Article

Five Year Field Evaluation of *Prosopis alba* Clones on pH 9–10 Soils in Argentina Selected for Growth in the Greenhouse at Seawater Salinities (45 dS m^{-1})

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Abstract: *Prosopis alba* seedlings, that grew at the 45 dS m^{-1} salinity level in a previous study of growth and survival of Argentine and Peruvian *Prosopis*, were propagated by rooting cuttings and established in a seed orchard/long term evaluation trial on soils with low salinity (EC 5.1–7.5 dS m^{-1}) but high pH (8.9 to 10.2). A pH gradient occurred in the field with values ranging from pH 9.4 in block 1 to pH 10.3 in block 5. After five years growth, almost all of the clones had a mean height greater than 4 m and one clone was more than 5 m. Ten of the 21 tested clones had significantly greater biomass growth than the three seed propagated check varieties. The broad-sense (*i.e.*, clone mean) heritability was estimated to be 0.45 for biomass, 0.53 for diameter and 0.59 for height indicating that strong genetic gains should be possible by selecting and vegetatively propagating the best genotypes. In the block with the highest pH values, two clones that appear to be *P. alba* × *P. ruscifolia* hybrids (*i.e.*, *P. vinallilo*) had the greatest biomass. Correlations between growth during the last two months in the high salinity hydroponic greenhouse selection system and growth in the field were significant ($R^2 = 0.262$) and positive, although the relationship was negative for putative *P. vinallilo* clones ($R^2 = 0.938$). The several fold increase in biomass of some of the clones over the three check varieties, suggests that the greenhouse screen was successful in identifying superior salt tolerant clones. Apparently

whether the greenhouse seedlings had lesser (~1 cm) to greater (~3 cm) height growth was not as important as just having a healthy live apical meristem. The observed salt tolerance of the putative *P. vinalillo* clones may prove useful as rootstocks for recently described high pod producing *P. alba* clones.

Keywords: agroforestry; arid; hardwood lumber; nitrogen fixation; sodic; salt tolerance

1. Introduction

According to FAO, approximately 400 million ha are affected by sodic or saline soils [1]. Among the recommendations for applied research by an FAO expert committee were development of salt-tolerant crop varieties and the use of *Salicornia, Atriplex, Salvadora* and *Prosopis* as a good alternative in salt-affected areas [2]. Nitrogen fixing trees of the genus *Prosopis* show promise for the rehabilitation of saline soils in subtropical regions because of their high salt tolerance and because they have economically useful products that can provide the economic incentive to drive the restoration of saline lands. The value of *Prosopis* is illustrated by the fact that in Argentina more than 100,000 tons of *Prosopis* logs were harvested annually and processed for furniture, flooring, door, window and shutter fabrication prior to about 2003 [3]. In 2004, the Argentinean Secretariat of the Environment and Sustainable Development, which includes the Forestry Department, issued a report which decried the deforestation and degradation of Argentine native forests, and in particular forests in the Chaco ecological zone where *Prosopis alba* (Griseb.) is located [4]. Several of the largest *P. alba* lumber, flooring, and furniture companies have ceased operations due to the severity of this degradation [5]. Flour from the milled pods is also being developed for human food uses [6]. In the USA there are numerous markets that offer mesquite (*Prosopis glandulosa* Torr. or *P. velutina* Woot.) lumber at prices greater than US$ 2,000 m^{-3}. Felker and Guevara [3] calculated that a sawn lumber price of US$ 800 m^{-3} will be required to achieve an internal rate of return of 11% for well managed plantations of genetically unimproved stock. Further, plantations on moderately saline soils (<20 dS m^{-1}) with high water tables would potentially provide rates of internal return in the range of 20% [3]. Clonal selections of *P. alba* for economically useful characters such as rapid growth rate, high pod production and pods with improved flavor (sweeter pods without bitter characteristics) have been made [7].

Various studies have reported high salinity tolerance for different *Prosopis* species [8–12]. A recent study compared the survival and growth of *Prosopis pallida* (Humb. & Bonpl. ex Willd.) Kunth from Peru and *P. alba* from Argentina in a greenhouse setting in which the salinity was increased from 10 to 45 dS m^{-1} over a period of four months and maintained at 45 dS m^{-1} for an additional two months [13].

From the more than 2,000 seedlings from 25 half-sib families in this hydroponic selection system that evaluated a broad based collection of African, Peruvian, Chilean and Argentinean *Prosopis* [13], 21 *P. alba* seedlings, from nine Argentine *P. alba* half-sib families that grew in the highest salinity treatment (45 dS m^{-1}) were repotted and clonally propagated to establish a clonal seed orchard on the grounds of the Universidad Catolica de Santiago del Estero field station in Fernandez, Argentina. None of the Peruvian accessions in this trial with high salt tolerances were evaluated due to their lack of cold hardiness in Argentina. The soil was slightly saline (EC 5.15 to 7.3 dS m^{-1}) but it had pH values ranging

from 8.9 to 10.2. These pH values are far more alkaline than a pH value of 8.0 where P, Mn, B, Cu and Zn availability begins to severely decline [14].

To capitalize on the high salt tolerance of these seedlings, it will be necessary to multiply them asexually. While rapid and reliable propagation by mini-grafting is possible [15], this will not be useful since the rootstocks would come from genetic stock with unknown salt tolerance.

Interspecific hybridization frequently occurs among *Prosopis* species [16]. In spite of the fact that specimens were selected of *P. alba* mother trees in accordance with Burkart [17], great segregation in morphological characters was observed in the progeny. As described in other studies [18], some clones have finely divided leaflets typical of the *P. alba* mother tree while other clones have much larger and more widely spaced leaflets typical of *P. vinallilo* (little vinal). Some taxonomists have suggested *P. vinallilo* could be a hybrid between *P. alba* and *P. ruscifolia* [17]. The weedy *P. ruscifolia,* with its 15 cm long thorns, is more common in the area where the *P. alba* was collected.

The main objective of this study was to determine if the greenhouse hydroponic screening assay was a useful genotype selection tool. Thus regressions between height growth in the greenhouse *versus* biomass estimates for five year old trees in the field were examined. Additionally it was important to examine these clones in a multi-year field trial for other positive and negative characteristics that could not have arisen in the highly controlled greenhouse environment. An unexpected result that required analyses/interpretation was the presence of interspecific hybrid progeny possessing superior tolerance to soils with pH 10.3.

2. Methodology

The previous hydroponic system evaluated 25 accessions of *Prosopis* from Peru (*P. pallida*), West Africa (*P. juliflora*), Chile (*P. alba/nigra*) and Argentina (*P. alba*, *P. flexuosa*, *P. ruscifolia)* in a randomized complete block design (RCBD) with four replicates. The Peruvian *P. pallida* species are not adaptable to Argentina due to lack of cold hardiness and *P. alba* is more amenable to lumber production than the *P. flexuosa* and *P. ruscifolia* evaluated in the hydroponic system. Thus for the clonal seed orchard described in this communication, we only selected clones from nine open pollinated half-sibling families of *P. alba* designated O1 to O9 that originated from a highly saline area (floodplain of the Rio Saladillo in Province of Santiago del Estero, Argentina 28°52'36.85"S: 63°58'46.36"W). An example of one of these *P. alba* trees near a home is presented in Figure 1. These mother trees fell within Burkart's [16] delineation of *P. alba*. A flow diagram of the genetic selection process is illustrated in Figure 2.

