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Distichlis palmeri: Perennial Grain Yields under Saline Paddy-style Cultivation of Grains on Seawater

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Abstract

All our major grain crops are glycophytic grasses that require irrigation with quality water, annual tillage and reseeding of non-salinized soils. Farming of perennial halophytic crops has the potential to circumvent these restrictive issues. The present study reports perennial yields and proximate analysis of *Distichlis palmeri* grain. *D. palmeri* is a C_4 dioecious halophytic grass endemic to the Sea of Cortez where native Cocopah gathered its 8 mm long grain as a wild harvest. We grew first generation *D. palmer* perennially over four consecutive crop years under experimental saline paddy-style conditions that reflect its natural tidal-flat habitat. Mean grain yield from our experimental system with a 1:3 male to female ratio was 176 g m^{-2} (SD = 28.2). Crude protein ranged from 7 – 12.5% with a net energy between 60 – 75%. Grain from Crop 2 germinated and grew under brackish conditions into fully flowering plants in two years. Our model in Tucson, Arizona demonstrates feasibility of developing paddy-style landscape based cropping systems for the domestication of *D. palmeri* as a perennial grain crop for human consumption. Cropping of *D. palmeri* may serve soil remediation and carbon sink functions for salinized soils and as a repurpose for saline waste water supplies.

Keywords: halophyte, perennial grain, paddy cultivation, *Distichlis palmeri*

1. Introduction

All our major grain crops are annual grasses, but a case has been made for developing energy-efficient non-tillage perennial grain crops (Glover et al., 2010; Van Tassel & DeHaan, 2013). Authors (Flowers, 2004; Fedoroff et al., 2010) state that population growth in conjunction with increasing salinity of agricultural lands poses a threat to global food security. While some authors discuss general genetic approaches (Flowers, 2004;Fedoroff et al., 2010) to increase salt tolerance in conventional crops, others make a case for domestication of existing halophytes (Fedoroff et al., 2010; Glenn et al., 1998; Glenn & Brown, 1999; Panta et al., 2014; Rozema & Flowers, 2008) and the potential to use saline waste streams (Bresdin et al., 2016; Glenn et al., 1998; Riley et al., 1997) or seawater (Glenn et al., 2013) as irrigation sources.

The present study reports yields and nutritional value of first generation *Distichlis palmeri* grain when grown perennially under saline paddy conditions that reflect its natural habitat. Our model suggests feasibility of developing landscape based cropping systems for the domestication of *D. palmeri* as a perennial grain crop that may serve remediation and carbon sink functions for salinized soils and water supplies in arid climates.

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Distichlis palmeri is a perennial rhizomous dioecious mudflat saltgrass with aerenchyma tissue and C4 photosynthesis (Bresdin and Glenn, 2016; Glenn et al., 1992). It is endemic to the Colorado River Delta and produces a grain for human consumption that was a wild harvested staple food of native Cocopah people (Felger, 1979, 2000; Pearlstein et al., 2012). Furthermore, it is extremely salt tolerant, growing on seawater with salinities of 38-42 g ml^{-1} under natural conditions (Felger, 2000). *D. palmeri* is distinguished from common saltgrass, *D. spicata,* by culm height (about 1 m versus 0.3 m) and large size of its grain. *D. spicata* caryopses are minute while the caryopsis of *D. palmeri* weighs about 10 mg and is 8 mm long (Bresdin and Glenn, 2016; Pearlstein et al., 2012). Because they are borne on compound racemes, the grains are easily harvested and processed and maybe why they were a major food source for the Cocopah. *D. palmeri* might have evolved from *D. spicata* in response to the annual summer floods from snow melt in the Rocky Mountains that entered the northern Gulf of California in the Colorado River (Felger, 1979, 2000). The relatively large seeds of *D. palmeri* germinate readily in brackish water and the plant is able to colonize large areas of shifting mudflats formed from sediments carried in the river.

