensitive with many accessions showing only an approximate 10% mortality loss from this treatment. The *P. ruscifolia* also had only a 10% drop in survival as opposed to the *P. juliflora*, *P. flexuosa* and *P. alba/nigra* species from both Catamarca and Chile. After the large mortality loss from 10 to $25\,dS\,m^{-1}$, in practically all the accessions there was only a gradual loss in mortality from the 25 to $45\,dS\,m^{-1}$ salinities.

As judged from the 95% confidence intervals, the mean survival of all 14 Peruvian accessions of 61.1% in the $45\,dS\,m^{-1}$ treatment was significantly greater than the mean survival 41.7% of the Argentine *P. alba* accessions. The three Peruvian accessions with the greatest survival, 422, 417 and UDEP1, were from different provenances and had greater than 80% survival. However, within both the Peruvian and Argentine accessions there was considerable variability in the survival. For example the Peruvian accessions ranged from 14% to 85% survival while the Argentine *P. alba* accessions ranged from 27% to 60% survival. The Argentine accession with the greatest survival was the weedy vinal, *P. ruscifolia*. While the number of accessions per Peruvian provenance was very limited, virtually all of the provenances had accessions with high and low survival and with the exception of provenance 1 from Contumaza (which had the greatest survival), there was no obvious provenance trend for survival.

It is important to distinguish between the seedlings that did not die at the high $45\,dS\,m^{-1}$ salinity level and while green were essentially dormant with no growth, from those which grew at this salinity level. For both the *P. alba* and *P. pallida* accessions, slightly more than half of the seedlings that survived were able to grow at the highest salinity level. Here again the mean of the *P. pallida* accessions which grew at the highest salinity level (37%) was significantly higher than the mean of the *P. alba* accessions (23%). Within *P. pallida* and *P. alba*, there were very large accession differences in percentage growth (0–61% and 11–33%, respectively) at the highest salinity treatment.

Surprisingly, the accessions which had the greatest survival at the high salinity level were not the accessions which had the greatest mean growth at the highest salinity level. For example *P. pallida* accession UDEP 1 had the greatest survival of

85% but only 34% of its seedlings grew at the high salinity level, while *P. pallida* accession 553 with 72% survival had the greatest percentage of seedlings which grew (61%). The same trend also occurred for *P. alba*. The accession with the greatest percentage growth at the 45 dS m^{-1} treatment (553) was collected in Piura.

The mean growth of the accessions and the maximum individual growth per accession at the three salinity levels are presented in Table 3. The *P. pallida* accessions dominated the mean and maximum individual growth rate at all three salinity levels. However, at the highest salinity levels *P. alba* accessions also were near the top in mean accession growth. *P. pallida* had the individual with the greatest individual growth of 5 cm, but no accessions with maximum height growth of 4 cm, while six *P. alba* accessions had individuals with maximum height growth of 4 cm. Surprisingly, even *P. alba* accession O6 with fifth from the lowest mean accession growth had one individual with a 4 cm maximum growth rate.

There was little consistency in the ranking among the families at the three salinity levels. For example, *P. pallida* 543 which ranked first at the lowest salinity level dropped to fifth and fourth from the worst accessions at the intermediate and highest salinity level and *P. alba* O9 which ranked third and fourth from the worst at the lowest and intermediate salinity levels rose to third from the top at the highest salinity level. Some accessions such as *P. juliflora* 44 and *P. pallida* 439 were within the top grouping at all salinity levels. The weedy *P. ruscifolia* which had the greatest overall percentage survival and for Argentina the greatest percentage of seedlings that grew at the highest salinity level, was intermediate in mean accession growth but had an individual with 3 cm maximum growth. It is fortunate that the much more desirable *P. alba* species had individuals with greater height growth than the *P. ruscifolia*. The *P. flexuosa* species from Catamarca, Argentina had much greater growth than the *P. alba/nigra* 1117 from the Chilean salt deserts.

