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Nipa (*Distichlis palmeri*): A perennial grain crop for saltwater irrigation

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ABSTRACT

The perennial saltgrass nipa (*Distichlis palmeri*, Poaceae) is endemic to northern Gulf of California tidal marshes flooded with hypersaline (38–42 g L^{-1}) seawater. Nipa was a wild harvest staple of the Cocopah people of the Río Colorado delta. We investigated the physiology, anatomy, chromosome number, and agronomic potential of nipa as a global food crop. Nipa seeds had 60–93% germination on salinities ranging from 0 to 30 g L^{-1}. Relative Growth Rates (RGR) on both flooded and aerobic conditions remained above 4% d^{-1} up to 30 g L^{-1}, about half the RGR on freshwater. Nipa grain (caryopses) had 7–8% protein, 8% sugar and 79% total digestible carbohydrates (mostly starch) and only 2% ash and 8% fiber, equal to conventional grains in apparent nutritional value. Shoots were low in ash and sodium, and compared favorably to alfalfa forage in protein, digestible carbohydrates and energy contents. Mature female stands in the Colorado River delta produced an estimated 1.25 t ha^{-1} of grain, but over two years in the greenhouse only partial flowering was observed. Nevertheless, *D. palmeri* appears to be worth developing as a perennial grain and forage crop, especially for salinized, flooded soils.

1. Introduction

1.1. Natural history of nipa

Nipa is a unique saltgrass (*Distichlis palmeri* (Vasey) Fassett ex I.M. Johnston) endemic to the shores of the northern Gulf of California, Mexico (Felger, 2000). It is distinguished from *D. spicata* (L.) Greene and other *Distichlis* species by morphological, anatomical, ecological, and molecular characteristics, and in particular by the large size of its grain (caryopsis), larger than any of the other 10 species of the genus (Bell, 2010; Bell and Columbus, 2008; Felger, 2000). The name nipa ("nee-pah") derives from the indigenous Cocopah name ($n^{y}pa$) for *D. palmeri* (Castetter and Bell, 1951; Felger, 2000, 2007) and we propose nipa as the internationally recognized vernacular name for this species.

Nipa is one of only a few species of grasses (Poaceae) entirely endemic to the Sonoran Desert (Felger, 2000). The greatest stands are on tidal mud flats at the delta of the Río Colorado, especially Islas Gore and Montague and the opposite delta shores of Baja California and Sonora (Felger, 2000; Glenn et al., 2005). Much of the delta area where it grows is flooded up to twice daily with hypersaline (38–42 g L^{-1}) seawater in an extreme desert environment (e.g., Sykes, 1937) with a mean annual precipitation of only 76 mm (Weather.com, 2010), and a tidal amplitude of up to 7–9+ m (http://www.sanfelipe.com.mx/weather/apr.html). In these places nipa forms extensive monospecific stands, often with 100% coverage. It also extends several kilometers inland in a patchy distribution on salt flats and into brackish water influenced by occasional seawater tidal flow, and in some of these stands it occurs with other halophytes. Elsewhere, farther south along the shores of the northern Gulf of California, *D. palmeri* generally occurs intermixed with other halophytes in disjunct tidal marshes, but these populations are much reduced in areal extent compared to those of the immediate Río Colorado delta region. This distributional pattern resembles that of a meta-population with the large Río Colorado delta stands forming the most significant or core population.

Ethnographic and historical accounts indicate that the Río Colorado delta population probably has become reduced since construction of the large upriver dams, diversions, and re-filling of the Salton Sea in the 20th century (Felger, 2000), yet there is no indication that *D. palmeri* has been appreciably diminished since the latter part of the 20th century. The core population has conservation protection since it is within the Zona Nucleo of the Reserva de la Biósfera Alto Golfo y Delta del Río Colorado of the Mexican federal

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government (Felger et al., 2007). However, other populations are vulnerable to coastal development (e.g., Glenn et al., 2005).

1.2. Botanical description

Distichlis is a genus of dioecious perennials with Kranz anatomy that predicts C4 photosynthesis. The leaves have bicellular microhairs that excrete salts, which commonly are seen on the leaf surfaces (e.g., Bell and Columbus, 2008). The stems (culms) of *D. palmeri* produce terminal panicles. Female panicles are usually 5–13.5 cm long; the spikelets break apart above the glumes and between the florets, and are 6–9-flowered, with the terminal one often a sterile rudiment. Our observations of naturally occurring populations indicate that most or often nearly all stems become synchronously reproductive, with flowering occurring in March and April and grain ripening in May.

The scientific literature on *D. palmeri* is limited. Bell and Columbus (2008) found it to be a sister species to the widespread *D. spicata* (L.) Greene based on strong molecular and morphological evidence including leaf morphology. The two species are clearly distinguished from each other by a number of key features including: staminate lemmas 7–9 mm long, pistillate lemmas 12–16 mm long, anthers 3.8–4.9 mm long, and mostly intertidal habitats for *D. palmeri*; staminate and pistillate lemmas 3–6 mm long, anthers 1.8–2.6 mm long, and not intertidal habitats for *D. spicata* (Felger, 2000). There are no known intermediate specimens although they occur in close parapatric proximity in the Río Colorado delta region (Felger, 2000). López Soto et al. (2009) showed that *D. palmeri* and the South American *D. australis* (Speg.) Villami differed in leaf anatomy from all other members of the genus including *D. spicata*. The results of a single determination (Gould, 1966) indicated that *D. palmeri* has a somatic chromosome number of 40, the same as *D. spicata*.

1.3. Use of nipa as a grain by the Cocopah people

The indigenous Cocopah people who lived along the lower Río Colorado harvested the grain as one of their major staples prior to the construction of upriver dams and disruption of their traditional culture. In May and probably early June, great quantities of grain-containing spikelets washed ashore and accumulated in tidal windrows, where it was easily gathered. Stems with intact "seed heads" (panicles) were also harvested while the grain was still green, then thrown into baskets, dried between fires, and threshed. The grain was ground into coarse flour and usually consumed as gruel (*atole*), or the flour was made into leavened or unleavened bread (Castetter and Bell, 1951; Felger, 2007).

1.4. Interest in D. palmeri as a modern grain crop

Interest in *D. palmeri* as a modern grain crop was sparked during the 1970s when the idea of developing biosaline crops from wild halophytes first received serious consideration (e.g., Felger and Moser, 1973; Felger and Nabhan, 1978; Glenn et al., 1999; Rozema and Flowers, 2008). All our major grain crops are annual grasses but a case has been made for energy-conserving non-tillage perennial grain crops like *D. palmeri* that do not require re-sowing each year (Glover et al., 2010). Among conventional grain crops, only rice is able to grow under anaerobic conditions as a paddy-style crop; the others require well-drained aerobic soils. A case has been made for increasing the range of crops that can be grown under hypoxic conditions typical of many salt-affected soils (Barret-Lennard, 2003; Colmer and Flowers, 2008), and *D. palmeri* grows in heavy, poorly drained soils in its natural habitat. Hence, *D. palmeri* combines several desirable traits that could expand food production into saline areas.

An attempt was made to commercialize *D. palmeri* from collections originating from wild plants as well as *D. spicata* (Yensen, 2006). While *D. spicata* selections have been successfully planted out as forage crops, the grain potential of *D. palmeri* was not successfully demonstrated in field trials in Australia (Leake, 2004). Only a few published scientific studies have attempted to evaluate the growth rate, salinity tolerance, flood tolerance, physiological adjustments, grain yield or nutritional values of *D. palmeri*, to determine if this plant could be a serious candidate as a modern perennial grain crop for saltwater irrigation (Glenn, 1987; Glenn and O'Leary, 1985; Miyamoto et al., 1996; Noaman, 2004; Noaman and El-Haddad, 2000; Yensen and Weber, 1986, 1987). Some halophytes are slow growing plants that would not be suitable crop plants, and grain production of the widespread *D. spicata* is usually sparse (Hanser, 2006) and the grain is too small to be of economic interest. We know of no ethnographic reports of the grain of any *Distichlis* species other than *D. palmeri* being used for food.

