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Saltgrass (*Distichlis spicata*) – A New Salt-Resistant Forage Crop for the Reclamation of Salinized Soils

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C. Executive Summary

The Aral Sea Basin, shared by several Central Asian Republics, has been affected strongly by severe salinity problems, a consequence of a long period (1920's – 1970's) of soil and water abuse. As a result, most arable lands were subjected to soil salinization and consequently abandoned. In a reclamation process of these lands, large quantities of water are required to leach the salt from plant roots. The high expenses involved in such a process can be partially compensated if accompanied by profitable salt-resistant crops, preferably fodder for the important livestock production typical to this region. However, most crops would not meet these requirements due to the harsh climate conditions of the Aral Sea basin. In the present project, we proposed to examine the potential of saltgrass (*Distichlis spicata*, Poaceae), an American salt marsh plant species, as a forage crop for saline environments, to select promising accessions, and to test their performance in Israel and in Turkmenistan. We have established a large collection of saltgrass accessions, some of which were identified as of considerable potential for high quality fodder production. Further examinations included the effects of salinity on the yields and the quality of the selected accessions. In Turkmenistan, although saltgrass accessions were brought there at a very early stage of the project, the plants did not produce sufficient biomass for further, large-scale field experiments and animal feed tests. This resulted from several unexpected reasons which included low propagation rates, severe climate conditions, and lack of infrastructure such as available water and electricity systems for field experiments and vehicles to reach trials at frequent intervals. However, in worldwide agricultural aspects, we have shed light on a plant species with a considerable potential to become a successful forage crop for saline environments.

D. Research Objectives

Almost 1 billion hectares of land, which accounts for 10% of the earth's surface, are affected by salinity and over 50% of all irrigated lands have a salinity problem (Flowers & Yeo 1995; Flowers & Flowers 2005). Most crop plants are glycophytes or non-halophytes that do not express their full genetic potential for growth, development, and productivity under salt stress, (Läuchli & Epstein

1990; Munns 1993). Salt-affected soils are most often the result of human activity, mainly through mismanagement of irrigation systems (Rhoades 1990). In irrigated lands, salinization of the soil occurs where evapo-transpiration rates exceed irrigation rates or where drainage capacity of the soil is low. In most arid and semiarid lands, deficit irrigation is practiced due to water scarcity. The diminishing supply of good-quality water for irrigation often results in the use of low quality water, high in salinity (Pasternak 1987). In addition, low awareness of these problems leads to poor drainage infrastructure, which in turn accelerates soil salinization processes (Rhoades 1990).

The environmental catastrophe of the Aral Sea basin has become a classic example of anthropogenic abuse of lands and waters. In this huge region, shared by the Republics of Central Asia, large areas, sometimes more than half of the farmlands, have been taken out of production due to severe salinization of soils and water sources, thus entailing serious food security problems (Micklin 1992). The reclamation of those saline lands must involve salt-resistant plants of economical value. Livestock production, mainly Karakul sheep, goats, and Asian camels, is of major economical importance in the Aral Sea basin. Based solely on grazing, animal performance can be low due to low crude protein and low metabolizable energy intake from the local herbage and shrubs (Degen *et al.* 2002). Salt-resistant forage crops can be introduced to salinized lands to serve as a primary stage of the reclamation of former agricultural lands and also provide alternative feeding sources for livestock.

Saltgrass (*Distichlis spicata* L., Poaceae) has been shown to have the potential of being such a crop (Ginzo *et al.* 1986; Brinzela *et al.* 1990; Pasternak *et al.* 1993). Saltgrass is a plant species native to coastal and inland highly saline environments in America. Unlike other halophytes, saltgrass avoids salt accumulation in aboveground tissues, and it has the potential to obtain considerable, good quality yields of fodder, even at high salinity levels (EC_i>10 $dS \cdot m^{-1}$). Yet, saltgrass is a wild plant species, which requires careful selection and domestication processes before it becomes a useful crop.

The objectives of this project were: 1) to evaluate a wide spectrum of *D. spicata* ecotypes for forage quality potential; 2) to study growth response of selected genotypes to salinity as an indicator of productivity in saline environments; 3) to examine the adaptability of saltgrass accessions to the

climatic and edaphic conditions in Turkmenistan; 4) to carry out field evaluation of forage productivity, quality, and feed value of the selected accessions under saline conditions in Turkmenistan.