Within the Peruvian germplasm, there were both similarities and differences within provenances. For example, Peruvian accession 553 with the greatest mean growth at the highest salinity level (2.1 cm) was collected in the same area as accession 556 with one of the lowest growth rates (0.1 cm). Also accessions 543 and 541 from the same area ranked 24th and fourth respectively. However, the two accessions from the Procedencia of Viru 417 and 425 and two accessions (423 and 424) from the Procedencia of Algarrrobal had similar mean and maximum growth rates.

While the mean growth rates of the unreplicated *P. alba* accessions grown with 128 individuals per accession were lower than those in the replicated portion, the growth rates of the individuals with the greatest performance were similar (Table 4). The lower mean growth rate is probably attributable to the fact that they were grown in the center portion of the hydroponic system surrounded on all four sides by the replicated portion of the trial. However, from this portion of 1152 initial individuals, 50 seedlings with active growth at the highest salinity level were selected and repotted in good soil for a selection intensity of < 5%.

In contrast to the almost 550 km distance between the Peruvian accessions, the *P. alba* accessions were all collected within 30 km of each other on the fringes of a hypersaline area in Argentina.

Table 3

Height growth (difference in height between salinities) of *Prosopis* families as a function of increasing salinity in randomized complete block design

25 dS m^{-1} salinity		35 dS m^{-1} salinity		45 dS m^{-1} salinity	
Accession	Growth mean (max) (cm)	Accession	Growth mean and (max) (cm)	Accession	Growth mean and (max) (cm)
543 (*P. pallida*)	9.0 (9)	439 (*P. pallida*)	2.07 (7)	553 (*P. pallida*)	2.12 (5)
439 (*P. pallida*)	8.6 (23)	44 (*P. juliflora*)	1.89 (4)	537 (*P. pallida*)	2.0 (2.0)
537 (*P. pallida*)	8.0 (8)	424 (*P. pallida*)	1.71 (9)	O9 (*P. alba*)	1.70 (4)
44 (*P. juliflora*)	6.7 (16)	556 (*P. pallida*)	1.57 (3)	541 (*P. pallida*)	1.28 (3)
O7 (*P. alba*)	6.6 (26)	425 (*P. pallida*)	1.56 (4)	44 (*P. juliflora*)	1.28 (4)
541 (*P. pallida*)	6.3 (15)	417 (*P. pallida*)	1.31 (14)	O2 (*P. alba*)	1.24 (4)
424 (*P. pallida*)	6.2 (22)	UDEP2 (*P. pallida*)	1.00 (1)	439 (*P. pallida*)	1.17 (3)
553 (*P. pallida*)	6.0 (17)	537 (*P. pallida*)	1.00 (1)	545 (*P. pallida*)	1.17 (3)
1117 (*P. alba/ nigra*)	6.0 (14)	553 (*P. pallida*)	0.92 (4)	424 (*P. pallida*)	1.03 (3)
425 (*P. pallida*)	5.1 (14)	O1 (*P. alba*)	0.81 (8)	423 (*P. pallida*)	1.00 (3)
O3 (*P. alba*)	5.1 (21)	423 (*P. pallida*)	0.80 (4)	422 (*P. pallida*)	0.96 (3)
417 (*P. pallida*)	5.0 (22)	VINAL (*P. ruscifolia*)	0.80 (6)	O7 (*P. alba*)	0.88 (3)
O8 (*P. alba*)	4.6 (20)	545 (*P. pallida*)	0.71 (3)	VINAL (*P. ruscifolia*)	0.85 (4)
422 (*P. pallida*)	4.4 (9)	UDEP1 (*P. pallida*)	0.67 (6)	UDEP1 (*P. pallida*)	0.53 (3)
423 (*P. pallida*)	4.3 (11)	CAT1 (*P. flexuosa*)	0.65 (3)	CAT1 (*P. flexuosa*)	0.44 (3)
O6 (*P. alba*)	4.3 (15)	422 (*P. pallida*)	0.57 (4)	O4 (*P. alba*)	0.38 (4)
556 (*P. pallida*)	4.3 (15)	O8 (*P. alba*)	0.52 (8)	O1 (*P. alba*)	0.37 (2)
UDEP2 (*P. pallida*)	4.0 (4)	O7 (*P. alba*)	0.42 (2)	O5 (*P. alba*)	0.36 (4)
VINAL (*P. ruscifolia*)	3.9 (17)	O2 (*P. alba*)	0.30 (3)	O3 (*P. alba*)	0.33 (2)
UDEP1 (*P. pallida*)	3.8 (12)	541 (*P. pallida*)	0.25 (2)	417 (*P. pallida*)	0.20 (3)
545 (*P. pallida*)	3.6 (8)	O3 (*P. alba*)	0.24 (3)	425 (*P. pallida*)	0.15 (1)
O2 (*P. alba*)	3.5 (9)	O6 (*P. alba*)	0.21 (4)	556 (*P. pallida*)	0.11 (1)
O4 (*P. alba*)	3.1 (16)	543 (*P. pallida*)	0.00 (0)	O6 (*P. alba*)	0.02 (4)
O5 (*P. alba*)	2.9 (11)	O9 (*P. alba*)	0.00 (2)	543 (*P. pallida*)	0.00 (0)
O9 (*P. alba*)	2.5 (20)	O5 (*P. alba*)	−0.20 (1)	1117 (*P. alba/ nigra*)	−0.17 (2)
O1 (*P. alba*)	2.0 (7)	O4 (*P. alba*)	−0.47 (3)	O8 (*P. alba*)	−0.61 (2)
CAT1 (*P. flexuosa*)	1.6 (11)	1117 (*P. alba/ nigra*)	−0.50 (1)	UDEP2 (*P. pallida*)	−4.00 (0)