Each replicate in the hydroponic screening trial consisted of a row of 16 seedlings. The codes for the clones are as previously described [12], e.g., O7B4P6(V) is half-sib family O7, block 4 and plant 6 of 16, and V to indicate a morphology similar to *P. vinalillo*. Due to lack of growth, no plants were selected from family O1 or O5 and only one plant was selected from family O2, O4, and O9. After the seedlings in the previous trial were kept for about four weeks at each of the 10, 25, 35 and 45 dS m^{-1} salinities [13], they were subjected to two additional months in the same 45 dS m^{-1} experimental condition. Those plants showing a very healthy appearance and/or a new live apical meristem were chosen for further experimentation. Overall plant growth was limited under this salinity level due to the small size of the root cell volume (50 cm tall plants in five cm deep cavity) that made them root

bound. Only 21 of the 576 (3.6%) best plants from the *P. alba* replicated portion of this trial were selected for further evaluation and asexually propagated by rooting cuttings using bottom heat, water soluble salts of plant hormones and *Agrobacterium rhizogenes* that can insert the indoleacetic acid synthesizing gene into plant roots and stimulate rooting [19]. The three check varieties in this field trial were seedlings originated form "plus trees", located near the Forestry Experiment Station of the Universidad Catolica de Santiago del Estero, in Fernandez, Argentina, that were used for seed for the one million seedlings produced per year for sale to establish plantations.

Figure 1. One of the *P. alba* mother trees (O7) collected near a home in the highly saline floodplain of the Rio Saladillo, Province of Santiago del Estero, Argentina whose seeds were used in the hydroponic salinity selection system in the greenhouse.

Figure 2. Flow diagram from the original collection of seed from trees in the saline areas of the Rio Saladillo, Argentina, to establishment of 21 clones in five replicates on high pH soils in Fernandez, Argentina.

Seeds were collected from **9** *Prosopis alba* mother trees several km apart in the Rio Saladillo flood plain.

↓

Sixteen seedings per replicate and 4 replicates of each of 9 mother trees were established in randomized complete block design in hydroponic salinity system that was flooded 2 times a day with saline nutrient solution and in which the last 2 months they were irrigated with 45 dS m^{-1} salinity.

↓

Twenty one of the trees (3.6%)(0 from family O1&O5, 1 from family O2, O4 & O9, 2 from family O8, 4 from families O3 & O6, and 8 from family O7) that survived with no apical tip mortality and/or that had 1-3 cm growth were repotted in non saline soil.

↓

When the 21 seedlings in the greenhouse reached 1.2 m height, cuttings were taken and rooted under mist producing 5 rooted cuttings for the 21 clones.

↓

The rooted cuttings were planted in the field in 5 blocks with a single rooted cutting per replicate.

Some of the progeny had intermediate leaf and thorn morphology between the *P. alba* female parent and a putative unknown *P. ruscifolia* male parent that is much more abundant in this region than *P. alba* and that only grows next to homes where this highly palatable species can be protected from herbivores. The seedlings with intermediate leaf morphology were presumed to be *P. vinalillo* and were designated by a V after the codes. The great differences in leaf/thorn morphologies of these three "species" can be seen in Figure 3.

Figure 3. Leaf, thorn and inflorescence morphology of thornless *P. alba* from seed (left), thornless putative *P. vinalillo* (O7B4P6V) with larger and more widely spaced leaflets (center) and putative *P. ruscifolia* (vinal) (right). Note that there are no thorns on these *P. alba* or putative *P. vinallilo* but long thorns on the putative *P. ruscifolia*. The ruler is in cm.

Due to the small size of these plants, only enough rooted cuttings were available to establish five single tree replications in a RCBD at the Universidad Catolica de Santiago del Estero Forestry Experiment Station in Fernandez, Argentina. This planting served as a clonal seed orchard, clonal evaluation trial and source of material for full sib crosses. This trial was established on 13 January 2004 (summer rainy season in Argentina) and five years after the 2–3 mm diameter rooted cuttings were planted. On 25 August 2009, measurements of height and diameter were made. Biomass estimates were obtained using a previously published model [7]: log(fresh wt) = −1.1085 + 2.7027 × log(diameter), where fresh weight and diameter are in kg and cm, respectively. After planting the trees were hand watered. The plants were flood irrigated every winter with 100 mm of water. The rainfall measured at the Research station in Fernandez was 709 mm in 2004, 402 mm in 2005, 750 mm in 2006, 674 mm in 2007, 678 mm in 2008 and 602 mm in 2009. Each plant was protected from rabbit damage by placing spiny branches around the plants. For the first three years, the insecticides cypermethrin and Lambda-cyhalothrin were used three times in the summer at rates on the label to control psyllids that are the leaf sucking insects. Weeds were controlled by mowing, hoeing, and spraying 1.5% glyphosate at the base of the trees. Samples for soil pH and salinity were taken at the 0–30 and 60–90 cm depths in each of the five blocks in August 2009. Soil samples were taken from

various locations in the block and pooled for a single sample per depth per block. Pruning of the trees began in 2005.

The Instituto Nacional de Tecnologia Agricultura (INTA) (Argentine federal government agricultural service) in Santiago del Estero, Argentina measured the electrical conductivity on a suspension of 10 g of dry soil and 25 mL of water and the pH was measured on a saturated paste using the method of Richards [20].

In plant breeding, probably the two most important breeding factors, besides the genetic control (*i.e.*, that a trait is heritable) are: (a) a high yield of the economic trait of interest (wood, grain, *etc.*) and (b) uniformity to facilitate harvest and to maintain product quality standards in the market. It is possible to have two families or clones with the same mean economic yield but that greatly differ in uniformity. For example clone A could have moderate photosynthetic assimilation over all blocks but not be susceptible to low yields in some blocks due to an unfavorable edaphic factor, *i.e.*, low P, pH, water logging, *etc.*, while clone B could have higher photosynthetic assimilation than clone A on favorable blocks but lower yields on blocks with unfavorable edaphic factors. Uniformity is typically measured by the variance or standard error of the mean value (*i.e.*, SEM) for a given genotype, or equivalently by obtaining confidence intervals (CIs). Here, it is desirable to have the smallest SEMs values or range of CIs.

Thus, in this study, as a first stage, a linear mixed model was fitted for the responses height, diameter and biomass, based on the following linear model:

$$y_{ij} = \mu + Block_i + Clone_j + e_{ij}$$

where μ corresponds to the overall mean, $Block_i$ to the fixed effect of block, $Clone_j$ to the fixed effect of clone, and e_{ij} is the residual term for the observation on the *i*th block *j*th clone, with $e_{ij} \sim N(0,\sigma_j^2)$. In addition, a different residual variance was estimated for each of the clones. A likelihood ratio test [21] was used to test for this homogeneity of variances. Means and SEMs were predicted for each of the genotypes and 95% confidence intervals were obtained based on the means of the five blocks.