The salinity problem in agriculture may be solved using huge amounts of fresh water in combination with extremely expensive investments in efficient drainage systems in order to leach the salt from the upper soil profile. Because of the large expense of such a project, the solution must evolve from the domestication of salt-resistant plant species or through the improvement of salt resistance among important crops (utilizing halophytes as the sole genetic source available for this trait) (Serrano 1996). The present project addresses this possibility directly and furthermore, it has been aimed at providing a practical answer to the crucial problem of the Aral Sea Basin.

E. Methods and Results

E.1. Phases of project implementation

The project was divided into several phases. The first one, which took place in 1998 before the embarking of the US-AID/CDR/CAR support, included saltgrass collection missions in the USA and South America, followed by genetic characterization of the collected accessions (Ram *et al.* 2004).

In the second phase, we evaluated a broad collection of saltgrass accessions as fodder crops in order to select candidates to be used for future breeding programs. Fodder quality of a crop is complex to determine, as it involves parameters such as yield and chemical composition, as well as aspects such as digestibility and palatability. Desirable traits, namely high crude protein (CP) content, metabolizable energy (ME), organic matter digestibility (OMD), and dry matter (DM), low ash content and neutral-detergent fiber (NDF), and a high rate of leafiness (LFS) were used to define a quality index (QI) in order to assess the potential of a saltgrass accession to become a forage crop. We also sought possible relationships between geographical origin and quality traits that might indicate to us specific collection missions aimed at expanding the available saltgrass germplasm.

In the third, phase, before large scale field experiments are carried out, the growth response at a wide range of salinity levels was used as an indication for the potential performance of selected saltgrass accessions in saline

environments. Outstanding accessions were propagated and further examined for their performance under various fertilization and salinity combinations at a small scale field experiment.

For the forth phase of the project, promising accessions were sent to Turkmenistan twice: at an early stage of saltgrass examinations (2002) as well as at a later stage (2004). This phase of research was aimed at testing the adaptability of the chosen accessions to the climatic and edaphic conditions in Turkmenistan and consequently, at carrying out field evaluation of forage productivity and quality, including animal feed trials with local Karakul sheep.

E.2. Phase 1 – Saltgrass collection and genetic characterization

Plants were collected from several locations in the USA and South America (Table 1). Most plants were collected from the wild on May and June 1998. In the USA, plants were collected from a few sites at distances ranging from 10^2 to 10^4 km. In California, several ecotypes were obtained from a collection of saltgrass from various known sites in the Central Valley (Dr. Dyer, USDA, Soil Conservation Service, Lockeford, CA). Several ecotypes that originated in other parts of the USA were obtained from a collection in Delaware (Prof. Gallagher, College of Marine Studies, University of Delaware, Lewes, DE). For South American ecotypes, each plant was the only representative of a region.

At each site, several (2-5) vital rhizomes were dug out, washed from soil and organic matter, wrapped with wet paper and kept in plastic bags in a cool-box (5-15°C) for 2-5 weeks before being planted in pots containing perlite and peat (1:1). Plants were kept in quarantine for 5 months before being transferred to a greenhouse at Ben-Gurion University of the Negev (Beer Sheva, Israel). When plants were established, one rhizome from each bucket representing a different location was cut and replanted individually in a pot and was considered as an accession, as confirmed by RAPD analysis (Ram *et al.* 2004).

Table 1: *Sampling sites of saltgrass ecotypes. I – Inland ecotypes; S – seashore ecotypes; TMS - tidal marsh on seashore; OU- uncertain origin; TE – tidal estuary; ISM – inland saline marsh or lake, seasonally inundated; RB- river or ditch bank, seasonally inundated*