1.5. Objectives of this study

The present study explored the potential of *D. palmeri* as a modern grain and forage crop for salt water irrigation. We first collected grain (caryopses) of *D. palmeri* from populations in the delta of the Río Colorado and estimated biomass and grain yield capacity in natural stands. In subsequent greenhouse experiments, we tested their capacity for germination and growth under saline and flooded conditions in short-term experiments in Tucson, Arizona. Then plants grown from seeds were held for two years in order to determine the flowering characteristics and ratio of male to female plants under greenhouse conditions. We also explored the plants' capacity for osmotic adjustment and accumulation of minerals in foliage and grain as a function of salinity, and conducted proximate analyses to evaluate the nutritional content of the foliage and grain in comparison to conventional forage and grain crops. Finally, we conducted scanning electron microscope (SEM) investigations of the rhizomes and leaves of *D. palmeri* and determined the chromosome number, to compare with *D. spicata*, the closest relative of *D. palmeri*. Based on these results, *D. palmeri* is seen as a distinct, large-grain species that has potential as a non-tillage, perennial grain crop for saltwater irrigation in salinized river deltas and irrigation districts along the world's coastal deserts and perhaps many inland regions. Furthermore, it is also likely to be a suitable grain crop for hot, non-desert regions with saline as well as freshwater conditions.

2. Materials and methods

2.1. Collection of D. palmeri plants and seeds

Plant material was collected at several locations in the delta of the Colorado River, Mexico, on May 13, 2009 (Appendix 1). Herbarium specimens from these collections are deposited at the University of Arizona Herbarium in Tucson, with duplicate specimens distributed to other institutions in Mexico and the U.S. Stand densities and other details of the collection sites were documented with 69 digital photographs taken during the collection process. The collection sites were subject to inundation by seawater, either daily or at highest tides. The estuary of the Río Colorado has mixed semidiurnal tides (two high tides per day), and some "inland" *D. palmeri* stands grow on hypersaline saturated soils inundated only by the highest tides. Although a small volume of agricultural drainage water sometimes flows to the sea, extreme tides dominate the hydrology of the estuary, and salinities of water flooding the *D. palmeri* stands are normally in the range of 38–42 $g\,L^{-1}$ throughout the year (Glenn et al., 2007).

Pistillate specimens were collected and placed in newspaper in a plant press and air-dried in order to keep the grain-bearing panicles intact for later analysis in the laboratory in Tucson. Each sample consisted of several dozen stems with their terminal panicles. Panicles that had not yet dropped grain-containing spikelets were selected to determine the number of grain produced per stem. In some cases many of the spikelets and/or florets had detached during transit, and for these samples, remaining intact fertile florets were combined with loose fertile spikelets and/or florets within the newspaper sheets, to determine the mean number and weight of grain (caryopses) per stem for that herbarium sample. Caryopses (grain) for germination and growth trials were also selected from the air-dried herbarium specimens, representing a mix across collection sites. Other caryopses were separated ("dehulled") by hand from their enclosing lemmas and paleas and used to determine the dimensions, weight, and nutritional composition of the grain. Biomass yields of grain and stems per m^2 of area in plant stands were estimated by multiplying the weight of stems and grain by the density of stems in plant stands, determined on photographs of plant stands taken in the field. A modification of the line-intercept method was used to estimate stem density from photographs (Bonham, 1989). The number of stems that intercepted a 25-cm bar on each photograph was multiplied by 4 to get stems per m, then squared to get stems per m^2.

2.2. Germination experiments

Preliminary experiments showed that most seeds germinated readily, with many seedlings emerging within 5–7 days after sowing. Dehulled grain (seeds) germinated a few days sooner than ones with intact hulls, but final germination percentages were similar. Some seeds had delayed germination, with some germinating as late as 84 days after sowing. Effects of salinity on germination were tested in a greenhouse experiment conducted over 35 days in November and December 2010, using the seed collected in May 2010. Ten seeds per pot were sown in 3.8 L plastic pots equipped with drain holes and containing soil consisting of 2 parts sand and 1 part potting soil by volume. Three pots were sown per salinity treatment (30 seeds per salinity treatment). The pots in each experiment were irrigated daily with 1 L water from the municipal supply containing 0 g L^{-1}, 5 g L^{-1}, 10 g L^{-1}, 20 g L^{-1}, or 30 g L^{-1} artificial sea salts (Crystal Sea® Marinemix, Marine Enterprises International, Baltimore, MD), consisting of approximately 83% NaCl, 10% $MgSO_4$, 3.5% $CaCl_2$, 3.1% KCl, plus trace amounts of other salts, similar to the composition of natural seawater. The irrigation volume was sufficient to produce copious drainage from each pot and to maintain the pot salinity within 10% of the treatment solution salinity. Germination was scored when a green coleoptile emerged from the soil. Pots were treated as the experimental units (3 replications per treatment) for statistical analyses.

2.3. Growth experiment under aerobic conditions

Salinity trials followed methods described in Glenn (1987) and Vasquez et al. (2005). Seeds were germinated under freshwater irrigation then transplanted as seedlings (1 per pot) into 16 cm diameter pots equipped with drainage holes containing approximately 1500 cm^3 of soil (2 parts sand and 1 part potting mix by volume). Seedlings were randomly assigned to treatments through the use of a random number table. We selected shoots for transplanting to the experimental pots (and those to estimate the initial dry weight) that were ca. 2 cm long. The pots were pre-irrigated with the appropriate test solution before seedlings were transplanted. The experiment tested salinities of 0, 5, 10, 15, 20, 25 and 30 g L^{-1} with 5 replicate pots per treatment (35 pots total). The pots were grouped into 7 rows of 5 pots each with each row representing a salinity treatment and with rims of adjacent pots nearly touching within and between rows. At the start of the experiment, shoots from 10 seedlings from identical pots, started at the same time, were harvested to determine the initial weight (grams dry weight, gdw) of shoots per pot (mean = 0.069 gdw, Std. Error = 0.003) to be used in calculation of relative growth rates (RGR) by the formula (Hoffman and Poorter, 2002):

$$\mathrm{RGR}\left(\%\,\mathrm{d}^{-1}\right) = [\ln(\text{final wt.}) - \ln(\text{initial wt.})]/\text{no. days} \times 100 \quad (1)$$

Test solutions were made up with freshwater from the municipal supply source plus artificial sea salts and 1 g L^{-1} of soluble fertilizer (Scotts Miracle-Gro Complete, Marysville, OH). The pots were irrigated daily with 500 ml of test solution, which produced copious drainage and kept salinities in pots within 10% of the irrigation salinities. The experiment was initiated July 5, 2009 and shoots were harvested by cutting plants to ground level on August 26, 2009 (52 days), at which time the shoots had densely filled the pots and reached 25–30 cm height on the lower salinity treatments. The number of stems per pot was recorded, and the plants in the pots were allowed to grow a second crop of shoots under continued irrigation until September 26, 2009 (31 days). Shoots from the second crop were harvested and then dried to a constant weight in a solar drying oven to determine dry weight.

2.4. Growth experiment under anaerobic (flooded) conditions

Growth under anaerobic conditions was measured for plants grown in 16-cm-diameter pots containing approximately 2800 cm^3 of sand plus potting mix. The plants were started as seedlings in pots, each with drainage holes, under daily freshwater irrigation until they had developed a root system and shoots had proliferated, and each plant weighed approximately 2–3 g fresh weight. Each pot was then placed in a plastic sleeve brought up over the lip of the pot, which prevented the pots from draining, and they were maintained under flooded conditions from October 9 to November 4, 2009 (26 days) under test salinities of 0, 5, 10, 20, 25, and 30 g L^{-1} with three replicates per treatment (18 pots total). Test solutions were made up the same as for the aerobic growth experiments. Shoots from three additional pots, treated the same as the test pots, were harvested to determine dry weight of shoots at the start of the experiment (mean = 0.81 gdw, Std. Error = 0.04). The test pots were grouped into a block and were kept flooded to a depth of 1–2 cm above the soil line by the addition of water of the correct test salinity for each pot during the experiment. This flooding led to a gradual increase in the salinity of the interstitial water in each pot, which was determined weekly by extracting water samples from the soil in the test pots and measuring salinity during the experiment. By the end of the experiment, salinities had increased by 5–15 g L^{-1} over the initial range of salinities, and salinities over the time span of the experiment were expressed as the mean of the starting salinity and the final salinity in each pot. Near the end of the experiment, the redox potential of the soil in each pot was measured at a depth of 2–3 cm below the soil surface with a redox potential electrode (Orion 3 Star pH Meter, Thermo Electron Corp., Beverly, MA) as described in Vandersande et al. (2001).

2.5. Longer-term growth observations

The ability of *D. palmeri* to grow over long periods under flooded conditions was tested by transplanting seedlings into 33 large plastic pots (30 cm diameter, 30 cm deep) with drainage holes, then

grouping the pots closely together in a shallow pool (1.7 m diameter) with water containing 10 ppt salts and 0.1 g L^{-1} soluble fertilizer. Each pot represented an individual plant started from a single seed, so that ratios of male to female plants could be determined. The plants were held in tanks for eighteen months (November 2009 to April 2011) with the water level maintained at 30 cm deep by the addition of freshwater as needed to compensate for evapotranspiration. In November 2010 plant heights were measured, and the number of stems per m^2 were estimated by randomly placing a 25 × 25 cm square frame at three points over the canopy and counting the number of stems within the frame. An additional 0.1 g L^{-1} of fertilizer was added and the plants were observed to document the flowering process, which occurs in spring in native populations. Shoot density and standing biomass were re-measured in April 20, 2011, after harvesting seeds, which had ripened by this date.