These floods were curtailed by construction of upstream dams and diversions of water for agriculture (Nelson et al., 2013). The river only occasionally reaches the sea today. Nevertheless, *D. palmeri* has persisted as the dominant halophyte in the Colorado River delta, forming self-regulating monoculture stands covering several thousand hectares along the banks of the river in its intertidal reach and on Montague Island at the mouth of the river (Nagler et al., 2006, 2009). Dense stands of *D. palmeri* on Montague Island yield an estimated 1250 kg ha^{-1} of grain (Pearlstein et al., 2012) overlapping the low end of cultivated grain crops. It is also the dominant halophyte in the low intertidal zone of the esteros south of the Colorado River delta but above the mangrove line (Nagler et al., 2009).

Interest in *D. palmeri* as a modern grain crop for saltwater agriculture began in the 1970s when the idea emerged of developing new crops for saline soils and waters from wild halophytes (Felger, 1979; Mudie, 1974; Somers, 1979). Several attempts have been made to introduce *D. palmeri* into cultivation as a grain crop. Yensen and Weber (1986, 1987) showed that *D. palmeri* grain had nutritional qualities similar to wheat and other grains. Yensen (2006) patented several selected lines of *D. palmeri* and *D. spicata* for grain and forage production. Short-term trials have shown good vegetative growth, but a low percentage of flowering stems resulted in low grain yields during two years of large-scale field trials in Australia (Leake, 2004).

Pearlstein et al. (2012) revived interest in *D. palmeri* by collecting new germplasm from the Colorado River delta and conducting greenhouse trials. The high salt tolerance, high biomass production under anaerobic soil conditions, and high nutritional value of the grains were confirmed by Pearlstein (2012), and Bresdin and Glenn (2016). However, in two years of greenhouse trials, grain production was very low, only 2% of female plants became reproductive, compared to nearly 100% each year in the natural stands from which the grain was collected. It was speculated that *D. palmeri* might require several years of vegetative growth before reaching its full reproductive potential. The longest growth trials in the greenhouse were two years from germination. In the present research, we continued to cultivate the wild gathered *D. palmeri* outdoors in saline (26-34 g L^{-1} synthetic sea salt solution) paddy-style conditions with undisturbed microbial mats, and over four consecutive crop cycles nearly all stems produced flowers and resulted in perennial grain yields similar to yields of annual domesticated grain.

2. Methods

D. palmeri seeds collected from the wild in May 2009 were sown by Pearlstein in November 2009 and were allowed to mature in a greenhouse under flooded conditions at 10 g L^{-1} salinity until March 2012 when the plants were relocated outdoors at the Environmental Research Laboratory, Tucson, AZ. Plant stock was divided and repotted into seven, 60 cm diameter, 60 cm deep pots (with drainage holes) containing a 2:1 ratio of sand to potting soil. Pots were set into a larger diameter, 60 cm deep tub and filled with flood irrigation water of 10 g L^{-1} synthetic sea salt (Crystal Sea® Marinemix, Marine Enterprises International, Baltimore, MD) consisting of 83% NaCl, 10% $MgSO_4$, 3.5% $CaCl_2$, 3.1% KCl and trace amounts of other salts (Figure 1). Initial absorbed and consumed water was replenished with 10 g L^{-1} irrigation water for the first nine months while outdoors. This allowed time for soil moisture and salinity to stabilize.

Irrigation was then changed to city water, approximately 0.7 g L^{-1}, to make up for water lost to evapotranspiration. Small splits of plants were transplanted and moved back into the greenhouse June 2014 and continued to receive the same irrigation regime as outdoor plants.