In order to estimate genetic variance components, the above model was re-fitted assuming the Clone term as a random effect, with $Clone_j \sim N(0, \sigma_{clone}^2)$. The expression to estimate broad-sense (clonal mean) heritability, H^2, corresponded to:

$$H^2 = \frac{\sigma_{clone}^2}{\sigma_{clone}^2 + \sigma^2}$$

where σ_{clone}^2 is the variance component associated with the clones and σ^2 is the mean residual variance obtained over all clones, and an approximate standard error was obtained using the delta method. All models were fitted using the software ASReml v. 3.0 [21].

3. Results

The soil pH and salinity of this trial are shown in Table 1 where it can be seen that the salinity was far less than the 45 dS m^{-1} level that was used to select the initial seedlings. In contrast, the pH values were extreme, ranging from 8.9 in block 1 to 10.2 in block 5. The pH values for the 60–90 cm depth ranged from 0.9 to 0.2 pH units lower than the surface 0–30 cm. Due to the gradient in soil pH, the

mean height, diameter and biomass of all blocks in addition to the values for block 5 which had the most extreme pH values is presented.

Table 1. Soil electrical conductivity and pH values for each block and their average in the field trial of *P. alba* clones selected for growth at high salinities.

Depth (cm)	Block 1	Block 2	Block 3	Block 4	Block 5
Conductivity dS m^{-1}					
0–30	6.90	7.45	7.10	7.30	5.15
60–90	5.75	6.45	11.35	5.85	10.10
Average	6.33	6.95	9.23	6.58	7.63
pH					
0–30	8.90	9.10	9.70	10.11	10.20
60–90	9.80	9.90	10.20	10.30	10.40
Average	9.35	9.50	9.95	10.21	10.30

Figure 4 presents the mean diameter, biomass and height growth values for all five blocks per genotype. Since biomass is exponentially related to diameter, greater differences among the clones are seen in biomass than diameter or height. In relation to the overall mean values (Figure 4), almost all of the clones had a mean height greater than 4 m, with the exception of one clone that was more than 5 m tall, which is exceptional at this high pH. One important observation is that the variability in biomass resulting from progeny of the same mother tree (within O3, O7, *etc.*) is apparently large. As observed by the individual 95% confidence intervals (as discussed below), there were 10 clones *i.e.*, O2B4P1, O3B1P13, O3B4P7(V), O6B1P13, O7B3P3, O7B4P4(V), 07B4P6(V), O8B3P14, O8B3P15 and O9B4P14 that had significantly greater biomass than the three check varieties. At the end of five years, the most rapidly growing clone O7B4P6(V) had a biomass of 106 kg per tree, which was more than four times the mean of the three checks, *i.e.*, 24.4 kg.

In comparing the mean values for the overall diameter, height and biomass (Figure 4) to the individual values for block 5 (Figure 5), where the most extreme pH values occurred, the comparative performance of two putative *P. vinallilo* (*i.e.*, O3B4P17(V) and O7B4P6(V)), and the *P. alba* clones O6B1P11, O8B3P14 and O9B4P14 over the three *P. alba* check half-sib families was especially notable.