The accession mean is ranked in decreasing mean growth.

It is useful to examine correlations which may be helpful in predicting future performance. Thus in Tables 5 and 6, for *P. alba* and *P. pallida*, respectively, we have examined correlations among the various parameters involved with growth and survival at the highest salinity level. Contrasting behaviors were observed for *P. alba*

Table 4
Mean and maximum height growth (difference in height between salinities) of nine *P. alba* families from Santiago del Estero, Argentina with 128 individuals per family in non-replicated design as a function of increasing salinity

Accession	Growth mean and (max) (cm)		
	$25\,dS\,m^{-1}$	$35\,dS\,m^{-1}$	$45\,dS\,m^{-1}$
O1	1.63 (17)	0.35 (9)	−0.42 (2)
O2	5.70 (27)	−0.03 (15)	−0.56 (1)
O3	3.16 (14)	−0.20 (4)	−0.40 (2)
O4	3.14 (21)	−0.80 (13)	−0.02 (4)
O5	3.73 (17)	−0.24 (10)	−1.82 (4)
O6	4.56 (20)	−0.24 (17)	−1.27 (2)
O7	5.74 (30)	0.81 (19)	−0.31 (3)
O8	2.90 (15)	0.36 (6)	−0.88 (3)
O9	3.02 (13)	0.66 (15)	0.30 (4)

Table 5
Correlations (square of the coefficient of determination) between survival and growth characteristics of nine *P. alba* families grown at $45\,dS\,m^{-1}$ ($n=9$)

	Percent survival	Percent with growth	Family mean of growth	Maximum individual growth
Percent survival				
Percent with growth	0.46*			
Family mean of growth	0.49*	0.01		
Maximum individual growth	0.26	0.07	0.24	

Significance of the correlations are indicated by *$p=0.05$, **$p=0.01$ and ***$p=0.001$.