Ecotype	Country / State	County / Location	I / S	Ecosystem
CT1,CT3	Connecticut	New London / Barn Island	S	TMS
CT2	"	New London / near Mystic	S	TMS
DE1,2,4,5	Delaware	Sussex / near Lewes	S	TMS
DE3	"	Lewes, UDE collection	S	UO
GA1-8	Georgia	McIntosh / Sapelo Island	S	TMS
AL1	Alabama	Mobile / Deer River	S	TE
AL2	"	Mobile / Dauphin Island	S	TMS
AL3-6	"	Mobile / Heron Bay	S	TMS
UT1	Utah	Goshen	I	ISM
UT2	"	Big Salt Lake	I	ISM
UT3	"	Vernon	I	RB
UT4	"	Jordan River	I	RB
CA1	California	Mendota	I	UO
CA2	California	Riverside / Salton Sea	I	ISM
CA3,13,16	"	Kings / Tulare Lake-bed	I	RB
CA4,14	"	S. Luis Obispo/Soda Lake	I	ISM
CA5,17	"	Fresno / Tranquillity	I	RB
CA6	"	S. Luis Obispo/Morro Bay	S	TE
CA7	"	Merced / Hartley Slough	I	ISM
CA8,15	"	San Joaquin	I	RB
CA9	"	San Diego / Mission bank	S	RB
CA10	"	Tulare / Alpaugh	I	RB
CA11	"	Monterey/Elkhorn Slough	S	TE
CA12,18	"	Kings / Kettleman city	I	RB
CH1	Chile	Tamarugal	I	ISM
CH2	Chile	Juta Valley	I	ISM
Arg1	Argentina	Trelew Chubut	I	RB, TE
Arg2	Argentina	Chinchina La Rioja	I	ISM

E.3. Phase 2 – Evaluation of Potential Forage Quality

Accessions were replicated and grown in pots containing perlite and peat (1:1), drip-irrigated with fresh water (1.5 $dS \cdot m^{-1}$) and on-line liquid fertilizer (Sheffer 5:3:8 (N:P:K, respectively), Fertilizers & Chemicals Ltd., Israel) supply at 50 ppm N. The above-ground portion of the plants was harvested on July 2002, before the occurrence of any signs of bloom.

Seeking tools to discriminate between saltgrass accessions and to evaluate their potential appeal for herbivores, we broadened 'leafiness' to define a new term - 'LFS', which included additional descriptive parameters. Shortly before harvest, leaf/whole-canopy ratio of each accession was determined and shoot softness, vitality, and lushness were ranked from 1 (low) to 10 (high). These evaluations were made among the saltgrass accessions, and not against other grass species.

Harvested plant material was dried in an oven at 50°C until constant mass

to determine dry matter content (%DM). Dry matter was ground to fine powder, which was used for further analyses. Samples were analyzed for nitrogen (N) content by the Kjeldahl method (Bradstreet 1965) and for ash by burning at 550°C (AOAC, 1990). Crude protein was calculated as 6.25·N. Neutral detergent fiber (NDF) content was determined as described by Van Soest *et al.* (1991), applying a Fiertec System M6 (tecator, Haganas, Sweden). All measurements were done in triplicate.

Organic matter digestibility and metabolizable energy yield of the saltgrass samples were estimated *in vitro* using the Hohenheimer Gas method (Menke *et al.* 1979; Menke and Steingass 1988). In this method, the gas produced in anaerobic fermentation of substrate is used to predict the nutritive value. Rumen liquor and particulate matter were collected before morning feeding via a rumen fistula from two sheep and two goats fed on a roughage diet of mainly poor quality wheat straw and some lucerne hay; the liquor was homogenized, strained and filtered through glass wool. Incubation media was prepared as described by Menke *et al.* (1979). Samples, each of 200 mg DM, were incubated in triplicate in 100 ml calibrated glass syringes in which 30 ml of the incubation media was added. The glassware was kept at 39°C and flushed with CO_2 before use and the mixture was kept stirred under CO_2 at 39°C. Gas production, as determined by piston movement, was measured over 24 h after correcting for gas production due to rumen fluid alone. Gas production (GP, ml/200 mg DM) and crude protein (CP, g/kg DM) were used to estimate organic matter digestibility (OMD) and *in vitro* metabolizable energy (IVME) yield as follows:

$$\text{OMD (\%)} = 24.91 + 0.722\ \text{GP} + 0.0815\ \text{CP}$$

and,

$$\text{IVME (kJ/g DM)} = 2.20 + 0.136\ \text{GP} + 0.0057\ \text{CP}$$

To rank the saltgrass accessions, a modified version of the method described by Conklin *et al.* (1991) was adopted. The mean of each quality parameter (DM, ash, CP, NDF, ME, and LFS) was subtracted from the value obtained by each accession and the result was divided by the SD of the mean to produce a specific parameter index. For example, the equation of the IVME index took the form:

$$IVMEIx = (\text{accession } IVME - \text{mean } IVME)/SD$$

In this case, calculations were made for sheep and goats separately, but the average of IVMEIx for sheep and goats was calculated (MEIx) and used for further determination of the quality index (QI). QI was calculated for each saltgrass accession as follows:

$$QI = DMIx - AshIx + CPIx - NDFIx + MEIx + LFSIx + 10$$

Statistical differences between groups of saltgrass accessions (country of origin) or between ecosystems of origin (inland *vs*. seashore) were determined using a one-factorial Analysis of Variance (ANOVA) followed by a Fischer's PLSD test. PCA was exercised on the covariance matrix of all quality parameters or on the most important two of them (CP and ME) with the MVSP package (Kovach, 1999) in order to obtain a two-dimensional ordination pattern of saltgrass accessions using various geographical or eco-geographical parameters of sorting.

Mean DM, ash, and NDF contents for all saltgrass accessions examined in the present study were 43.1, 6.83, and 72%, respectively (Table 2). Crude protein content averaged 11.6% and ranged from a low of 6.9% to a high of 18.9%. *In vitro* metabolizable energy yields and organic matter digestibility were higher in sheep rumen fluid than in goat rumen fluid for all saltgrass accessions. Mean metabolizable energy yield for sheep was 6.30±0.66, ranging between 4.71 and 8.63 kJ/g DM, whereas in goats, the mean was 5.61±0.75, ranging from 3.21 to 6.79 kJ/g DM. Mean organic matter digestibility (OMD) was 50.6±2.64 % for sheep and 47.8±2.55 % for goats. LFS averaged 6.96 with large differences between accessions within and, especially, among groups from distinct origins.

Table 2: *Dry matter (DM), ash, crude protein (CP) and neutral detergent fiber (NDF) contents, leafiness (LFS), and in vitro metabolizable energy (ME) and organic matter digestibility (OMD) yields (using sheep and goat rumen fluids) of 48 saltgrass accessions originated from various regions (states; df=6) or ecosystems (seashore vs. inland; df=1) of the American continent.*

Origin	n	DM	Ash	CP	NDF	ME-sheep	ME-goats	ME average	OMD	LFS	QI
		(%)	(% DM)			(kJ/g DM)			(%)		
GA	8	43.9	6.10	9.56	73.6	6.65	6.44	6.55	50.5	8.38	11.6
S. Amer	4	40.5	8.06	14.39	72.8	7.03	6.52	6.64	53.8	7.25	10.4
DE	5	43.2	7.15	13.03	71.2	6.44	5.45	5.95	49.3	5.60	9.4
AL	6	45.4	6.73	9.84	77.7	6.22	5.90	5.88	46.6	8.33	9.8
CT	3	43.7	7.24	14.27	76.0	5.98	5.36	5.67	44.5	7.67	9.1
CA	18	42.8	6.78	11.96	69.3	6.22	5.37	5.79	48.0	6.33	9.9
UT	4	41.1	6.73	12.70	70.0	5.47	5.20	5.34	46.2	5.75	8.6
		NS	NS	***	NS	*	***	**	**	***	*
Seashore	27	43.7	6.56	10.96	72.8	6.35	5.82	6.09	49.0	7.32	10.2
Inland	21	42.4	7.10	12.70	70.5	6.23	5.52	5.88	48.8	6.40	9.8
		NS	NS	**	NS	NS	NS	NS	NS	*	NS
Total mean		43.1	6.83	11.57	72.0	6.30	5.63	5.92	48.5	6.96	10.0
SD		3.47	1.06	2.22	5.29	0.66	0.75	0.71	3.81	1.30	2.04

NS, not significant.

*, **, and *** indicate for significant differences at P<0.05, 0.01, and 0.001, respectively.

PCA analyses (that included all quality parameters) of all saltgrass accessions were carried out to reveal possible clustering patterns according to countries or ecosystem (seashore or inland) of origin. GA accessions clustered separately whereas all other accessions were scattered randomly (Fig. 1A). No separate clustering of inland or seashore accessions could be observed, in spite of the segregation of the seashore GA group from the generally mixed scattering (Fig. 1B).