Figure 4. Means of five blocks for height (m), basal diameter (cm), and biomass (kg) of 21 clones from *P. alba* parents selected for growth in a greenhouse hydroponic system at 45 dS m^{-1} electrical conductivities. Note the clones are arranged by families O2, O3, *etc.* Biomass values were estimated with regression equations as described in the text. The error bars are 95% confidence intervals.
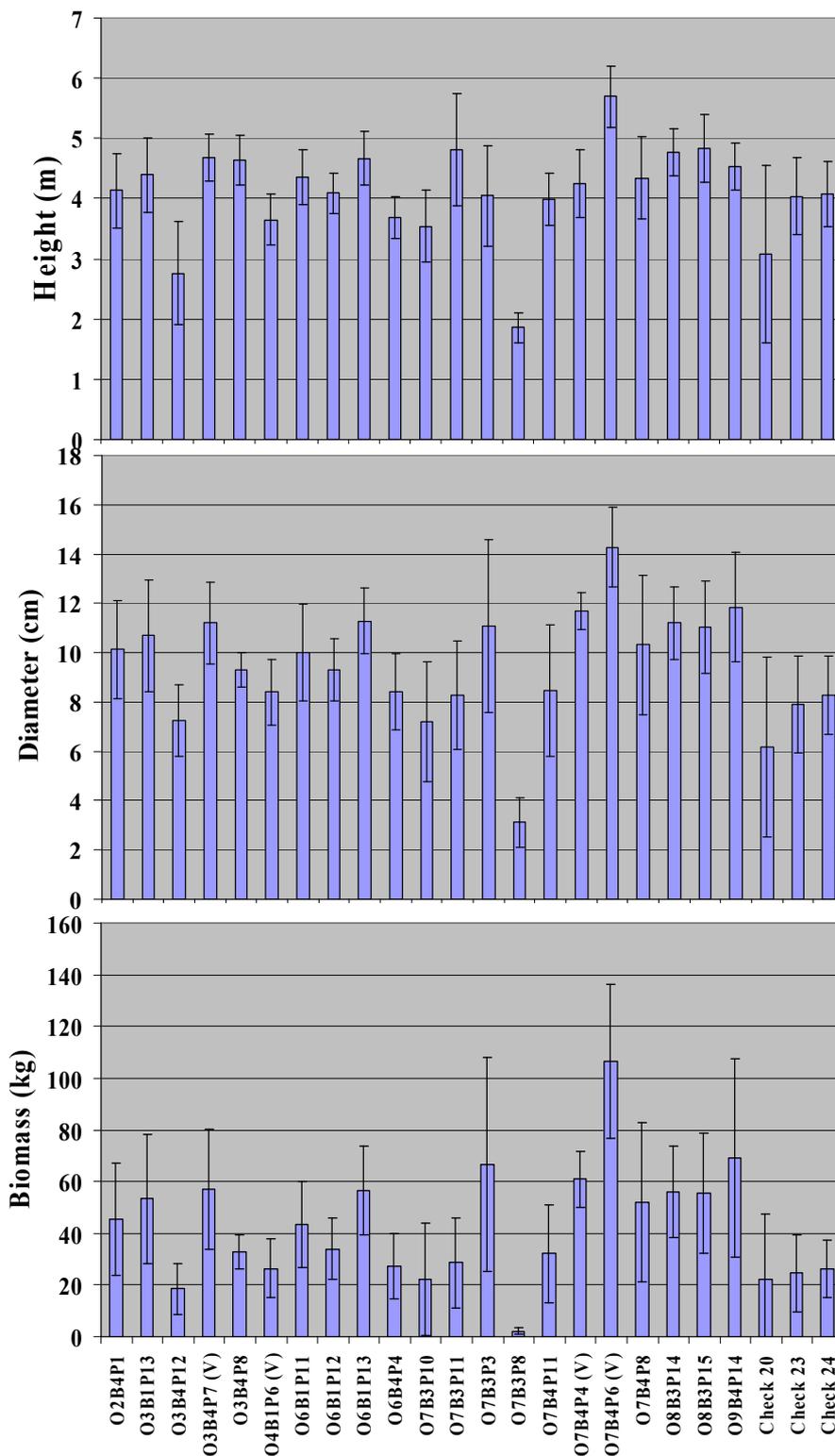
Figure 5. Individual values for block 5, which had most extreme pH value (*i.e.*, average 10.3), for height (m), basal diameter (cm), and biomass (kg) of 21 clones from *P. alba* parents selected for growth in a greenhouse hydroponic system at 45 dS m^{-1} electrical conductivities. Biomass values were estimated with regression equations as described in the text.
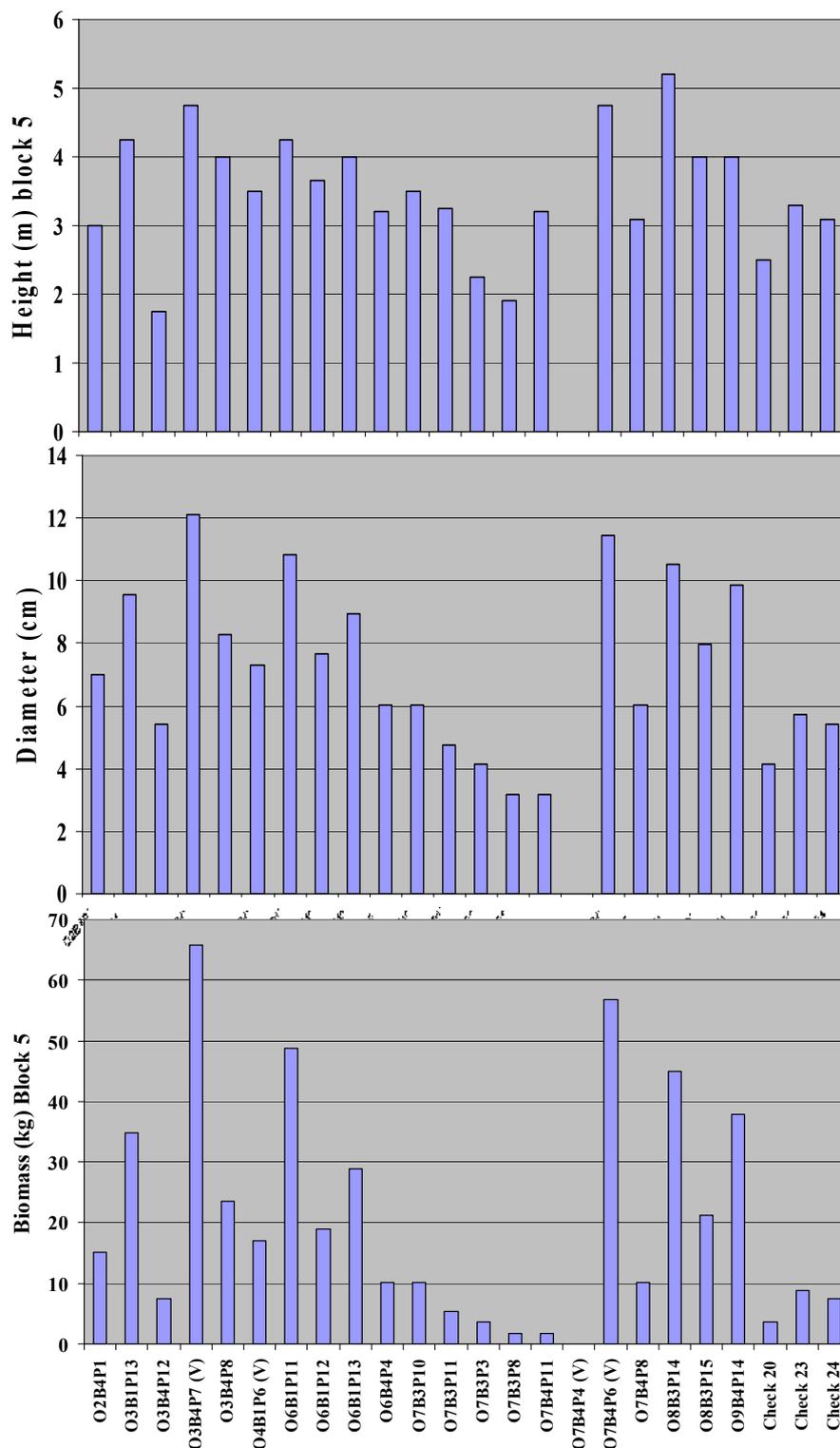
Table 2 shows the *p*-values of evaluating the significance of clonal differences together with the observed broad-sense heritability. For all traits analyzed, there are significant differences among the clones, and the amount of genetic control of these traits, as indicated by the heritability is moderate, where the largest values correspond to total height. Therefore, relevant genetic gains can be obtained by selecting and vegetatively propagating the best genotypes.

Table 2. P-values of evaluating the significance of fixed clonal effects and broad-sense heritability for biomass, diameter and height for 21 clones from *P. alba* mother trees tested on an alkaline site in Argentina. These were obtained from two different fitted linear models. Standard errors are presented in parenthesis.

	Biomass	**Diameter**	**Height**
Clone	0.030	<0.001	0.009
H^2	0.45	0.53	0.59
	(0.10)	(0.01)	(0.09)

In order to study further the uniformity, and therefore variability, of the different clones, a simple linear regression was obtained between the estimated residual variance, as a response, and the predicted mean, as an explanatory variable. This was done for each of the traits using all 21 clones.

No significant relationships, which were tested by fitting a simple linear regression, were obtained for total height and diameter, with correlations of −0.089 and −0.051, respectively. However, a significant association ($p = 0.003$), was detected for biomass with a correlation of 0.587. This result, when all clones are pooled, indicates that as the mean biomass of a given clone gets larger an increase on the variability is observed. Similar results have been reported in other biological situations [22]. The model fitted in this case was (SEM clonal Biomass) = 3.42 + 0.1272 × (mean clonal Biomass).

With regard to evaluating clones for uniformity of response on sites with contrasting edaphic factors, it was hypothesized that if a clone existed that did not decrease in biomass on block 5 with the greatest edaphic stress, then it would be expected to have a lower coefficient of variation. To test this, we have plotted the 95% confidence intervals (for the five blocks for each clone), divided by the mean of the five blocks, against the mean biomass of block 5, as shown in Figure 6 below.

Figure 6 shows that clones, which had the greatest biomass in the block with the most adverse edaphic conditions, also had the smallest confidence interval equivalent of the coefficient of variation. This significant relationship ($p < 0.002$) would imply that there is a genetic basis for the lower coefficient of variation and provides justification for using the 95% confidence intervals for each clone rather than pooled variances across all clones. The clone with the highest mean biomass of more than 100 kg in Figure 4, had a lower SE than other clones with values around 60 kg because this clone was not depressed as much in yield in block 5 as the others. Figure 6 suggests that several clones, such as O7B4P6(V), will not only have greater biomass than other clones but also greater uniformity.