Table 6
Correlations (square of the coefficient of determination) between survival and growth characteristics of 14 *P. pallida* families grown at $45\,dS\ m^{-1}$ ($n=14$)

	Percent survival	Percent with growth	Family mean of growth	Maximum individual growth
Percent survival				
Percent with growth	0.70***			
Family mean of growth	0.11	0.43*		
Maximum individual growth	0.53***	0.77***	0.49**	

Significance of the correlations are indicated by *$p=0.05$, **$p=0.01$ and ***$p=0.001$.

and *P. pallida*. For *P. alba*, the percentage of seedlings which survived at the highest salinity level had a much lower correlation ($r^2=0.46$) with the number of seedlings which grew at that salinity level than for *P. pallida* ($r^2=0.70$). In contrast, the correlation between percentage survival at the highest salinity level and family mean

of growth was greater for *P. alba* ($r^2 = 0.49$) than for P. *pallida* ($r^2 = 0.11$). The correlation between mean family growth and maximum individual was lower for *P. alba* ($r^2 = 0.24$) than for *P. pallida* ($r^2 = 0.49$). Within *P. alba*, it appears as if the potential for fast growth rate in individuals at high salinity levels is almost located at random among *Prosopis* families and that with a sufficiently high number of seedlings, it would be possible to find individuals with rapid growth rates in most families.

4. Discussion

The continued exploration for highly salt-tolerant *Prosopis* is important for both theoretical and applied considerations. Within the family Fabaceae to which *Prosopis* belongs, virtually all of the important annual legumes that belong to the subfamily Papilonoideae, i.e. soybeans, common beans, peas are highly salt sensitive and only alfalfa among the commercial legumes has a moderate degree of salt tolerance. Thus, identification and understanding of the mechanism of salt tolerance in *Prosopis* may have relevance to current commercially important legumes. The majority of the molecular work characterizing salt tolerance has been conducted on Arabidopsis, salt water algae, and yeast mutants (Bohnert et al., 1999: Hasegawa et al., 2000) although Winicov (1998) has reported a transcriptional regulator for gene expression in salt-tolerant alfalfa. *Prosopis* is not a halophyte in that it is not able to grow with facility at 40–60 dS m^{-1} or to absorb and then secrete sodium salts on the leaves. Under sodic (but not saline) conditions, we have found that micronutrient additions, particularly zinc, are required to permit active growth and to decrease leaf sodium contents in stressed leaves from 2.0% of dry weight to 0.5% in healthy plants (Cline et al., 1986). This would suggest that an active energy pump such as described by Hasegawa et al. (2000), possibly activated by zinc, is important for salt regulation in *Prosopis*. As clonal propagation of *P. pallida* by stem cuttings is moderately easy, this could be a useful model system for salt tolerance in the legume family. It would be interesting to search for cDNAs in highly salt-tolerant *Prosopis* clones that were not present in the low-salt-tolerant clones in the hope that this information would be relevant to common annual legumes.

With regard to other studies on salt tolerance in *Prosopis*, this work builds on our earlier work screening *Prosopis* for growth at seawater salinity levels (Felker et al., 1981; Rhodes and Felker, 1988). In particular, it confirms the earlier growth of Hawaiian *P. pallida* (Felker et al. 1981), *P. juliflora* 44 and some *P. alba* at seawater salinities (Rhodes & Felker, 1988). Ahmad et al. (1994) who examined two *P. juliflora*, one *P. pallida* and two *P. glandulosa* accessions found that the *P. glandulosa* accessions did not survive beyond 20 dS m^{-1}, and that the *P. juliflora* from Brazil was the most salt tolerant and able to grow at 40 dS m^{-1}. Baker et al., (1995) examined the effect of salinity up to 0.6 M NaCl on nitrogen fixation in *P. juliflora* germplasm from Honduras with rhizobia and with and without VAM mycorrhizae. These authors found that at 0.3 M NaCl (EC about 26 dS m^{-1}), *Prosopis* still had nodules but at 0.6 M salinity (about 52 dS m^{-1}), no nodules or N

fixation occurred but the seedlings were still alive. In the light of these results, these authors suggested that limiting factor in N fixation at these salinities was not the *Prosopis* but rather lack of sufficient salt tolerance in the rhizobia. In this regard, it is important to point out that the rhizobia strain found to effectively cross inoculate 13 *Prosopis* species (Felker and Clark, 1981) was later shown by Hua et al. (1982) to possess exceptional salt tolerance for a rhizobia in being able to be grown with facility at 0.5 M NaCl.