2.6. Measurement of osmolality of shoot, rhizome, and root tissues

Shoot tissue samples were collected from plants in each pot at the first and second harvests in the aerobic growth experiment, and from rhizome sections with attached roots (called "rhizomes with roots" throughout the text and figures) at the end of that experiment. The samples were kept frozen until measured. Osmotic potential was measured in extracts as described in Vasquez et al. (2006). It was necessary to dilute tissue samples with distilled water (2 ml per gram fresh weight tissue) in order to obtain a liquid sample that could be assayed. The samples were crushed in a plastic test tube with a metal plunger and a 0.01 ml aliquot was assayed for osmolality with a Wescor Vapor Pressure Model 5130C osmometer calibrated with standard solutions of 100, 290, and 2000 mOsm kg^{-1}. *Distichlis* spp. excrete excess salts in the leaf mesophyll onto their leaf surfaces through salt glands and these are presumably not osmotically active in the leaves. The contribution of leaf tissue alone (without excreted salts) to osmolality was estimated by wiping leaf samples from the first harvest (3 per salinity trial) with tissue paper moistened with distilled water to remove surface salts prior to measurement of osmolality in the leaf tissue.

2.7. Salinity measurements, ion analyses and proximate analyses

Salinities were measured with a Markson Model 15/16 Specific Conductance Meter (Henderson, NC) and with a hand-held refractometer (American Optical), both of which were calibrated with the artificial sea salts mixtures used in the growth experiments. Salinities were measured in the irrigation solutions, drainage solutions from pots, and interstitial water samples from pots at irregular intervals during the growth experiments to monitor any deviations from the intended treatment salinities during each experiment. Cations and anions in samples of shoots and rhizomes with their roots were analyzed in tissues from the second harvest of the aerobic growth experiment. Pooled samples across replicate pots were analyzed by the Soil, Water and Plant Analytical Laboratory of the Department of Soil, Water and Environmental Science, University of Arizona, Tucson. Three samples of the grain were analyzed for sugars, crude protein, ash and mineral content, and a pooled sample of shoot material from 15 ppt salinity treatment was analyzed for complete nutritional content by Litchfield Analytical Services, Litchfield, MI.

2.8. Determining chromosome numbers

Root tips were collected from four individual greenhouse-grown plants and treated as described in Reid (2001) with colchicine to arrest mitosis, 0.7% acetocarmine to stain, and Macerozyme R-10 and cellulase "Onozuka" R-10 (both from Yakult Honsha Company, Tokyo, Japan) to digest cell walls. Chromosome spreads were made with the squash method of Reid (2001) and were examined under a 100× oil objective on a Zeiss Photomicroscope II equipped with phase contrast. Spreads were photographed on Kodak T-Max 100 film (Eastman Kodak Company, Rochester, NY).

2.9. Statistical methods

Salinity trials were subjected to one-way analysis of variance (ANOVA) in which salinity treatment was the independent variable and germination, RGR, soil redox potential and osmotic adjustment were the dependent variables.

3. Results

3.1. Field observations and biomass and grain yield estimates

D. palmeri at the Río Colorado delta occurs in dense, mostly monospecific, stands on mudflats and along tidal channels with plant heights ranging from 15–45 cm (Appendix 2, electronic version only). Male and female stands of this dioecious species grow in a patchwork pattern, suggesting that the observed plants tend to spread by clonal propagation of rhizomes rather than by seed dispersal. At the time of collection in May 2009 nearly every mature stem of *D. palmeri* bore a terminal male or female panicle. Among female stands, the grain was ripe and in the massive population on Isla Montague in the delta, many spikelets and florets had fallen and were seen in windrows at the high tide line or in small mats on top of the plant stands. Many empty husks (florets without the caryopsis and its partially enclosing palea) were also present in the windrows and mats. Despite the prolific seed production, no new seedlings were observed in the plant stands.

Stem density in female stands ranged from 700–1100 stems per m^2. A selection of intact field-collected female specimens were separated into stems ($n = 235$) and caryopses ($n = 2726$) for a mean of 11.6 ripe caryopses per stem. Nearly all stems examined had a terminal panicle. On intact panicles, 49.4% of florets had a caryopsis (grain) and 50.6% were empty, mainly those of terminal spikelets of the panicles. On the other hand, empty husks (florets without a caryopsis) dominated the material that had shattered from the plants and that made up the windrows observed in the field. Stems weighed a mean 3.02 grams (dry weight) and caryopses per stem mean was 0.139 g for a harvest index (ratio of grain to total biomass) of 0.044. Based on a stem density of about 900 per m^2 estimated from field photographs, extrapolated biomass and grain yields would be 2.72 kg m^{-2} and 0.125 kg m^{-2}, respectively, in dense female stands. Caryopses mean weight was 11.2 mg (Std. Error = 0.3, $n = 82$) and measured 6.38 mm (Std. Error = 0.12 mm, $n = 77$) by 1.68 mm (Std. Error = 0.04, $n = 77$). Greater variation, however, might be expected since Felger (2000) found that caryopses measured 7.6–8.5 mm long from a range of herbarium specimens collected between 1889 and 1993.

3.2. Germination at different salinities

Increasing salinity slowed but did not prevent germination of seeds on salinities up to 30 g L^{-1} (Fig. 1). After 35 days seeds on 0 g L^{-1} salinity had 93% germination compared to 70% for seeds on 10 and 20 g L^{-1} and 60% for seeds on 30 g L^{-1} ($F = 3.96$, $P = 0.013$, df = 3, 11).

3.3. Relative growth rates, RGR, at different salinities under flooded and non-flooded conditions

Under daily irrigation in freely drained pots, RGR at the first harvest was highest on 5 g L^{-1} (ca. 8% d^{-1}) and decreased to 4.5% d^{-1}

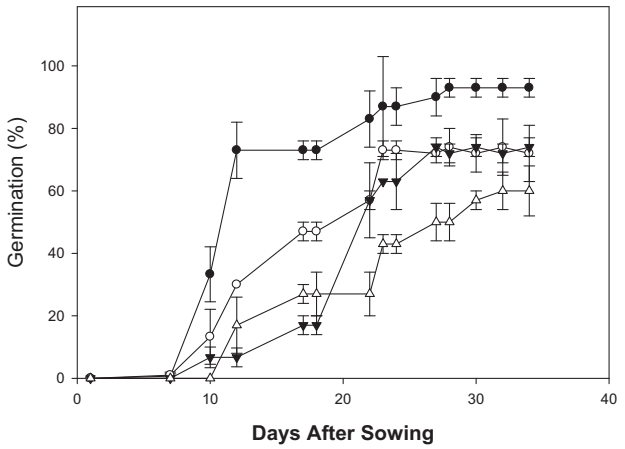

Fig. 1. Percentage seed germination versus time on salinities: 0 g L^{-1} (closed circles); 10 g L^{-1} (open circles); 20 g L^{-1} (closed triangles); 30 g L^{-1} (open triangles). Bars are standard error of means ($n = 30$).

on 30 g L^{-1} (Fig. 2A) ($F = 6.61$, $P < 0.001$, df = 6, 24). RGR values over the two harvests combined followed the same trend as the first harvest with respect to salinity ($F = 52.5$, $P < 0.001$, df = 6, 24) but were about 20% lower than RGRs values obtained in the first harvest. Since plants were cut to ground level in the first harvest, a recovery time was needed for plants to resume full growth. Salinities in flooded pots were higher than in non-flooded pots due to the accumulation of salts over time, especially at the two higher salinities (Fig. 2A). RGR values for plants in flooded pots ranged from 6.5% d^{-1} to 4.5% d^{-1} over the salinity range of 0–30 g L^{-1} ($F = 8.26$, $P = 0.001$, df = 6, 14) similar to results under unflooded conditions; however RGR showed a marked decrease at salinities of 35 g L^{-1} and above. Flooded pots had negative redox potentials ranging from −200 mV to −380 mV (mean = −265, Std. Error = 20) and redox potentials did not differ significantly by salinity ($F = 1.7$, $P = 0.203$, df = 6,14). These negative redox values are typical of flooded wetland soils (Vandersande et al., 2001). Shoot densities ranged from 4400 shoots m^{-2} on freshwater to 900 shoots m^{-2} on 30 g L^{-1} based on results after the first harvest in unflooded pots (Fig. 2B).