Figure 6. Growth of *P. alba* and putative *P. vinallilo* clones in block 5 with highest soil pH of 10.3 *versus* 95% confidence intervals/mean biomass of all five blocks.
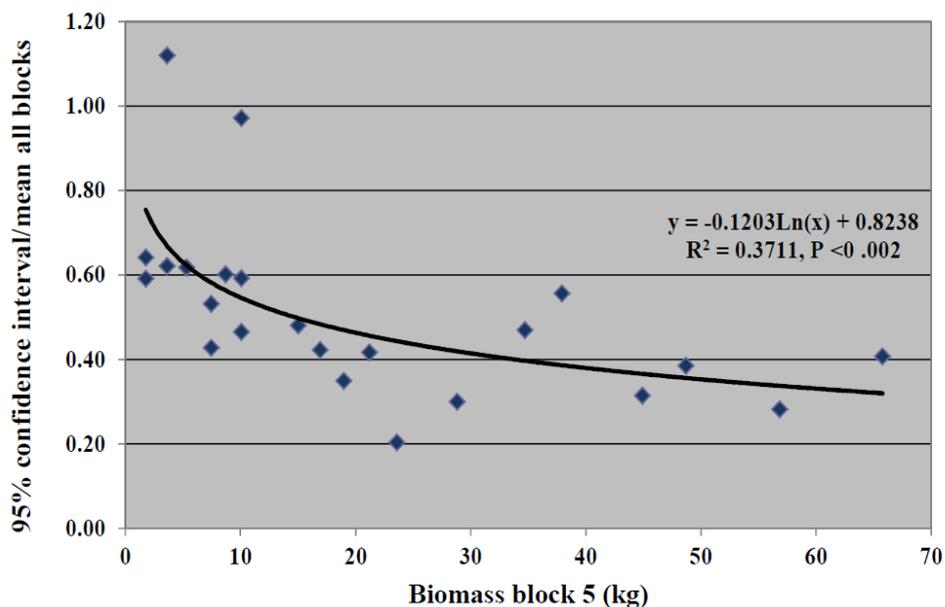
Regardless of the interpretation whether or not the variances can be pooled, the next step, as described below, would seem to be to make crosses between the high biomass producing putative *P. vinallilo* clone (O7B4P6V) and *P. alba* clones to examine segregation ratios and recombination frequencies.

Simple linear regressions were examined between the growth in greenhouse salinity selection system to five year old growth in the field. For *P. alba* trees over all five blocks this correlation had an R^2 of 0.108 and for *P. vinallilo* the correlation was 0.445. This information is presented graphically in Figure 7 for block 5 that had the most severe soil pH. For *P. alba*, there was a weak positive correlation ($R^2 = 0.262$) that for 17 values would be significant at a level of 5%. In contrast, for putative *P. vinallilo*, there was a negative correlation with an R^2 of 0.938 that is significant. However, with only four values for the putative *P. vinallilo* the negative correlation between growth at high salinity in the greenhouse, and high pH/moderate salinity in the field is not very clarifying. There is a possibility that selection at high salinity may be useful for selection at high pH values. At least the four *P. alba* clones with the greatest growth at pH 10 were also the ones with the greatest growth at the high salinity values, *i.e.*, selection of *P. alba* clones with >3 cm growth in the sand culture identified the *P. alba* with the greatest growth in the field. What is not presented here are the growth of the other 97% of the trees that had severe dieback, dead apical meristems, or were entirely dead and thus not evaluated in this field trial [13]. Given the several fold increase in biomass of some of the clones over the three check varieties for the means of all five blocks, and the even larger increase in biomass over the checks in block 5, it is clear that the greenhouse screen was successful in identifying superior salt tolerant clones. Apparently whether the greenhouse seedlings had, 1, 2 or 3 cm growth was not as important as just having a healthy live apical meristem.

Figure 7. Regressions between *Prosopis* biomass at the end of five years growth in field on high pH soils for block 5 *versus* growth and the height growth (in cm) for the last two months in a greenhouse hydroponic system at 45 dS m^{-1}.
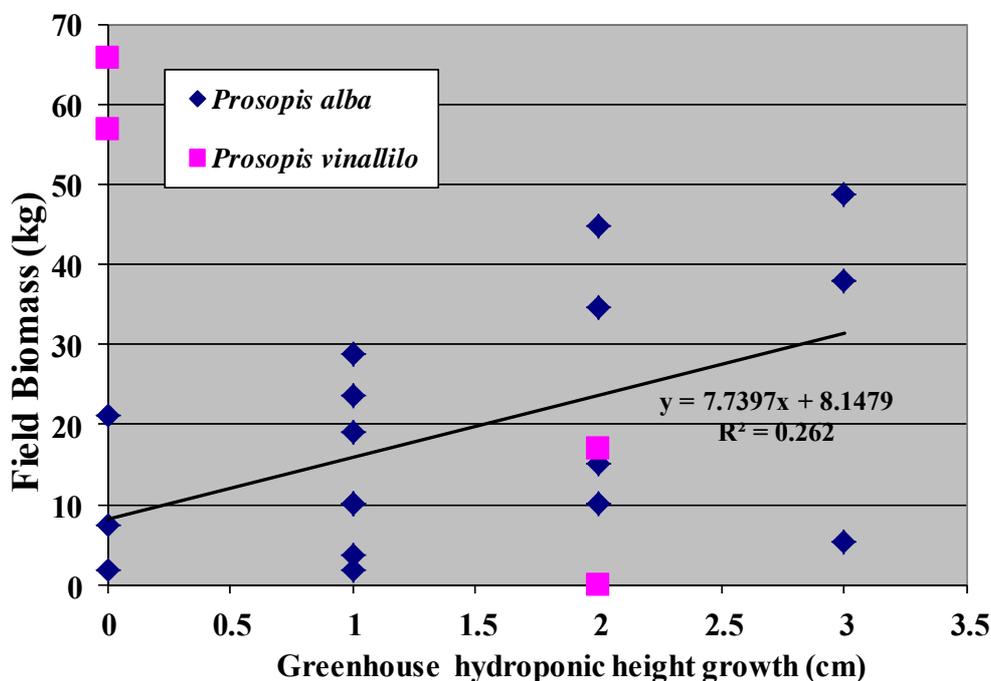
As described earlier there was great variability among the clones for ability to produce roots from cuttings [18]. Fortunately, some of the highest biomass producing clones also had high rooting percentages. For example, in block 5 the biomass and rooting percentage for the test clones were: O3B1P13 (35 kg/100%), O3B4P7(V) (66 kg/40%), O6B1P11 (49 kg/60%), O6B1P13 (29 kg/100%), O7B4P6(V) (57 kg/100%), O8B3P14 (45 kg/100%) and O9B4P14 (38 kg/60%).