Cony and Trione (1998) found considerable intraspecific variation in the salt tolerance of seed germination in the Argentine *Prosopis* species *P. flexuosa* and *P. chilensis*. Even though seed germination may not possess resistance to salinity, it is possible that whole plants may be more resistant. Since seedlings produced in nurseries will be used to establish plantations, it will be important to continue these studies on established plants.

In an effort to examine morphological mechanisms for salt tolerance in *Prosopis*, Serrato-Valenti et al. (1996) conducted a histochemical study on the roots of *P. juliflora*, *P. tamarugo* and *P cineraria* as a function of salinity (from 0 to 0.6 M NaCl). As the salinity increased, the cortex thickened and the tissues became increasingly lignified, which they suggested was an important salinity-tolerance mechanism for *Prosopis*.

While the *Prosopis* in these trials has been shown to have much greater salt tolerance than annual legumes there are other tree species and nitrogen-fixing trees with even greater salt tolerance. In a genetic improvement trial, El-Lakany and Luard (1982) found that *Casuarina glauca*, *C. obesa* and *C. equisetifolia* all could grow at salinities of 0.55 M NaCl and Ng (1987) confirmed that *C. equisetifolia* fixed nitrogen at salinities of 0.5 M NaCl. Van der Moezel et al. (1988) who examined the salinity tolerance of various *Eucalyptus* and *Casuarina* species under combinations of saline, saline waterlogged and waterlogged conditions found that *Eucalyptus* also could actively grow at salinities of 42 dS m^{-1} and that *E. spathulata* had the greatest growth at the highest salinity levels. In a study that examined use of salt-tolerant tree species to reuse saline drainage irrigation waters, Grieve et al. (1999) found that the best eucalyptus clone suffered a 50% drop in biomass growth at a salinity of 30 dS m^{-1}. The most salt trees known to us are the Australian *Acacias* that continued to grow at salinities of 70 dS m^{-1} and that did not exhibit 50% population mortality at salinities of 95 dS m^{-1} which is close to double the salinity of ocean water (Craig et al., 1990).

Of all the tree species capable of growing at approximately seawater salinities, *Prosopis* probably has the greatest potential for economically sustainable plantations due to 35% sugar 9% protein pods that are palatable to humans and livestock and to its furniture quality lumber. In fact, *Prosopis* lumber is more dimensionally stable than such luxury timbers such as teak (*Tectona grandis*), mahogany (*Swietenia macrophylla*) and the rosewoods (*Dalbergia retusa* and *latifolia*) (Felker, 2000). If *Prosopis* is priced equivalent to the World Bank benchmark prices of Malaysian sawnwood of $740 m^{-3} (www.worldbank.org/html/ieccp), at a specific gravity of $750 m^{-3} the timber has a value of $990 ton^{-1}. Under dryland conditions, annual sawn lumber increments of 0.5 m^{-3} ha^{-1} $year^{-1}$ have been estimated for *Prosopis* trees (1.2 cm diameter increment for 100 ha^{-1}) that yielded an internal rate of return

of 9.3% (Felker, 2000). However, if abundant brackish water were available in the 20–30 dS m^{-1} range where elite clones grow rapidly, rates of return much greater than 9.3% should be achieved.