3.4. Longer term growth and flowering observations in the greenhouse

When held for a year (November 2009 to November 2010) under flooded conditions at 10 g L^{-1} salinity, the plants had well developed rhizome and root systems, which completely filled the pots and emerged through the drain holes in pots to proliferate within the external solution. Shoots grew to form a closed canopy within the tank, with a mean stem height of 72 cm and a stem density of 3125 m^{-2}.

The plants did not flower during the first year after sowing, but some plants flowered in the second year (under greenhouse conditions, which are almost always different from field conditions). Male flowers first emerged in late December 2010 and female flowers first emerged several weeks later, in January 2011. Among 33 plants, 11 were male, 9 female, and 13 did not flower. Caryopses were ripe by April 15, 2011, about the time of early ripening among wild populations. Female plants produced a mean 95 caryopses per plant, with a mean of 4.75 per fertile stem; however only 2.42% of stems of female plants became reproductive. By April 2011 stem density had increased to 9104 m^{-2} and biomass had increased to 3.54 g m^{-2} among plants in pots.

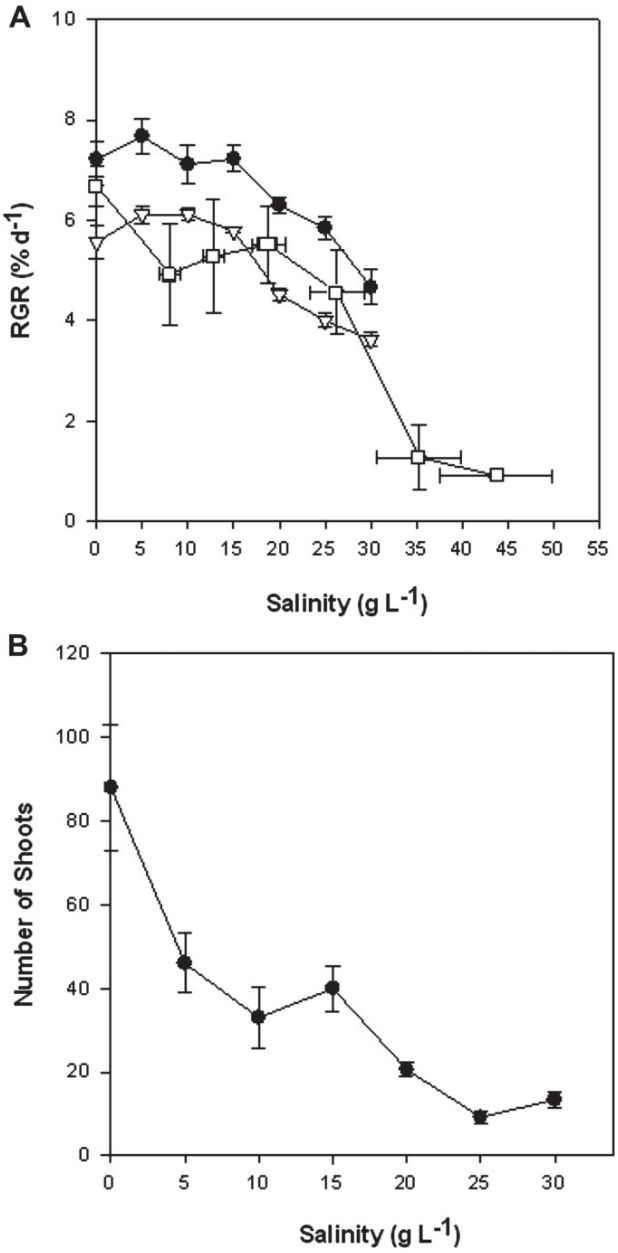

Fig. 2. (A) Relative growth rates (RGR) of *D. palmeri* as a function of salinity in greenhouse experiments showing RGR of plants under aerobic conditions; first harvest (closed circles), first and second harvests combined (open triangles) and flooded (anaerobic) conditions (open squares). (B) Number of shoots versus salinity for plants from first harvest under aerobic conditions. Bars are standard errors of means.

3.5. Ion content and osmotic adjustment

Cation and anion contents of rhizomes with their roots, and shoots, are shown in Fig. 3A and B, respectively. Chloride and sodium increased in response to salinity up to 20 g L^{-1} in rhizomes with roots, and decreased markedly at higher salinities. Chloride and sodium also increased somewhat in response to salinity in shoots, but peaked at 10–15 g L^{-1} salinity, and levels were much lower in shoots than in rhizomes with roots. Salts might have leaked into the aerenchyma tissues of rhizomes but were excluded from the shoots by the suberized epidermal layer around the stele. Levels of sulfate,

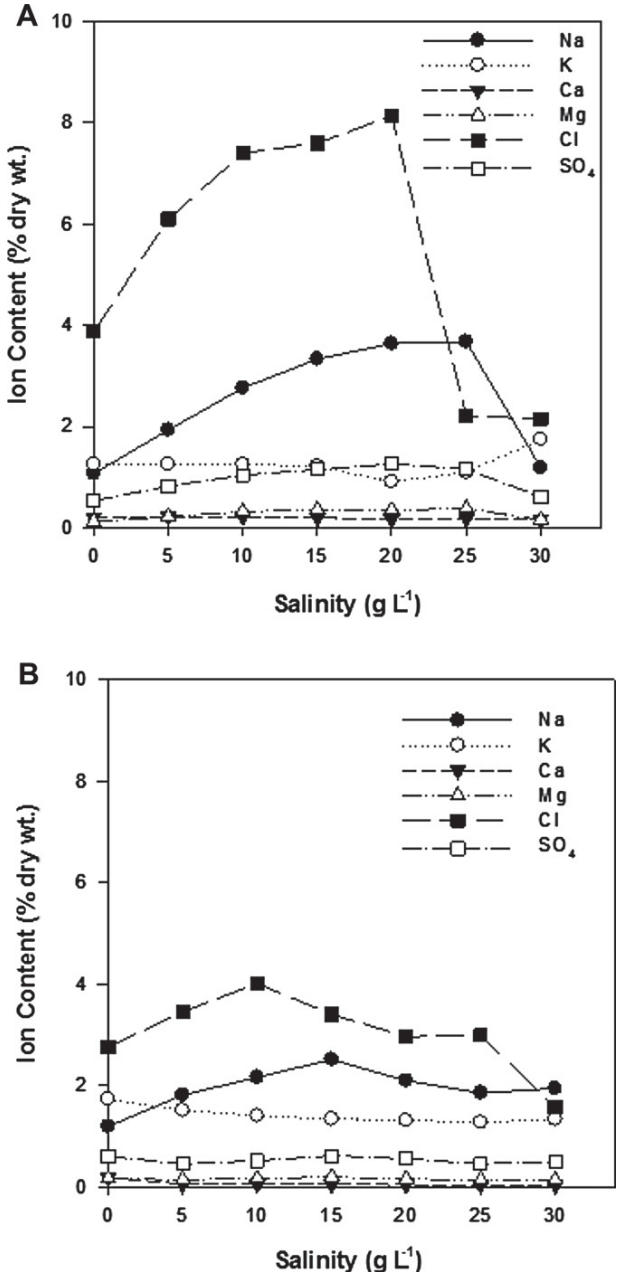

Fig. 3. Cation and anion content of *D. palmeri* rhizomes with roots (A) and shoots (B). Results are analyses of composite samples across replicates at each salinity.