In a simpler analysis, where only two quality parameters (CP and ME) were used (Fig. 2A), most GA accessions grouped together at the upper left quarter of the array (negative CPIx and positive MEIx). In the same analysis, 3 of 4 South American accessions were located at the upper right quarter, indicating positive CPIx and MEIx. All AL accessions were at the left half of the CP axis (negative CPIx). The CA accessions that comprised the largest group scattered randomly among all quarters of the array, as well as the other accessions (Fig. 2A). When analyzed according to inland or seashore origin, about 70% of

seashore accessions were left of the CP axis (negative CPIx), while 70% of the inland were at the opposite side (Fig. 2B). However, the ME axis did not have any discriminating effect on either of the two groups.

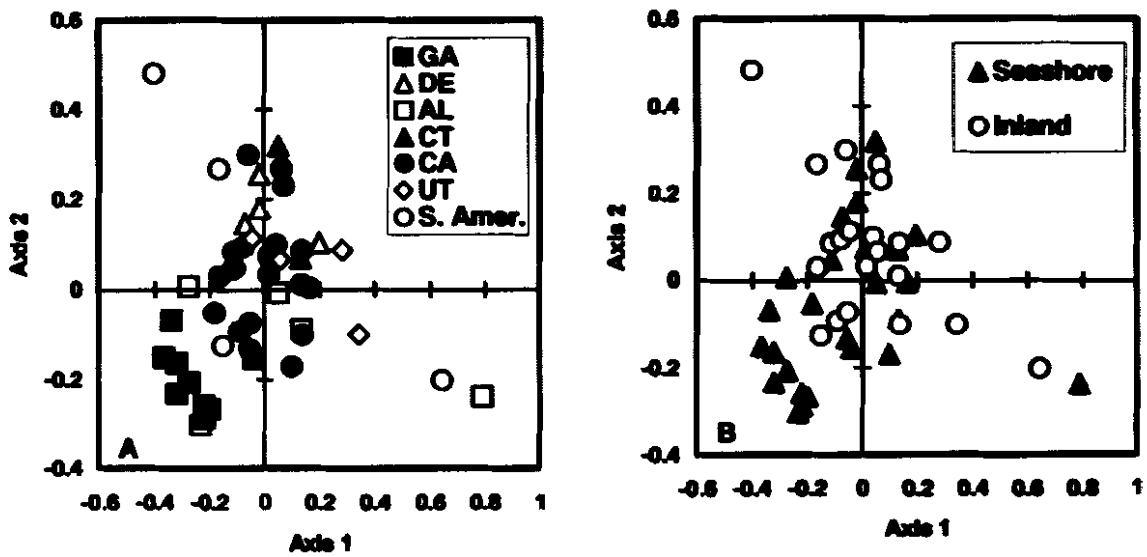

Fig. 1. PCA analyses of fodder quality results (all quality parameters included) of 48 saltgrass accessions. A. Accessions are sorted according to country of origin. B. accessions are labeled according to their ecosystem of origin.

Dissection of all quality criteria according to the country of origin revealed no significant differences for DM, ash, and NDF contents (Table 2). However, significant differences were found for CP, ME, OMD, and LFS. When ranked by quality index (QI), the GA group was significantly better than all groups except for the S. American group (Table 2). The GA group obtained the highest LFS, had reasonably high ME and OMD but the lowest CP mean, while the S. American group had the highest CP, ME, and OMD but a relatively low LFS. All other groups had QI values lower than 10, indicating relatively poorer quality (Table 2), although some obtained quite good values for one parameter or another. Dissection for inland *vs.* seashore accessions showed a significant advantage in LFS for seashore ecotypes, higher CP content for inland accessions, and no significant difference for all other quality parameters (Table 2). The top six ranked saltgrass accessions, according to QI, are presented in Table 3.