Five of the clones (O3B4P7(V), O6B1P11, 07B4P6(V), O8B3P14 and O9B4P14) in the high pH block 5 had diameters of approximately 10 cm or greater. The largest diameters were obtained by the putative *P. vinallilo* clones *i.e.*, 11 cm for O7B4P6(V) and 12 cm for O3B4P7(V). This corresponds to an annual increment diameter growth of about 2 cm, for these trees that were planted five years earlier as several mm diameter rooted cuttings. Felker and Guevara [2] examined the rates of internal returns for *Prosopis* plantations as a function of level of genetic improvement and management in which they assumed that trees would be harvested when they attained 40 cm in diameter at 10×10 m spacing. The diameter growth of 2 cm year^{-1} reported in this paper corresponded to a rotation age of 20 years. For the Argentina scenario, assuming a wholesale price of US$ 800 m^{-3} for the sawn lumber, Felker and Guevara [3] calculated an internal rate of return of 11.8% for a 24 year rotation and 19.8% for a 17 year rotation. Thus, the diameter growth reported in this paper would correspond to an internal rate of return of about 15%.

4. Discussion

4.1. Worldwide Distribution of High pH Tolerance in Prosopis

While there are a considerable number of reports of *Prosopis* growing in highly saline conditions, there are much fewer reports of *Prosopis* growing in high pH environments. Cline *et al.* [23] measured the biomass of *P. alba* and *P. glandulosa* growing in artificial soil mixes from pH values of 6.0 to 8.9 with and without phosphorus and micronutrient additions. The study observed that *P. alba* growth was very strongly inhibited at pH 8.9 and that P and micronutrient amendments (in particular Zn) were critical to achieving some growth. Velarde *et al.* [24], who were working with *P. alba* on pH 8.5 soils in Argentina, found that applications of 200 g elemental S tree^{-1} decreased the soil pH near the tree about 0.3 pH units, and that the treatment containing S, P and micronutrients had a 42% biomass increase over the control. Mishra and Sharma [25] reported 10 years growth on high pH soils of *Eucalyptus tereticornis*, *Dalbergia sissoo* and *Prosopis juliflora*, and found that *P. juliflora* proved more effective than *E. tereticornis* and *D. sissoo* in its ability to enrich a sodic soil with organic matter and establishing better soil–water characteristics. Singh's group [26–29] at the Central Soil Salinity Research Institute (CSSRI) in Karnal, India has extensively studied recuperation of saline/sodic soils with *Prosopis* species. On soils with pH 10.3 that were similar to block 5 in this study, for *P. juliflora*, they reported 63% survival without amendments of gypsum or manure, but 100% survival with these amendments. Singh [30] found that *P. juliflora* grew well without amendments up to pH 9.0, but for greater pH's amendments with gypsum, barnyard manure and/or Zn were important for maintaining good growth. In contrast to these trials, some of the clones in block 5 grew very well at pH 10 without any additional amendments. In Indian field trials on soils with pH > 9, *P. alba* has been much less tolerant of high pH than *P. juliflora* [30].

4.2. Genetic Aspects of High pH Tolerance in Prosopis

The high broad-sense heritabilities for height, biomass and diameter growth implies these increases are tightly genetically controlled and suggests that further selection and possible hybridization with these clones is warranted.

The two clones with the greatest biomass accumulation under the most alkaline conditions were of the putative *P. vinalillo* species, indicating a possible *P. ruscifolia* parent. In a previous paper [18], based on the growth at high salinity levels, it was suggested that none of these putative *P. vinalillo* progeny had significant growth advantages over *P. alba* and perhaps the greater ecological adaptability of the *P. ruscifolia* (parent in the *P. vinallilo*) over *P. alba* was due to the much greater palatability to goats of *P. alba* than *P. ruscifolia*.

It would appear that some of the clones with high biomass accumulation could have high pH tolerance genes from a *P. ruscifolia* parent. As noted in Figure 3, putative *P. ruscifolia* had 15 cm long thorns but there were no thorns in some putative *P. vinallilo*. This would suggest that genetic recombination has occurred between the *P. ruscifolia* and *P. alba* for at least thorn and leaf characteristics. Given the fact that these characters segregated in one generation, there would appear to be a low genetic linkage between the long thorns and high pH tolerance. This suite of genes seems especially promising for use in the existing clones, for crossing into other *Prosopis* species, and for

cloning for insertion in other plant families. Indeed George and Parida [31] have recently expressed a salt tolerant gene from *P. juliflora* in tobacco and demonstrated superior salt tolerance of the resulting transgenic tobacco.

While the wood quality of the putative *P. vinallilo* hybrids is not known, it is reasonable to assume that the properties would be intermediate between the putative *P. alba* and *P. ruscifolia* parents that have been recently reported [32]. As mentioned earlier, *P. alba* is a truly exceptionally stable wood with a volumetric shrinkage of only 4.8% and a tangential/radial shrinkage ratio of 1.82. While *P. ruscifolia* has higher values of 7.3% for volumetric shrinkage and a tangential/radial shrinkage of 1.98, it is still superior to fine hardwoods such as cherry (*Prunus serotina Ehrh*), walnut (*Juglans nigra L.*) and white oak (*Quercus alba L.*). However, as evidenced by the much lower organic extractive values in *P. ruscifolia* (5.9%) than *P. alba* (16.5%), the *P. ruscifolia* wood is much lighter in color than *P. alba*.

P. ruscifolia and *P. alba* have several contrasting characters, *e.g.*, 15 cm thorns *versus* no thorns, light colored wood *versus* dark brown/purplish wood, bitter pods *versus* sweet pods, and non-palatable foliage for livestock *versus* foliage highly palatable to livestock. To estimate number of genes involved, possible dominance/recessive relationships and begin a genetic map based on recombination frequencies, it would be of interest to examine segregation of these characters in the progeny of a *P. alba* × *P. ruscifolia* cross or a cross of the *P. vinallilo* clones in this trial with the recently described *P. alba* clones with high production of very palatable pods [7].

In addition to *P. ruscifolia,* there is another potentially interesting combination between the diploid *P. pallida* from Peru and *P. alba*. Previous work identified seven *P. pallida* clones with sweet very palatable pods, high biomass productivity, erect form [33], a greater percentage of seedlings surviving seawater salinity than *P. alba* [13] and fast growing, erect trees with virtually no spines [33–35]. One detracting growth trait for *P. pallida* (for use in temperate regions) is its complete lack of tolerance to freezing weather [36]. Thus, it would be interesting to cross elite *P. pallida* [33] and *P. alba* [7] types to evaluate the progeny in temperate regions (such as Argentina, Mexico, India) for new combinations of resistance to soil salinity, soil pH, frost tolerance and desirable pod and lumber characteristics. Crosses among elite *P. alba* clones has been made, and perhaps these techniques could be used for a *P. alba* by *P. pallida* cross.

4.3. Use of High pH Tolerant Prosopis Germplasm as Rootstock for High Pod and Lumber Producing P. alba *Clones*

Ewens and Felker [7] recently reported results from five year old field trials in which *P. alba* clones produced more than 50 kg pods tree^{-1} year^{-1}, which at the 100 tree ha^{-1} planting density, corresponds to more than 5,000 kg ha^{-1} yr^{-1} of pods. Unfortunately, some of the trees produced from cuttings were damaged by high winds (*i.e.*, windthrow) but were still alive, and developed a new vertical trunk or trunks and produced pods (the trees would still be useful for pod production but not lumber). However, none of the seed propagated check trees blew over. Among the trees produced from cuttings, not all trees of the same clone blew over and one clone in particular, *i.e.*, B7F6T4, was not susceptible to windthrow. Thus, there appears to be both genetic and silvicultural alternatives to control this problem. It would be useful to have a source of highly salt/sodic tolerant seedlings to serve

as rootstock for the superior pod producing clones, especially if the rootstock provided stability against windthrow.