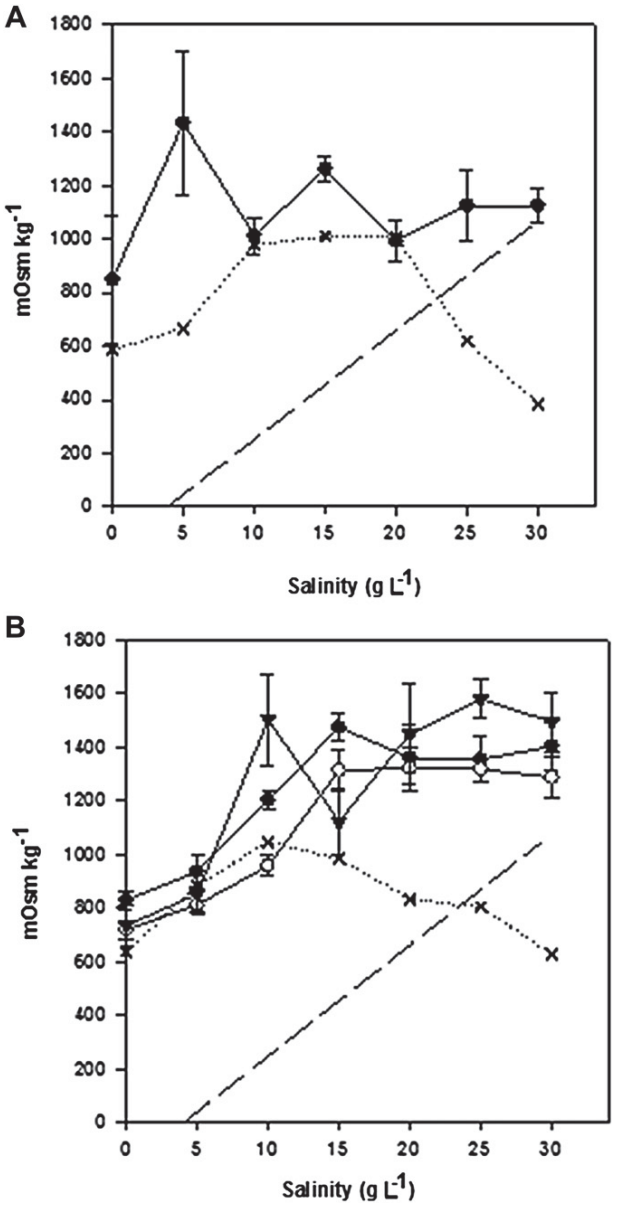

Fig. 4. Tissue osmolality of rhizomes with roots (A) and shoots (B) of *D. palmeri* measured in tissue extracts by osmometer. Dashed lines show the approximate omolality of the external solution; dotted lines shows the osmolality attributed to cations + anions. In (B) closed circles are results from the first harvest; open circles are results from the second harvest; and closed triangles are results from samples wiped to remove surface salts if present, from the second harvest. Bars are standard errors.

potassium, calcium, and magnesium were low and relatively constant across salinities in both shoots and rhizomes with roots.

Osmolalities measured in rhizomes with roots, and shoots, are shown in Fig. 4A and B, respectively. Rhizomes with roots maintained an osmolality of 850–1450 mOsm kg^{-1} with no significant trend across salinities ($P > 0.05$). Rhizome with root osmolality on 30 g L^{-1} was only slightly higher than the calculated osmolality of the external solution. The osmolality that could be attributed to the sum of cations and anions was lower than measured rhizome with root osmolality (Fig. 4A). On 30 g L^{-1}, cations and anions could account for only 30% of measured osmolality. Osmolality of the shoots was significantly affected by salinity ($P < 0.05$). Those osmolalities ranged from 800 mOsm kg^{-1} to 1600 mOsm kg^{-1} and showed a clear increase with salinity up to 15 g L^{-1}, followed by a leveling off up to 30 g L^{-1} (Fig. 4B). However, osmolality exceeded the calculated osmolality of the external solution across the salinity range. As with rhizomes plus roots, cations and anions in the shoots could not account for the extent of osmotic adjustment, especially at salinities above 10 g L^{-1}. At 30 g L^{-1}, cations and anions could account for about half the osmotic adjustment.

3.6. Analyses of grain and shoots

Proximate analyses of *D. palmeri* grain (caryopses) and shoots are in Table 1. *D. palmeri* grain had 6.7–8.7% crude protein and very

Table 1
Proximate analyses of *D. palmeri* shoots and grain from the present study and the literature. Alfalfa results are included for comparison with *D. palmeri* shoots.

Constituent	*D. palmeri* shoots[a]	Alfalfa hay[b]	*D. palmeri* grain[a]	*D. palmeri* grain[c]
Crude protein (%)	16.26	12.9	6.7–8.4	8.7
Acid detergent fiber (%)	31.86	44.0	–	–
Crude fiber (%)	25.49	37.7	–	8.4
Non-fiber (digestible) Carbohydrates (%)	24.39	37.4	–	71.1
Sugars (%)	–	–	4.5–6.3	–
Ash (%)	9.45	7.5	–	1.6
Fat (%)	0.01	1.3	0.02–0.29	1.8
Total digestible nutrients (%)	49.47	50.0	–	79.9
Digestible energy (Mcal/kg)	2.18	2.21	–	3.11
Phosphorous (%)	0.21	0.25	0.21–0.23	0.20
Calcium (%)	0.21	1.40	0.03–0.04	0.08
Potassium (%)	1.38	2.45	0.48–0.54	–
Sodium (%)	1.69	0.25	0.11–0.13	0.30
Magnesium (%)	0.19	0.14	0.07	0.03

[a] Results from the present study.
[b] Results for mature alfalfa hay from National Research Council (1986).
[c] Results from Yensen and Weber (1986).

low ash and sodium contents. Fiber content was only 8.4% and digestible carbohydrates (starch and sugars) were the main constituents of the grain. *D. palmeri* shoots were high in protein and digestible carbohydrates, had less than 10% ash, and relatively low sodium content. They compared favorably to alfalfa hay in protein, energy, and mineral contents (Table 1).

3.7. Anatomical observations and chromosome number

D. palmeri and its sister species *D. spicata* show vegetative anatomical similarities (e.g., Bell and Columbus, 2008). Rhizome sections of *D. palmeri* (Fig. 5) have scattered vascular bundles, a suberized endodermis, and a layer of aerenchyma tissue between the epidermis and stele, typical of *D. spicata* (Hansen et al., 1976). Adaxial leaf surfaces of *D. palmeri* have alternating long and short epidermal cells arranged in a ridge and furrow system, exposed-type stomata in the furrow portion of the leaf with knob-like protrusions from each guard cell, and bicellular microhairs (salt glands) in the furrows and on the sides of ridges, also typical of *D. spicata*. We did not observe silica saddle cells or trichomes in *D. palmeri*, but these can be absent from greenhouse-grown *D. spicata* plants as well (Hansen et al., 1976). Root tips from four separate plants grown from seeds collected for this study had somatic chromosome counts of 40 during mitosis, the same as the initial report by Gould (1966) and for *D. spicata*.

4. Discussion

4.1. Flowering and seed yield potential

Based on the greenhouse observations, at least several years might be needed for *D. palmeri* plants to achieve their full flowering potential. Whereas plants in wild populations are reproductive annually, and most stems produce an inflorescence, none of the greenhouse plants flowered the first year. Only 20 out of 33 flowered the second year, and only a small percentage of female stems bore flowers and fruits. Although the sample size was small, the ratio of males to females of 11:9 suggests that this dioecious species might produce equal numbers of male and female plants.

Stands of female plants at the Río Colorado delta had projected grain yields of 1.25 t ha^{-1}. Yensen (2006) reported grain yields of 2–4 t ha^{-1} for *D. palmeri* grown under field conditions, although details of the field trials were not given, and field trials in Australia were not able to replicate those positive findings (Leake, 2004). Greenhouse plants are being maintained under flooded conditions at 10 g L^{-1} salinity to determine the number of years needed for them to reach their full flowering and yield potential under optimal growth conditions.

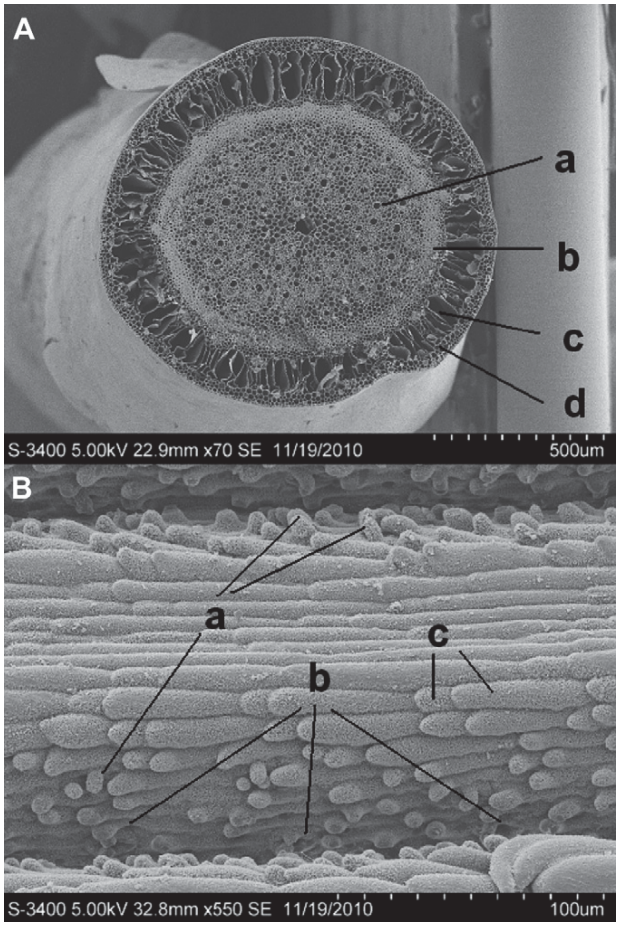

Fig. 5. Scanning electron microscope (SEM) views of *D. palmeri* rhizome and leaf. (A) Rhizome from a plant grown under flooded conditions, showing a vascular bundle (a), the suberized endodermis (b), ring of aerenchyma tissue (c) and epidermis (d). (B) An upper leaf surface showing bicellular salt glands (a), exposed-type stomata with knob-like protrusions on guard cells (b) and alternating long and short epidermal cells forming a ridge-and-furrow leaf morphology (c).