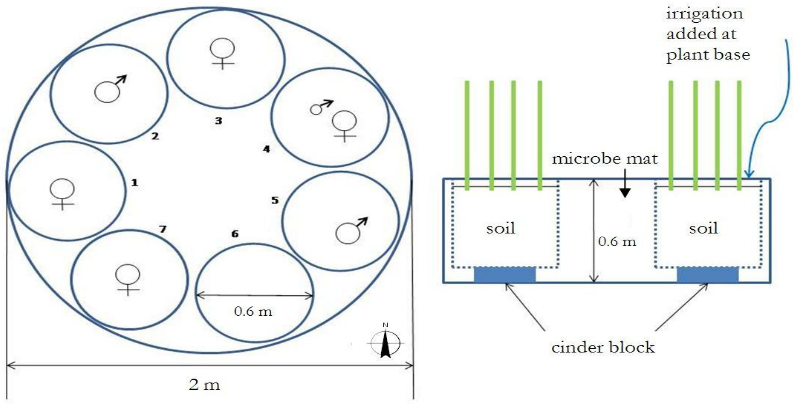

Figure 1: Shows design of the paddy-style cropping system; essentially a water-bath. Plastic pots with drainage were supported on cinder block inside a large tub of saline water. Mosquito fish were added for pest control and a volunteer microbial mat was allowed to self-regulate. Water was replenished as needed with city water added at the base of the plant; an attempt to simulate natural river flow and flush the soil. Numbers are pot numbers for ID purposes. Water salinity (g L^{-1}) was measured with a Traceable probe (VWR® Traceable® Portable Conductivity Meter, model: 23226-505) weekly during the flowering season from mid-February through harvest in May during Crop 2 and 3. Probe calibration was with synthetic sea salt mix and NaCl solutions. Stem density during Crop 2 was counted in two, 10 cm^2 areas per pot, averaged and translated to total stems per m^2.

D. palmeri grain from outdoor pots was harvested over four crop cycles, 2013 to 2015. Crop 1 was harvested May 9, 2013 by cutting all stems in each pot at the base. Total female and male stems per pot were counted. Crop 2 was harvested May 12, 2014 by cutting only mature grain containing stems high on the culm. Percent grain yield per spikelet was determined by weight of grain obtained from manual removal of chaff (Figure 2D) divided by weight of the intact spikelet. Proximate analyses of grain and stems were conducted by Litchfield Analytical Services (Litchfield, MI). A sample of seed from Crop 2 was germinated in 8L pots fall 2014 and maintained in the greenhouse until all plants were moved outdoors March 16, 2015. Crop 3 was harvested April 21, 2015 by cutting only mature, grain containing stems high on the culm. Stem density was not counted for Crop 3. To provide a consistent approach for all crop years, total weight of harvested spikelets was used to calculate field yield estimates for all crop years (Table 2). Tubs were either drained or left to go dry starting late fall 2015 and were dispersed early 2016 due to closing of the Environmental Research laboratory. A few small pots were kept by the authors prior to drying. Bresdin kept two 8 L pots, one female and one male each in a 19 L bucket of saline water that was replenished with tap water at the base of the plant. Grain from the female pot was harvested April 21, 2016 to determine if grain production was sustained over four crop cycles.

3. Results

Under outdoor conditions in Tucson, flowering began in mid-February with males emerging about seven days prior to females on outdoor plants. Unlike previous greenhouse trials, nearly all stems in each outdoor pot were reproductive for crops 1-4. Pots were dominated by female plants (Table 1) to give an overall male to female ratio of approximately 1:3. At an thesis, versatile stamens were a greenish cream and stigmas were purple giving the stand a purple cast. Re-growth of females after Crop 1 harvest was slow because plants were cut back to ground level and had not fully recovered by Crop 2. For subsequent harvests, grain heads were cut high on the culm to encourage re-growth of stems.

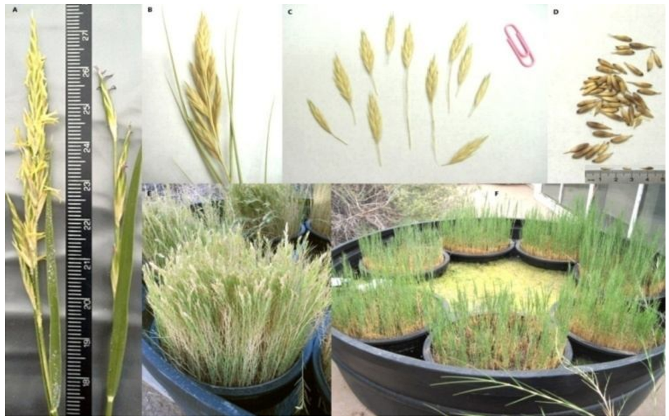

Figure 2: Plant material from outdoor stock grown in 26-34 g L^{-1} TDS paddy with microbial mat: A) male flower (left) female flower (right), cm scale; B) compound raceme; C) spikelets from raceme A; D) mature caryopses from spikelets C indicates purity used for yield by % weight; E) female pot #6, Crop 1 nearing harvest; F) outdoor paddy setup showing the light green algal mat three months after Crop 1 harvest at base of culm, April 2013. Photos taken by Cylphine Bresdin.