Since *Prosopis* is self-incompatible and insect/bee pollinated [37], and since the trees were planted as single tree replicates in the five blocks, these trees should intermate and their seeds should consist of hybrids of the 21 clones. However, this will imply that new offspring is used instead of the original individuals. These seeds should provide more saline/sodic tolerance than random unselected seedlots, although realized genetic gains will depend on narrow-sense heritability (*i.e.*, additive genetic variance) on the trait that was not estimable on this study. Seeds could be bulked from all 21 clones, bulked from a subset of the 21 clones that all had significantly greater than the mean growth of the check varieties, or selected from individual elite clones in which case the seeds would consist of a half-sib family. The almost four fold biomass increase of the fastest growing clone over the mean biomass of the three check half-sib families (that are currently being used to provide seed for commercial plantings), is a very significant improvement and the high clone-mean heritability suggests that large genetic gains can be made using vegetative propagation and clonal deployment.

4.4. Asexual Propagation of High pH Tolerant Prosopis Germplasm

As reported in an earlier paper [19], the highly variable rooting percentage among clones does not seem to be related to the female parent, whereas salinity tolerance does seem to be consistent within a half-sib family [13]. This would suggest a genetic improvement strategy of first screening many parent trees for growth at high salinity and then doing a second intensive screen within the most rapidly growing families for high rooting percentages and high root numbers per cutting.

While rooting of cuttings techniques have been described for *Prosopis* [19], in this study, it was found that there is too much variability in rooting responses, even for the easiest to root *P. alba* clones (*P. pallida* is the exception in being much easier to root), to be commercially viable. The highest rooting percentages obtained for a broad collection of clones [19] occurred when *A. rhizogenes* was included in the rooting hormone mix. *A. rhizogenes* is particularly interesting for rooting cuttings as it can insert the RolB gene into the roots of susceptible species that results in indoleacetic acid (IAA) synthesis and the formation of adventitious roots [38]. Permission from APHIS/PPQ has been obtained to transplant *P. alba* cuttings rooted with the genetically unmodified wild type "Tiger" strain of Strobel and Nachimas [39] into the field in California, USA [40]. Insertion of *Agrobacterium* genes from the related *A. tumenfaciens* is strongly influenced by pH (optimum 5.2–5.8), log *versus* stationary growth phase, vir gene induction period [40], optical density of inocula [41], glucose concentration (0.5% optima), temperature (about 20 °C) and acetosyringone concentration (10−20 μM) [42,43]. It would seem important to examine these variables for their influence on *A. rhizogenes* stimulation of *P. alba* cuttings. Alternatively, great variability in rooting among clones suggests that selection or classical breeding could be used to combine high rooting with desirable economic traits for pods and lumber.

5. Conclusions

In summary, this paper reports growth in height, diameter and biomass accumulation for 21 *P. alba* related clones, originally selected in a greenhouse hydroponic trial, five years after establishment on a moderately saline but highly alkaline soil. Growth of most clones had a mean height significantly than

the mean of the three commercial checklots. At the end of this period, the most rapidly growing clone (O7B4P6(V)) had a mean individual tree biomass of 106 kg, which was more than 3.8 times greater than the mean of the three checks indicating the utility of greenhouse hydroponic screening in the selection of clones for salinity tolerance. Apart from other salt tolerant trees such *Eucalyptus* and *Casuarina* that do not have high-value wood, the wood of *Prosopis* commands very high international demand for fine furniture, flooring and interior components [3]. Five of the clones in the high pH block had annual diameter growth increments of 2 cm or greater; under operational planting conditions this could result in a 20 year rotation and stem diameters averaging about 40 cm. Due to the high price for high quality lumber, this annual diameter increment would correspond to an internal rate of return of about 15%. The exceptional growth rates on this high pH soil of putative interspecific *P. alba* by *P. ruscifolia* hybrids, suggests the need to measure the linkage between thorn and high pH tolerance in full sib crosses of *P. ruscifolia* and *P. alba*, and to clone the high pH tolerance genes in *P. ruscifolia.* Rooted cuttings and/or seedlings from the best clones in this trial should be examined for use as rootstock on recently described *P. alba* clones selected for high production of pods with superior taste properties.

Acknowledgements

Conflict of Interest

The authors declare no conflict of interest.

References and Notes

1. Munns, R. The impact of salinity stress. CSIRO Division of Plant Industry: Canberra, ACT, Australia. Available online: http://www.plantstress.com/Articles/salinity_i/salinity_i.htm (accessed on 11 March 2012).
2. *Advances in the Assessment and Monitoring of Salinization and Status of Biosaline Agriculture: Report of an Expert Consultation, Dubai, United Arab Emirates, 26–29 November 2007*; World Soil Series Reports; Food and Agriculture Organization of the United Nations: Rome, Italy, 2009; Volume 104.
3. Felker, P.; Guevara, J.C. Potential of commercial hardwood forestry plantations in arid lands—An economic analyses of *Prosopis* lumber production in Argentina and the United States under varying management and genetic improvement strategies. *Forest Ecol. Manage.* **2003**, *186*, 271–286.
4. Montenegro, C.; Gasparri, I.; Manghi, E.; Strada, M.; Bono, J.; Parmuchi, M.G. *Informe sobre Deforestación en Argentina*; Dirección de Bosques, Unidad de Manejo del Sistema de Evaluación Forestal, Secretaría de Ambiente y Desarrollo Sustentable de la Nación: Buenos Aires, Argentina, 2004.

5.   Ewens, M. Estacion Forestal Experimental, Universidad Catolica de Santiago del Estero, Argentina. Unpublished work, 2010.

6   Felker, P.; Grados, N.; Cruz, G.; Prokopiuk, D. Economic assessment of production of flour from *Prosopis alba* and *P. pallida* pods for human food applications. *J. Arid Environ.* **2003**, *53*, 517–528.

7.   Ewens, M.; Felker, P. A comparison of pod production and insect ratings of 12 elite *Prosopis alba* clones in a 5 year semi arid argentine field trial. *Forest Ecol. Manage*. **2010**, *260*, 378–383.

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

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

10. Cony, M.A.; Trione, S.O. Inter and intraspecific variability in *Prosopis flexuosa* and *P. chilensis*: Seed germination under salt and moisture stress. *J. Arid Environ.* **1998**, *40*, 307–317.

11. Felker, P.; Clark, P.R.; Laag, A.E.; Pratt, P.F. 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 Soil* **1981**, *61*, 311–317.

12. Rhodes, D.; Felker, P. Mass screening *Prosopis* (mesquite) seedlings for growth at seawater salinity. *Forest Ecol. Manage.* **1988**, *24*, 169–176.