4.2. Growth response to salinity

Germination response to salinity is similar for *D. palmeri* and *D. spicata* (e.g., Shahba and Qian, 2008), with the majority of seeds germinating even on 30 g L^{-1} salinity. However, for at least some populations of *D. spicata* the seeds are dormant when first produced and need to be chilled or the grain scarified to break dormancy (Shahba and Qian, 2008) whereas most *D. palmeri* seeds germinated readily without pre-treatment. Our results for *D. palmeri* showed that salinity had a positive effect on biomass production up to 5–10 g L^{-1}, but a negative effect at higher salinities. RGR on both flooded and aerobic conditions remained above 4% d^{-1} up to 25 g L^{-1}, about half the RGR on freshwater. Similar results were reported by Miyamoto et al. (1996) and Noaman and El-Haddad (2000) and for *D. palmeri* grown in pots in greenhouse experiments. Salt tolerance was similar to that for *D. spicata*, which

also showed about 50% reduction in RGR on 30 g L^{-1} water relative to freshwater controls (Marcum et al., 2007). However, *D. palmeri* RGR values were at the high end of those reported for *D. spicata* (Sargent et al., 2008) or other halophytic grasses (Flowers and Colmer, 2008; Glenn, 1987). High RGR values were maintained over two harvests, indicating that *D. palmeri* could be subjected to multiple cuttings if grown as a forage crop, since it readily produced new shoots from rhizomes even when cut back to the soil line. Like *D. spicata* (Hansen et al., 1976), *D. palmeri* developed aerenchyma tissues under flooded conditions, allowing it to keep the roots aerated even in highly anaerobic soils.

Salinity had a negative effect on shoot (stem) densities in the greenhouse study, which ranged from 900–4000 shoots per m^2 over the salinity range of 0–30 g L^{-1}, with low-end values typical of field values in the Río Colorado delta. These densities are similar to densities reported for *D. spicata*, which produced 2600 shoots per m^2 in dense stands (Hanser, 2006).

Biomass production for *D. palmeri* in the greenhouse experiments was consistent with field observations. Glenn and O'Leary (1985) reported a biomass yield of 2.6 kg m^{-2} over two years growth in plots irrigated with 38 ppt seawater at Puerto Peñasco, Mexico, similar to the standing biomass of 2.7 kg m^{-2} estimated in natural stands in the present study. Glenn and O'Leary (1985) concluded that annual biomass yields of 1.3 kg m^{-2} could be achieved on seawater irrigation by partially harvesting the standing crop each year as shown also by the rapid regrowth of cut plants in our greenhouse experiment. On 10 g L^{-1} salinity, a yield of 2.36 kg m^{-2} yr^{-1} was observed in our greenhouse. Similarly high rates of biomass production have been reported for *D. spicata* grown on saline water (Bustan et al., 2005; Sargent et al., 2008).

4.3. Mineral content and osmotic adjustment

Halophytes adjust to the low external water potential of saline soils by accumulating osmotically active substances in their cytoplasm and vacuoles (Flowers and Colmer, 2008). In dictot (i.e., eudicot) halophytes, the central vacuole occupies most of the cell volume and is the site of mineral accumulation (mainly NaCl) for osmotic adjustment; foliage from these plants can consist of up to 50% mineral content, which is mostly NaCl when seawater is used for irrigation (Glenn and O'Leary, 1985). Monocot halophytes such as grasses accumulate lower levels of NaCl due to smaller vacuoles and the greater accumulation of organic compounds such as sugars and amino acids for osmotic adjustment (Flowers and Colmer, 2008). *D. palmeri* shoots achieved osmolalities up to 1400 mOsm kg^{-1} yet had ash contents under 10% and Na^+ content under 2% under salt water irrigation (Al-Shorepy et al., 2010; Bustan et al., 2005).

4.4. Chromosome number and affinity to other Distichlis spp.

Distichlis is a member of the subfamily Chloridoideae, which has base chromosome numbers of $x = 9$ and $x = 10$ (Roodt and Spies, 2003). The *Distichlis* species for which chromosome numbers have been determined are polyploid, having somatic chromosome numbers of either 40 (*D. palmeri*, *D. littoralis* (Engelm.) H.L. Bell & Columbus and coastal *D. spicata*) (Löve, 1984) or 38 (*D. eludens* (Soderstr. & H.F. Decker) H.L. Bell & Columbus and most inland *D. spicata*) (Harrington et al., 2009; Reeder, 1967). This genus thus potentially involves basic genomes of both $x = 9$ and $x = 10$. Evidence from meiosis (Reid, 2001) and from molecular analysis (Harrington et al., 2009) suggests that both the 40-chromosome and the 38-chromosome members of the *D. spicata* sensu lato complex are allopolyploids. Based on chloroplast haplotypes, the 38-chromosome and 40-chromosome groups within *D. spicata* sensu lato appear to have different maternal parents (Harrington et al., 2009). A hypothesis of allopolyploid speciation can be constructed in which multiple related genomes combine in various dosages to produce the distinct members of *Distichlis* in much the same way that the A, B, C, and D genomes of wheat (*Triticum* spp.) are known to have combined to produce *Triticum monococcum* (einkorn wheat) (AA), *Triticum turgidum* var. *durum* (durum wheat for spaghetti) (AABB), *Triticum aestivum* (bread wheat) (AABBDD), and *Aegilops cylindrica* (jointed goatgrass) (CCDD).

If the allopolyploid hypothesis of speciation in *Distichlis* is correct, *D. palmeri* and coastal *D. spicata*, each having a chromosome number of $2n = 40$, may be related by a shared genome, although further cytological work must be done to determine the genetic constitutions of these and other members of the genus. The absence of intermediate forms in areas where *D. palmeri* and *D. spicata* occupy adjoining ground suggests that hybrids do not form in nature. Whether this is the result of environmental requirement or pre-zygotic or post-zygotic barriers remains to be determined. However, 38-chromosome and 40-chromosome members of the *D. spicata* sensu lato complex can be crossed in the greenhouse, in spite of their apparent failure to cross in nature (Harrington et al., 2009). This leaves open the possibility that *D. palmeri* can be crossed with other species in the genus to broaden the range of environmental adaptation and to improve the palatability of the plant as forage.

4.5. Potential as an agronomic crop

Based on the present results and those from previous analyses reported by Yensen and Weber (1986, 1987), *D. palmeri* grain has nutritional values equivalent to that of conventional grain crops such as wheat and rice, with lower fiber and high digestible carbohydrate contents, and very low ash and sodium contents. Size (ca. 6.8 mm length) and weight (ca. 11 mg) of the grain is similar to that of short grain rice. The high food value of *D. palmeri* can also be inferred from its use as a staple food source by the Cocopah people, who applied their word for *D. palmeri*, nipa, to wheat when it was introduced to them (Castetter and Bell, 1951). A theoretical grain yield of 1.25 t ha^{-1} in wild stands overlaps the low end of cultivated grain yields (e.g., http://www.fao.org/news/story/en/item/53813/icode/) and considerable improvement of *D. palmeri* yields could be expected under cultivation. However, it might take several years for full production to be reached.

The forage (leaf biomass) component has high nutritional value in terms of protein content and low Na^+ content. Biomass yield and forage quality of *D. palmeri* were similar to values for alfalfa (National Research Council, 1986). *D. spicata* can develop tough stems that are difficult for livestock to digest, but if cut regularly the plants can be managed to produce good-quality forage (Al-Shorepy et al., 2010; Bustan et al., 2005). Based on our greenhouse results in which plants were cut then allowed to regrow, we reach a similar conclusion with regard to *D. palmeri*.

The ability of *D. palmeri* to grow under flooded conditions might be a distinct advantage in its use as a land reclamation crop. Many of the world's delta irrigation districts have severe salinization problems, due in part to rising sea levels, including, as examples, those at the mouths of the Colorado (Glenn et al., 1996), Ganges (Seraj and Salam, 2000), Indus (Qureshi et al., 2008), Murray (Nordblom et al., 2010), and Nile (Bohannon, 2010), rivers. Paddy rice is often planted in salinized portions of these districts as part of the soil restoration process. While rice is salt-sensitive, it is possible to flood soils and leach salts effectively in flood-plots planted with rice if freshwater is available and soils are not too saline (Seraj and Salam, 2000). *D. palmeri* would have obvious immediate value in those districts where soil conditions are too saline for rice, or where only brackish drainage water is available for irrigation. Other

applications for a halophyte grain and forage crop such as *D. palmeri* could be developed for secondary or primary salinized pasturelands, irrigation districts, and coastal deserts around the world.