The tantalizing scenario of growing *Prosopis* in coastal deserts with seawater irrigation as originally suggested by Epstein et al. (1979) for other plants appears to be in the realm of possibility since (1) a few *P. alba* and *P. pallida* grew at seawater salinity, (2) since only very limited selections from the germplasm base have been examined and (3) since other field-management techniques (e.g. provision of divalent cations Ca and Mg, P and critical micronutrients) may alleviate the stress. Due to the pressing human needs in deserts where *P. pallida* is adapted, i.e. Mauritania, Somalia, Ethiopia, etc., it seems reasonable to extensively collect *P. pallida* with the objective of finding clones possessing good growth rates, and similar production of large numbers of highly palatable sweet pods as reported by Alban et al. (2001).

It is important to highlight the fact that many of the Peruvian *Prosopis* accessions in this trial have proven exceptionally useful in arid lands in Haiti, Cape Verde and India. In Haitian field trials after 46 months, individual trees from accessions 419 and 420 from the Procedencia of Poroto, Prov Trujillo, Depto Libertad were cloned due to exceptional erect form, lack of spines and good height growth (Wojtusik et al., 1993). It is unfortunate that seeds of these accessions were exhausted for this salinity trial. Harsh et al. (1996) found that accession 424 had the greatest height growth of all the Peruvian accession (3.73 m after 4 years) and the second greatest height growth of 200 families of *P. alba*, *P. chilensis*, *P. flexuosa* and *P. nigra* in a dryland progeny trial in Jodhpur, India. In non-irrigated *Prosopis* progeny trials with many species in Cape Verde, Prosopis accession 423 was found to have the greatest stem basal diameter among 35 *Prosopis* accessions at two sites (Harris et al., 1996). In this salinity trial, 423 was in the middle range for mean growth and maximum growth. As 423 and 424 were both from Algarrobal (Proc), Contumaza (Prov), Cajamarca (Depto) it would seem important to more intensively sample this population for combinations of growth, pod characters and salinity tolerance.

The 30 *P. alba* and the 20 *P. pallida* with the greatest individual height growth were transplanted to larger containers with commercial high organic matter potting soil to use for propagation material for cuttings. No *P. ruscifolia* were selected for further multiplication due to the long thorns (25 cm) and weedy habit of this species. Within several weeks of transplanting, many of the *P. pallida* seedlings began to loose leaves, the stem became shriveled as if due to lack of water and the seedlings died. Pathologists S. Hongn and S. Weht of the Universidad Nacional de Tucuman have identified *Fusarium rigidiusculum* from the dying plants. Treatments used to counter this *Fusarium* wilt in young *P. alba* cuttings, i.e. drenches and foliar sprays with methylthioallophanate and later increasing the salinity to 10,000 mg l^{-1} NaCl to counter the putative *Fusarium* have had limited success. Virtually all of the elite *P. pallida* seedlings suffered from these symptoms while none of the *P. alba* seedlings were affected, although they were all grown in the same area using the same potting soil.

Later the remaining 150 *P. pallida* (albeit with less rapid growth) that survived the high salinity were successfully transplanted to a 100% sand mixture in another

greenhouse. Both the elite *P.* alba and *P. pallida* individuals are in the process of being transplanted to the field to produce a saline-resistant seed orchard and to make a second round of selections based on form, growth rate and pod characters. The *P. alba* will be planted near Santiago del Estero (27°45′S; 64°15′W) while the *P. pallida* will be planted about 370 km north in a tropical zone since *P. pallida* suffers virtually complete mortality at temperatures of −5°C (Felker et al., 1982).

In conclusion, this limited survey of two of the world's most valuable *Prosopis* species, i.e. *P. pallida* from Peru that has applications in truly tropical semi-arid regions, and *P. alba* from Argentina with applications in subtropical regions, found individual plants that grew in seawater salinities and these individuals are in the process of being multiplied asexually and in seed orchards. Previous genetic improvement programs from these species employed high selection intensities (>95%) to identify trees that all contained good form, fast growth, high pod production and pods with a palatable flavor (Alban et al., 2001; Felker et al., 2001). Larger screening programs will be necessary to identify thousands of individuals with high salt tolerance to be included in a second round of high-intensity selection for all these desired characters. In the meantime, the individuals identified here will provide much needed improvement over non-selected seed sources for saline arid regions. In carefully selected sites, the high-value lumber production should provide a sufficiently high internal rate of return to justify reforestation solely based on the economic potential.