Plants that had been split and moved into the greenhouse flowered at equal density to outdoor plants, but about ten days earlier. Male and female flowers emerged concurrently in the greenhouse during Crop 3 (2015); consequently females were receptive prior to pollen maturity and were not fertilized in the absence of wind in the greenhouse. Five random stems from an all-female pot (#1) were used to determine number of caryopsis per spikelet. Number of spikelets per raceme ranged from 5 -7 (SD. = 1). Number florets per spikelet ranged from 2-6 (SD. = 0.9). Number of caryopsis per floret was 1. Percent grain yield per spikelet was 0.67 (SD. = 0.04). Mean individual grain weight was 10 mg (ranged, 6-14 mg, SD. = 2.3). Mean grain size was 8 mm (rang = 6-9 mm, SD. = 0.9). Mean number of grain per raceme was 23 (rang = 18-30, SD. = 5). Mean culm density for Crop 2 was 4791 stems per m^2.

Table 1 shows comparison of grain yield determined by total harvested spikelet weight, 413 g, 466 g, 603 g and 32.5 g, respectively. Mean yield for the four crop years with a 1:3 male to female ratio was 176 g m^{-2} (SD = 28.2). The mean for just female pots was 248 g m^{-2} (SD = 40).

Table 1: Raw crop data per year with/out extrapolation to field yield

		spikelet (g)			
Pot #	Sex	2013	2014	2015	2016
1	Female	123.2	93.1	151.5	32.5
2	Male	0.0	0.0	0.0	0.0
3	Female	88.3	119.1	123.3	
4	Female/Male	45.9	66.5	85.4	
5	Male	23.6	0.0	0.0	
6	Female	77.7	83.9	127.9	
7	Female	54.5	104.3	115.0	
Total		413.4	466.8	603.1	32.5
g grain		276	311	402	22
# grain		27,572	31,138	40,227	2,168
g grain m-2		147	166	214	178

Paddy water salinity increased from 26 g L^{-1} to 32 g L^{-1} during the reproduction phase, with a general trend to increase as ambient temperature increased from February through April (Figure 3).