13. Velarde, M.; Felker, P.; Delgano, C. Evaluation of Argentine and Peruvian *Prosopis* germplasm for growth at seawater salinities. *J. Arid Environ.* **2003**, *55*, 515–531.

14. Lucas, R.E.; Davis, R.F. Relationships between pH values of organic soils and availabilities of 12 plant nutrients. *Soil Sci*. **1961**, *92*, 177–182.

15. Ewens, M.; Felker, P. The potential of mini-grafting for large scale commercial production of *Prosopis alba* clones. *J. Arid Environ.* **2003**, *55*, 379–387.

16. Hunziker, J.H.; Poggio, L.; Naranjo, C.A.; Palacios, R.A. Cytogenetics of some species and natural hybrids in *Prosopis* (Leguminosae). *Can. J. Genet. Cytol.* **1975**, *17*, 253–262.

17. Burkart, A. A monograph of the genus *Prosopis* (Leguminosae subfam. Mimosoideae). *J. Arnold Arbor.* **1976**, *57*, 219–249, 450–525.

18. Felker, P.; Ewens, M.; Velarde, V.; Medina, D. Initial evaluation of *Prosopis alba* griseb clones selected for growth at seawater salinities. *Arid Land Res. Manag.* **2008**, *22*, 334–345.

19. Felker, P.; Medina, D.; Soulier, C.; Velicce, G.; Velarde, M.; Gonzalez, C. A survey of environmental and biological factors (*Azospirillum spp*, *Agrobacterium Rhizogenes*, *Pseudomonas aurantiaca*) for their influence in rooting cuttings of *Prosopis alba* clones. *J. Arid Environ.* **2005**, *61*, 227–247.

20. Richards, L.A. *Saline and Sodic Soils*; USDA Salinity Handbook No 54; U.S. Department of Agriculture, Salinity Lab: Riverside, CA, USA, 1954.

21. Gilmour, A.R.; Gogel, B.J.; Cullis, B.R.; Thompson, R. *ASReml User Guide Release 3.0*; VSN International, Ltd: Hemel Hempstead, UK, 2009.

22. Snedecor, G.W.; Cochran, W.G. *Statistical Methods*, 8th ed.; Iowa State University Press: Ames, IA, USA, 1989.

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

24. Velarde, M.; Felker, P.; Gardiner, D. Influence of elemental sulfur, micronutrients, phosphorus, calcium, magnesium and potassium on growth of *Prosopis alba* on high pH soils in Argentina. *J. Arid Environ*. **2005**, *62*, 525–539.

25. Mishra, A.; Sharma, S.D. Influence of forest tree species on reclamation of semiarid sodic soils. *Soil Use Manage*. **2010**, *26*, 445–454.

26. Singh, G.; Abrol, I.P.; Cheema, S.S. Agroforestry on alkali soil: Effect of planting methods and amendments on initial growth, biomass accumulation and chemical composition of mesquite (*Prosopis juliflora*) with inter-space planted with and without Karnal grass (*Diplachne fusca*). *Agroforest. Syst*. **1988**, *7*, 135–160.

27. Singh, G.; Abrol, I.P.; Cheema, S.S. Effects of gypsum application on mesquite (*Prosopis juliflora*) and soil properties in an abandoned sodic soil. *Forest Ecol. Manage*. **1989**, *29*, 1–14.

28. Singh, G.; Abrol, I.P.; Cheema, S.S. Effects of spacing and lopping on a mesquite (*Prosopis juliflora*)-Karnal grass (*Leptochloa fusca*) agroforestry system on an alkaline soil. *Exp. Agric*. **1989**, *25*, 401–408.

29. Singh, G. An agroforestry practice for the development of salt lands using *Prosopis juliflora* and *Leptochloa Fusca*. *Agroforest. Syst*. **1995**, *29*, 61–75.

30. Singh, G. Central Soil Salinity Research Institute, Karnal, India. Personal communication, 2003.

31. George, S.; Parida, A. Over-expression of a rab family GTPase from phreatophyte *Prosopis juliflora* confers tolerance to salt stress on transgenic tobacco. *Mol. Biol. Rep.* **2011**, *38*, 1669–1674.

32. Pometti, C.L.; Pizzo, B.; Brunetti, M.; Macchioni, M.; Ewens, M.; Saidman, B.O. Argentinean native wood species: Physical and mechanical characterization of some *Prosopis* species and *Acacia aroma* (Leguminosae; Mimosoideae). *Bioresource Technol*. **2009**, *100*, 1999–2004.

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

34. Harris, P.J.C.; Pasiecznik, N.M.; Vera-Cruz, M.T.; Bradbury, M. *Prosopis* genetic improvement trials in Cape Verde. In *Prosopis*: *Semi—Arid Fuelwood and Forage Tree. Building Consensus for the Disenfranchised*, National Academy of Sciences, Washington, DC, USA, 13–15 March 1996; Felker, P., Moss, J., Eds.; Center Semi-Arid Forest Resources: Kingsville, TX, USA, 1996.

35. Harsh, L.N.; Tewari, J.C.; Sharma, N.K.; Felker, P. Performance of *Prosopis* species in arid regions of India. In *Prosopis*: *Semi-Arid Fuelwood and Forage Tree. Building consensus for the Disenfranchised*, National Academy of Sciences, Washington DC, USA, 13–15 March 1996; Felker, P., Moss, J., Eds.; Center Semi-Arid Forest Resources: Kingsville, TX, USA, 1996.

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

37. Simpson, B.B. Breeding systems of dominant perennial plants of two disjunct warm desert ecosystems. *Oecologia* **1977**, *27*, 203–226.

38. Collier, C.; Fuchs, B.; Walter, N.; Lutke, W.K.; Taylor, C.G. *Ex vitro* composite plants: An inexpensive, rapid method for root biology. *Plant J*. **2005**, *43*, 449–457.

39. Strobel, G.A.; Nachimas, A. Agrobacterium rhizogenes promotes the initial growth of bare root stock almond. *Microbiology* **1985**, *131*, 1245–1249.
40. Felker, P. Estacion Forestal Experimental, Universidad Catolica de Santiago del Estero, Santiago del Estero, Argentina. Unpublished work, 2010.
41. Stachel, S.E.; Nester, E.W.; Zambryski, P.C. A plant cell factor induces *Agrobacterium tumefaciens* vir gene expression (virulence gene expression/vir gene induction/plant-synthesized vir-inducing factor/bacterial-plant cell recognition). *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 379–383.
42. Turk, S.C.H.J.; Melchers, L.S.; den Dulk-Ras, H.; Regensburg-Tuink, A.J.G.; Hooykaas, P.J.J. Environmental conditions differentially affect vir gene induction indifferent Agrobacterium strains. Role of the VirA sensor protein. *Plant Mol. Biol.* **1991**, *16*, 1051–1059.
43. McCullen, C.A.; Binns, A.N. Agrobacterium tumefaciens and plant cell Interactions and activities required for Interkingdom macromolecular transfer. *Annu. Rev. Cell Dev. Biol.* **2006**, *22*, 101–127.