Acknowledgements

References

Ahmad, R., Ismail, S., Moinuddin, M., Shaheen, T., 1994. Screening of mesquite (*Prosopis* spp) for biomass production at barren sandy areas using highly saline water for irrigation. Pakistan Journal of Botany. 26, 265–282.

Alban, L., Matorel, M., Romero, J., Grados, N., Cruz, G., Felker, P., 2001. Cloning of elite, multipurpose trees of the *Prosopis juliflora/pallida* complex in Piura, Peru. Agroforestry Systems 54, 173–182.

Ayers, R.S., Westcott, D.W., 1985. Water quality for irrigation. FAO Irrigation and Drainage Paper #29. FAO, Rome.

Baker, A., Sprent, J.I., Wilson, J., 1995. Effects of sodium chloride and mycorrhizal infection on the growth and nitrogen fixation of *Prosopis juliflora*. Symbiosis, 19, 39–51.

Bohnert, H.J., Hua, S., Shen, B., 1999. Molecular mechanisms of salinity tolerance. In: Shinozaki, K., Yamaguchi-Shinozaki, K. (Eds.), Molecular Responses to Cold, Drought Heat and Drought Stress in Higher Plants. R.G. Landes Company, Austin, TX, pp. 29–60.

Cony, M.A., Trione, S.O., 1998. Inter and intraspecific variability in *Prosopis flexuosa* and *P. chilensis*: seed germination under salt and moisture stress. Journal of Arid Environments 40, 307–317.

Cline, G., Felker, P., Rhodes, D., 1986. Micronutrient, phosphorus and pH influences on growth and leaf tissue levels of *Prosopis alba* and *Prosopis glandulosa*. Forest Ecology and Management 16, 81–93.

Craig, G.F., Bell, D.T., Atkins, C.A., 1990. Response to salt and waterlogging stress of ten taxa of acacia selected from Naturally saline areas of Western Australia. Australian Journal of Botany 38, 619–630.

El-Lakany, M.H., Luard, E.J., 1982. Comparative salt tolerance of selected Casuarina species. Australian Forest Research 13, 11–20.

Epstein, E., Kingsbury, R.W., Norlyn, J.D., Rush, D.W., 1979. Production of food crops and other biomass by seawater culture. In: Hollaender, A. (Ed.), The Biosaline Concept. Plenum, NY, 391pp.

Felker, P., 2000. An investment based approach to *Prosopis* agroforestry in arid lands. Annals of Arid Zone 38, 383–395.

Felker, P., Clark, P.R., 1981. Nitrogen fixation (acetylene reduction) and cross inoculation in 12 prosopis (mesquite) species. Plant and Soil 57, 177–186.

Felker, P., Clark, P.R., Laag, A.E., Pratt, P.F., 1981. Salinity tolerance of the tree legumes mesquite (*Prosopis glandulosa*, var *torreyana*, *P. velutina* and *P. articulata*) algarrobo (*P. chilensis*) kiawe (*P pallida*) and tamarugo (*P tamarugo*) grown in sand culture on nitrogen free media. Plant and Soil 61, 311–317.

Felker, P., Clark, P.R., Nash, P., Osborn, J.F., Cannell, G.H., 1982. Screening *Prosopis* (Mesquite) for cold tolerance. Forest Science 28, 556–562.

Felker, P., Lopez, C., Soulier, C., Ochoa, J., Abdala, R., Ewens, M., 2001. Genetic evaluation of *Prosopis alba* (algarrobo) in Argentina for cloning elite trees. Agroforestry Systems 53, 65–76.

Flowers, T.J., Hajibageri, M.A., Clipson, N.J.W., 1986. Halophytes. Quarterly Review of Biology 61, 313–337.