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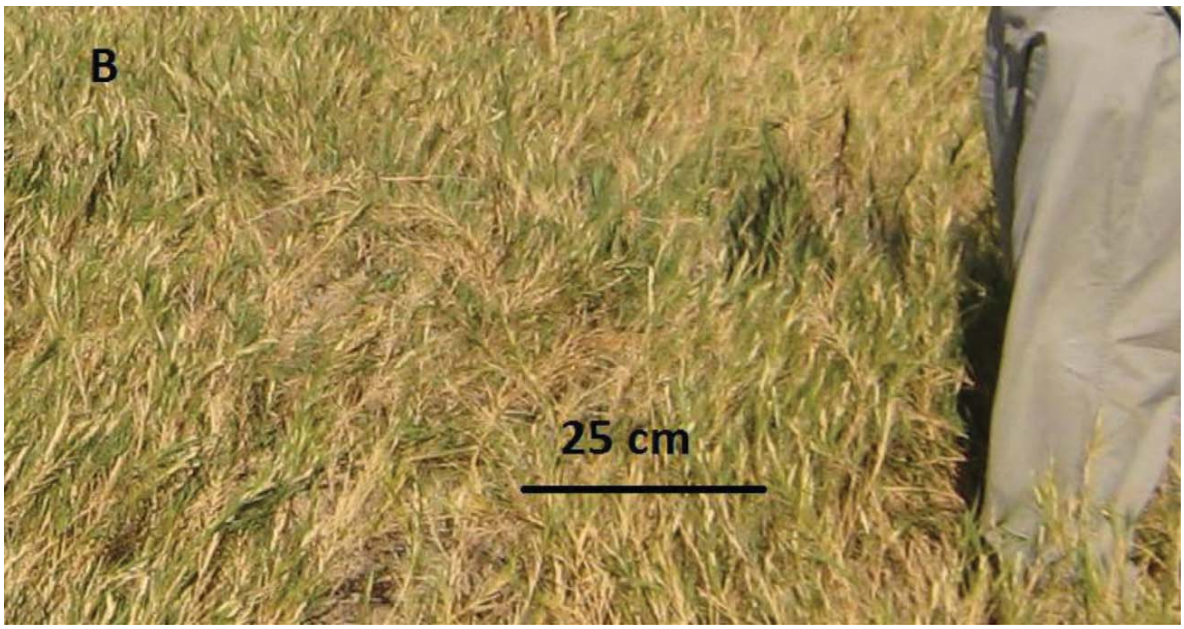
Appendix 1. *Distichlis palmeri* pistillate specimens used as sources material for this study

Mexico: delta region of the Río Colorado. Coordinates recorded in WGS 84 datum. First set of specimens deposited at the University of Arizona Herbarium (ARIZ):
Baja California: Municipio de Mexicali, Río Hardy drainage, ca. 10 km westward from the Sonora border, near NW edge of the Cienega de Santa Clara, 31.97631°N, 115.03699°W, ca. 1 m elev; barren mud flats with *D. palmeri* in patches along canal margins; localized patches of 100% coverage, roots in black, wet mud, soil surface caked in salt; leaf surfaces with salt crystals, nearly every mature stem reproductive, fruits ripe and readily falling; local name is *zacate espinosa*; *Felger 09-42*, with Juan Butrón-Mendez, Alejandra Calvo-Fonseca, Eduardo Soto-Montoya, Benjamin T. Wilder; 13 May 2009; duplicates to ASU, BRIT, CAS, DES, HCIB, IEB, MEXU, MO, NY, RSA, SD, TEX, UNM, UC, US, USON.
Sonora: Municipio de San Luis Río Colorado, NW margin of Cienega de Santa Clara, 32.02779°N, 114.91821°W, near sea level; stagnant, shallow brackish water, muddy anaerobic substrate, and salt crust on dry surface areas between pooling water, but subsurface soil is wet/saturated, with *Allenrolfea occidentalis, Distichlis spicata, Polypogon monspeliensis, Ruppia maritima, Salicornia subterminalis, Spergularia marina, Scirpus maritimus, Tamarix chinensis, Typha domingensis; D. palmeri* locally common; *Felger 09-52*, with Juan Butrón-Mendez, Alejandra Calvo-Fonseca, Eduardo Soto-Montoya, Benjamin T. Wilder, 13 May 2009.
Sonora: Municipio de San Luis Río Colorado, Estero Santa Isabel, ca.1 km inland from the sea, 31.75691°N, 114.58266 °W, sea level; mud flats and tidal sloughs with *D. palmeri* abundant, forming 100% coverage along tidal canals, no other plants seen here; *Felger 09-63*, with Eduardo Soto-Montoya, Benjamin T. Wilder; 13 May 2009; duplicates to CAS, HCIB, IEB, MEXU, MO, RSA, SD, USON.
Río Colorado delta: Isla Montague, Estero El Choyo, E side of island, 31.69229°N, 114.69092°W (WGS 84), sea level; "meadows" of 100% coverage of *D. palmeri* (no other plants seen here), large quantities of fruits (spikelets and florets) in tidal windrows and also numerous mats of detached fruits stranded on top of *Distichlis* stands; *Felger 09-65*, with Eduardo Soto-Montoya, Benjamin T. Wilder, 13 May 2009; duplicates to MEXU, MO, RSA, SD, USON.
Río Colorado Delta. Isla Montague, vicinity of lighthouse at S shore of island, 31.68404°N, 114.72025°W, ca. sea level; extensive *concheras*—vast tidal middens of shell, mostly clams; extensive "meadows" of 100% monospecific coverage of *D. palmeri*, with large quantities of fruits in tidal windrows and also numerous mats of detached fruits stranded on top of *Distichlis* stands; fruits ripe and recently fallen, *Felger 09-67*, with Eduardo Soto-Montoya, Benjamin T. Wilder, 13 May 2009; duplicates to: MEXU, MO, RSA, SD, USON.

Appendix 2. Growth characteristics of *D. palmeri* at the Río Colorado delta. (A) Ben Wilder collecting specimens (Felger 09-67) on Isla Montague (B) Close-up of from 1A showing plants with panicles bearing ripe grain. Photos by RSF, May 13, 2009.



References

Al-Shorepy, S.A., Alhadrami, G.A., El Awad, A.J., 2010. Development of sheep and goat production system based on the use of salt-tolerant plants and marginal resources in the United Arab Emirates. Small Ruminant Research 91, 39–46.

Barret-Lennard, E.C., 2003. The interaction between waterlogging and salinity in higher plants: causes, consequences and implications. Plant and Soil 253, 35–54.

Bell, H.L., 2010. A new species of *Distichlis* (Poaceae, Chloridoideae) from Baja California, Mexico. Madroño 57, 54–63.

Bell, H., Columbus, J.T., 2008. Proposal for an expanded *Distichlis* (Poaceae, Chloridoideae): Support from molecular, morphological and anatomical characters. Systematic Botany 33, 536–551.

Bohannon, J., 2010. The Nile Delta's sinking future. Science 327, 1444–1447.

Bonham, C.B., 1989. Measurements for Terrestrial Vegetation. Wiley Interscience, Hoboken.

Bustan, A., Pasternak, D., Pirogova, I., Durikov, M., Devries, T.T., El-Meccawi, S., Degen, A.A., 2005. Evaluation of saltgrass as a fodder crop for livestock. Journal of the Science of Food and Agriculture 85, 2077–2084.

Castetter, E.F., Bell, W.H., 1951. Yuman Indian Agriculture: Primitive Subsistence on the Lower Colorado and Gila Rivers. University of New Mexico Press, Albuquerque.

Colmer, T.D., Flowers, T.J., 2008. Flooding tolerance in halophytes. New Phytologist 179, 964–974.

Felger, R.S., 2000. Flora of the Gran Desierto and Río Colorado of Northwestern Mexico. University of Arizona Press, Tucson.

Felger, R.S., 2007. Living resources at the center of the Sonoran Desert: native American plant and animal utilization. In: Felger, R.S., Broyles, B. (Eds.), Dry Borders: Great Natural Reserves of the Sonoran Desert. University of Utah Press, Salt Lake City, pp. 147–192.

Felger, R.S., Broyles, B., Wilson, M.F., Nabhan, G.P., Turner, D.S., 2007. Six grand reserves, one grand desert. In: Felger, R.S., Broyles, B. (Eds.), Dry Borders: Great Natural Reserves of the Sonoran Desert. University of Utah Press, Salt Lake City, pp. 3–26.