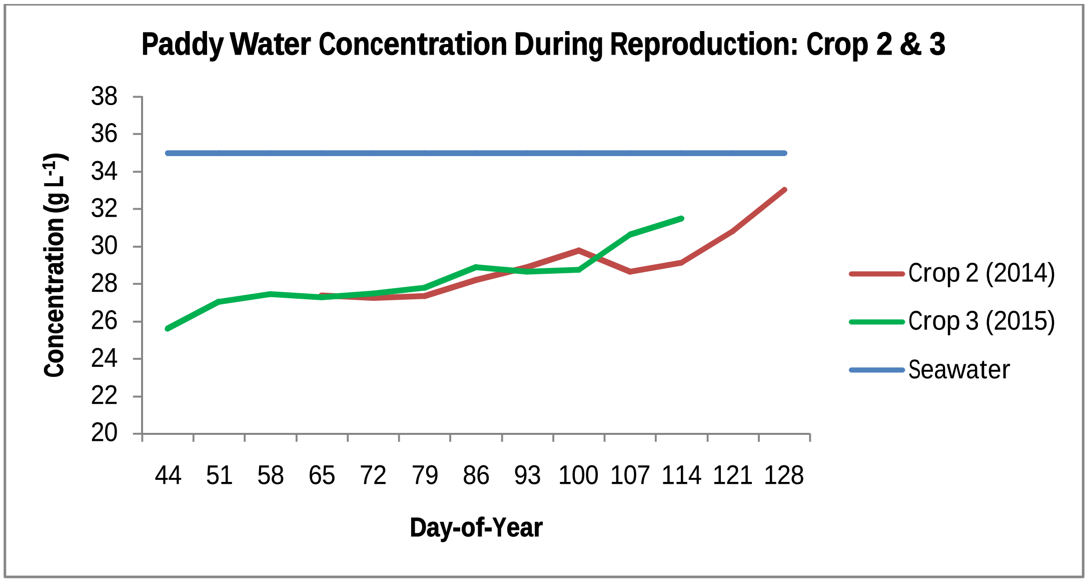

Figure 3: Salinity of water-bath was read as g L^{-1} at the surface and bottom of the tub on a weekly basis through the reproductive period from mid-February through April. The line chart represents the average of surface and bottom readings. There was a minor increase in salinity from Crop 2 to Crop 3, most notable as air temperature rises. Seeds from Crop 2 (2014) were fertile; they germinated and grew outdoors under saline conditions into fully flowering plants in two years. Proximate analysis of *D. palmeri* grain (Table 2) showed 7-12.5% crude protein with an effective net energy between 60-75%, low ash and sodium content, and high iron. Since thrashed biomass has potential as feedstock, stems were analyzed. Digestible carbohydrates were the main constituents of both grain and stems. Crude fiber was 38% of the stem material which was higher in calcium, sodium and magnesium than the grain.

Table 2: Proximate analysis of *Distichlis palmeri*.

Constituent	2013	2014	USDA Nutritional Database			
	grain	grain	quinoa	Rice	Wheat (durum)	stems
Crude protein (%)	7.22	12.54	14.12	7.94	13.68	6.04
Acid detergent fiber (%)	4.61	5.68	-	-	-	37.98
Crude fiber	3.68	4.54	7.00	3.50	-	30.38
Digestible carbohydrates (%)	68.22	71.29	64.16	77.24	71.13	45.85
Ash (%)	1.06	2.38	-	-	-	4.29
Fat (%)	1.68	1.33	6.07	2.92	2.47	1.26
Total digestible nutrients (%)	77.88	85.75	-	-	-	67.33
Effective net energy (%)	67.27	75.11	-	-	-	56.81
Digestible energy (Mcal/kg)	0.71	0.78	0.37	0.37	0.34	0.61
Phosphorus (%)	0.26	0.36	0.46	0.33	0.51	0.15
Calcium (%)	0.04	0.06	0.05	0.23	0.03	0.33
Potassium (%)	0.68	0.79	0.56	0.22	0.43	0.47
Sodium (%)	0.09	0.1	-	-	-	0.49
Magnesium (%)	0.07	0.09	0.20	0.14	0.14	0.18
Iron ppm	116	28	-	-	-	107

[1]Grain from Crop 1, and grain and biomass from Crop 2 grown outdoors under saline paddy style agronomic conditions with 20-34 g L^{-1} synthetic sea water are compared to values published for traditional grains (USDA nutritional database, http://ndb.nal.usda.gov/ndb).

4. Discussion

Based on comparison to conventional grain crops, nutritional data and grain size of *D. palmeri* is most similar to rice. According to ricepedia.org, rice (an annual crop) yields averaged 2 t ha^{-1} in 1960 and rose to over 4 t ha^{-1} in 2000 due to the Green Revolution and genetic modifications. With a 1:3 male: female ratio of non-modified and unfertilized stock, our average yield over four perennial crop years was 1.76 metric ton ha^{-1}. If we increase the number of females to males, we could obtain higher yields per area. The optimum ratio needs to be determined. The grain yield from female pots alone was equivalent to 2.48 metric ton ha^{-1}. Our yields tended to increase over time. This could be due to any combination of: increase in system salinity, restriction of roots, plant maturation or maturation of symbiotic relationships with microbial components. Increase in salinity was less than expected over the four years of outdoor paddy culture, suggesting that cultivation of *D. palmeri* with high nutrient waste saline waters might turn a nuisance into an asset. Salinities of the water were in the range of seawater, another possible irrigation source.

5. Conclusion

D. palmeri is a perennial salt water grass with high agricultural potential that flourishes under saline-hydric soils and anaerobic conditions in arid climates. It has a future as a staple grain producing perennial crop and potential to restructure soil and sequester carbon through a constructive use of waterlogged salinized lands as well as a potential to reduce volumes of saline waste streams.

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