Grieve, C.M., Guzy, M.R., Poss, J.A., Shannon, M.C., 1999. Screening eucalyptus clones for salt tolerance. Hortscience 34, 867–870.

Harris, P.J.C., Pasiecznik, N.M., Vera-Cruz, M.T., Bradbury, M., 1996. *Prosopis* genetic improvement trials in Cape Verde. In: Felker, P., Moss, J. (Eds), ''*Prosopis*: Semi-arid Fuelwood and Forage tree. Building Consensus for the Disenfranchised'' at the US National Academy of Sciences, March 1996. Center Semi-Arid Forest Resources Publ, Kingsville, TX. www.tamuk.edu/webuser/symposium.

Harsh, L.N., Tewari, J.C., Sharma, N.K., Felker, P., 1996. Performance of *Prosopis* species in arid regions of India. In: Felker, P., Moss, J. (Eds), ''*Prosopis*: semi-arid fuelwood and forage tree. Building Consensus for the Disenfranchised'' at the US National Academy of Sciences, March 1996. Center Semi-Arid Forest Resources Publ. Kingsville, TX. www.tamuk.edu/webuser/symposium.

Hasegawa, P.M., Bremen, R., Zhu, J.K., Bohnert, H.J., 2000. Plant cellular and molecular responses to high salinity. Annual Reviews Plant Physiology 51, 463–499.

Hua, S.T., Tsai, V.Y., Lichens, G.M., Noma, A.T., 1982. Accumulation of amino acids in *Rhizobium* sp. strain WR1001 in response to sodium chloride salinity. Applied and Environmental Microbiology 44, 135–140.

Morello, J.H., Crudelli, N.E., Saraceno, M., 1971. Los vinalares de Formosa (Republica Argentina) Instituto Nacional de Tecnologia Agropecuaria (INTA). Series Fitogeografica 11, 113.

Ng, B.H., 1987. The effects of salinity on growth, nodulation and nitrogen fixation of *Casuarina equisetifolia*. Plant and Soil 103, 123–125.

Rhodes, D., Felker, P., 1988. Mass screening *Prosopis* (mesquite) seedlings for growth at seawater salinity. Forest Ecology and Management 24, 169–176.

Richards, L.A., 1954. Saline and sodic soils. USDA Handbook 54, Riverside, California.

Serrato-Valenti, G., Cornara, L., Corallo, A., Riveros, F., 1996. Structural and histochemical changes in hypocotyl cortex of *Prosopis* species caused by salinity. Phyton 59, 13–24.

Smart, R.M., Barko, J.W., 1980. Nitrogen nutrition and salinity tolerance of *Distichlis Spicata* and *Spartina alterniflora*. Ecology 61, 630–638.

Somers, F.G., 1979. Natural halophytes as a potential resource for new salt-tolerant crops: some progress and prospects. In: Hollaender, A. (Ed.), The Biosaline Concept. Plenum, New York, NY, 391pp.

Sun, D., Dickinson, G., 1993. Responses of salt stress of 16 *Eucalyptus* species, *Grevillea robusta*, *Lophostemon confertus* and *Pinus caribea var.*. hondurensis. Forest Ecology and Management 60, 1–14.

Van der Moezel, P.G., Watson, L.E., Pearce-Pinto, V.N., Bell, .D.T., 1988. The response of six *Eucalyptus* species and *Casuarina obesa* to the combined effect of salinity and waterlogging. Australian Journal of Plant Physiology 15, 465–474.

Winicov, I., 1998. New approaches to improving salt tolerance in crop plants. Annals of Botany 82, 703–710.

Wojtusik, T., Felker, P., Russell, E.J., Benge, M.D., 1993. Cloning of erect, thornless, non-browsed nitrogen fixing trees of Haiti's principal fuelwood species (*Prosopis juliflora*). Agroforestry Systems 21, 293–300.