Felger, R.S., Moser, M.B., 1973. Eelgrass (*Zostera marina* L.) in the Gulf of California: discovery of its nutritional value by the Seri Indians. Science 131, 355–356.

Felger, R.S., Nabhan, G.P., 1978. Agroecosystem diversity: a model from the Sonoran Desert. In: Gonzalez, N.L. (Ed.), Social and Technological Management in the Dry Lands, AAAS Selected Symposium 10. Westview Press, Boulder, pp. 128–149.

Flowers, T.J., Colmer, T.D., 2008. Salinity tolerance in halophytes. New Phytologist 179, 945–963.

Glenn, E.P., 1987. Relationship between cation accumulation and water content of salt-tolerant grasses and a sedge. Plant, Cell & Environment 10, 205–212.

Glenn, E.P., Brown, J.J., Blumwald, E., 1999. Salt tolerance and crop potential of halophytes. Critical Reviews in Plant Sciences 18, 227–255.

Glenn, E.P., Flessa, K.W., Cohen, M.J., Nagler, P.L., Rowell, K., Zamora-Arroyo, F., 2007. Just add water and the Colorado River still reaches the sea. Environmental Management 40, 1–6.

Glenn, E.P., Lee, C., Felger, R., Zengel, S., 1996. Effects of water management on the wetlands of the Colorado River delta, Mexico. Conservation Biology 10, 1175–1186.

Glenn, E.P., Nagler, P.L., Brusca, R.C., Hinojosa-Huerta, O., 2005. Coastal wetlands of the northern Gulf of California: inventory and conservation status. Aquatic Conservation: Marine and Freshwater Ecosystems 16, 5–28.

Glenn, E.P., O'Leary, J.W., 1985. Productivity and irrigation requirements of halophytes grown with seawater in the Sonoran Desert. Journal of Arid Environments 9, 81–91.

Glover, J.D., Reganold, J.P., Bell, L.W., Borevitz, J., Brummer, E.C., Buckler, E.S., Cox, C.M., Cox, T.S., Crews, T.E., Culman, S.W., DeHaan, L.R., Eriksson, D., Gill, B.S., Holland, J., Fu, F., Hulke, B.S., Ibrahim, A.M.H., Jackson, W., Jones, S.S., Murray, S.C., Paterson, A.H., Ploschuk, E., Sacks, E.J., Snapp, S., Tao, D., Van Tassel, D.L., Wade, L.J., Wyse, D.L., Xu, Y., 2010. Increased food and ecosystem security via perennial grains. Science 328, 1638–1639.

Gould, F.W., 1966. Chromosome numbers of some Mexican grasses. Canadian Journal of Botany 44, 1683–1696.

Hansen, D.J., Dayanandan, P., Kaufman, P.B., Brotherson, J.D., 1976. Ecological adaptations of salt marsh grass, *Distichlis spicata* (Gramineae), and environmental factors affecting its growth and distribution. American Journal of Botany 63, 635–650.

Hanser, A.S., 2006. Distichlis spicata. In: Fire Effects Information System (on-line). U.S. Department of Agriculture, Forest Service Fire Science Laboratory. http://www.fs.fed.us/database/feis/ (accessed 15.07.11).

Harrington, J., Reid, S., Black IV, W.C., Brick, M., 2009. Was Rydberg right? Evidence for *Distichlis stricta* as a species distinct from *D. spicata*. Botany & Mycology 2009, Snowbird, Utah, July 25–29, 2009. Abstract. http://2009.botanyconference.org/engine/search/index.php?func=detail&aid=159 (accessed 15.07.11).

Hoffman, W.A., Poorter, H., 2002. Avoiding bias in calculations of relative growth rate. Annals of Botany 90, 37–42.

Leake, J., 2004. NyPa "Wild Wheat" Proving Trials, Final Report. NyPa Australia Limited, Adelaide, South Australia.

López Soto, M.M., Koch, S.D., Flores-Cruz, M., Engelmann, E.M., 2009. Anatomía comparada de la lamina foliar del género *Distichlis* (Poaceae). Acta Botanica Mexicana 89, 1–23.

Löve, A., 1984. Chromosome number reports LXXXII. Taxon 31, 126–134.

Marcum, K.B., Yensen, N.P., Leake, J.E., 2007. Genotypic variation in salinity tolerance of *Distichlis spicata* turf ecotypes. Australian Journal of Experimental Agriculture 47, 1506–1511.

Miyamoto, S., Glenn, E.P., Olsen, M.W., 1996. Growth, water use and salt uptake of four halophytes irrigated with highly saline water. Journal of Arid Environments 32, 141–159.

National Research Council, 1986. United States–Canadian Tables of Feed Composition: Nutritional Data for United States and Canadian Feeds, third ed. National Academies Press, Washington, DC.

Noaman, M.N., 2004. Effect of potassium and nitrogen fertilizers on the growth and biomass of some halophytes grown under high levels of salinity. Journal of Agronomy 3, 25–30.

Noaman, M.N., El-Haddad, E.-S., 2000. Effects of irrigation water salinity and leaching fraction on the growth of six halophyte species. Journal of Agricultural Science 135, 279–285.

Nordblom, T.L., Christy, B.P., Finlayson, J.D., Roberts, A.M., Kelly, J.A., 2010. Least cost land-use changes for targeted catchment salt load and water yield impacts in south eastern Australia. Agricultural Water Management 97, 811–823.

Qureshi, A.S., McCormick, P.G., Qadir, M., Aslam, Z., 2008. Managing salinity and waterlogging in the Indus Basin of Pakistan. Agricultural Water Management 95, 1–10.

Reeder, J.R., 1967. Notes on Mexican grasses. VI. Miscellaneous chromosome numbers. Bulletin of the Torrey Botanical Club 93, 1–17.

Reid, S.D., 2001. Chromosome races and polyploid cytoforms in *Distichlis spicata*. M.S. thesis, Colorado State University, Fort Collins.

Roodt, R., Spies, J.J., 2003. Chromosome studies in the grass subfamily Chloridoideae. I. Basic chromosome numbers. Taxon 52, 557–566.

Rozema, J., Flowers, T., 2008. Crops for a Salinized World. Science 322, 1478–1480.

Sargent, M.R., Tang, C., Sale, P.W.G., 2008. The ability of *Distichlis spicata* to grow sustainably within a saline discharge zone while improving the soil chemical and physical properties. Australian Journal of Soil Research 46, 37–44.

Seraj, Z.I., Salam, M.A., 2000. Growing rice in saline soils: Biotechnological approaches for Bangladesh. Biotechnology Directory (on-line). http://www.apctt.org/publications/tm_dec_grow.pdf (accessed 15.07.11).

Shahba, M.A., Qian, Y.L., 2008. Effect of seeding date, seeding rate, and seed treatments on saltgrass seed germination and establishment. Crop Science 48, 2453–2458.

Sykes, G., 1937. The Colorado River Delta. American Geographical Society Special Publication, no. 19. American Geographical Society, New York.

Vandersande, M.W., Glenn, E.P., Walworth, J.L., 2001. Tolerance of riparian plants from the Lower Colorado River to salinity, drought and inundation. Journal of Arid Environments 49, 147–159.

Vasquez, E.A., Glenn, E.P., Brown, J.J., Gutenspergen, G.R., Nelson, S.G., 2005. Salt tolerance underlies the cryptic invasion of North American salt marshes by an introduced haplotype of the common reed *Phragmites australis* (Poaceae). Marine Ecology Progress Series 298, 1–8.

Vasquez, E.A., Glenn, E.P., Gutenspergen, G.R., Brown, J.J., Nelson, S.G., 2006. Salt tolerance and osmotic adjustment of *Spartina alterniflora* (Poaceae) and the invasive M haplotype of *Phragmites australis* (Poaceae) along a salinity gradient. American Journal of Botany 93, 1784–1790.

Weather.Com, 2010. Average weather for Mexicali, Baja California. http://www.weather.com/outlook/travel/businesstraveler/wxclimatology/monthly/graph/MXBC0004?from=month_bottomnav_business (accessed 15.07.11).

Yensen, N.P., 2006. Halophytes uses for the twenty-first century. In: Khan, M.A., Weber, D.J. (Eds.), Ecophysiology of High Salinity Tolerant Plants. Springer, New York, pp. 367–396.

Yensen, S.B., Weber, C.W., 1986. Composition of *Distichlis palmeri* grain, a saltgrass. Journal of Food Science 51, 1089–1090.

Yensen, S.B., Weber, C.W., 1987. Protein quality of *Distichlis palmeri*, a salt grass. Nutrition Reports International 35, 863–872.