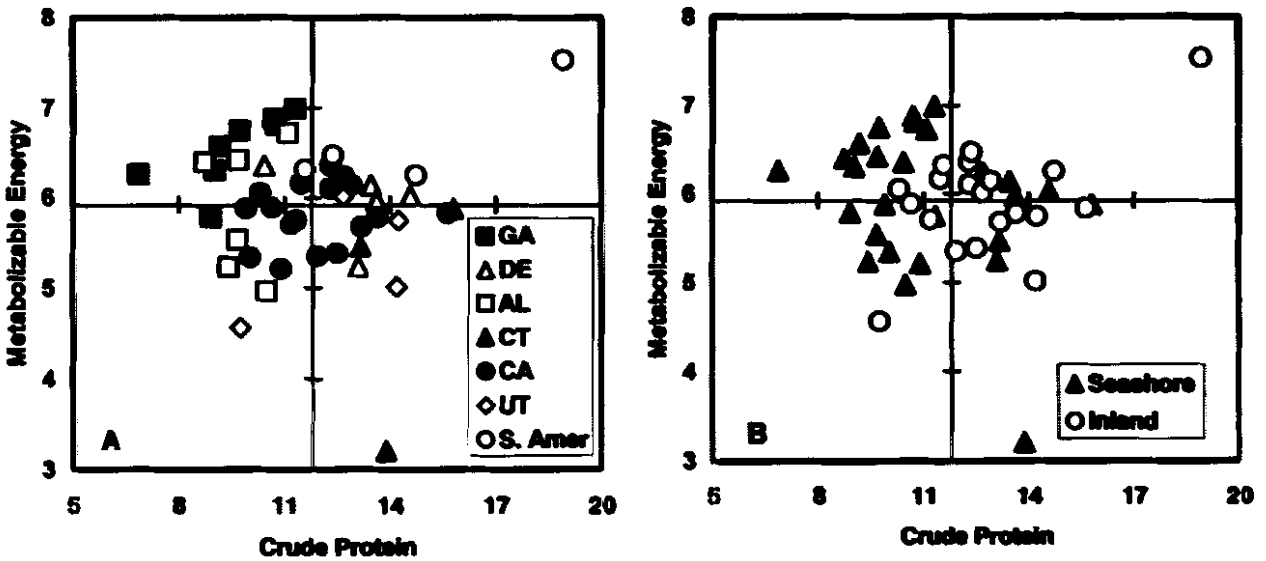

Fig. 2. Cluster analyses of 48 saltgrass accessions according to crude protein *vs.* metabolizable energy contents. Accessions are labeled according to country of origin (A) or ecosystem (B).

Table 3: *Forage quality properties of the best six saltgrass accessions, ranked according to the quality index (QI) described in the text.*

Acc.	Origin	DM	Ash	CP	NDF	ME	OMD	LFS	QI
		(%)	(% from DM)			(kJ/g DM)	(%)		
CH1	Tamarugal, Chile	41.9	7.7	18.9	70.6	7.53	58.4	7	14.65
GA2	Sapelo Island, GA	43.6	6.0	11.3	67.9	6.99	53.6	9	14.59
GA6	Sapelo Island, GA	44.9	7.0	10.7	66.7	6.88	52.8	9	13.85
GA7	Sapelo Island, GA	43.6	6.1	9.2	70.1	6.57	50.4	9	12.53
DE3	Lewes, DE	50.1	7.2	14.6	67.2	6.02	50.5	5	12.50
CA17	Tranquility, CA	43.7	7.0	13.6	64.8	5.78	48.8	7	12.03

E.4. Phase 3 - Growth and Quality Responses to Salinity

Ramets of each accession were cut and rooted in water culture. Accessions that displayed high rates of rooting and of vegetative growth resumption were further examined for their growth response to salinity. Uniform plants (n = 36) of each selected accession were transferred to a glasshouse under full sunlight to

grow in water culture in 1-L containers. Salinity level was gradually increased to produce six salinity treatments (1.5, 10, 20, 30, 40, 50 $dS \cdot m^{-1}$) obtained by $NaCl:CaCl_2$ solution at 3:1 ratio, respectively. The growing solution contained fertilizers (N:P:K, 5:3:8, respectively, at 50 ppm N), was maintained at a constant volume by adding fresh water when necessary, and was replaced weekly. Aeration with a bubbling system did not have any considerable influence on growth and, therefore, was discarded. Plant growth (whole plant fresh weight) was recorded weekly during 10 weeks in summer. The relative growth rate (RGR) became stable towards the 6th week and thereafter, and the average RGR of weeks 6-8 was chosen as an indicator for growth response to salinity.

Most saltgrass accessions that were examined under six different levels of water salinity displayed a similar pattern of RGR response to salinity. The highest RGR was obtained when grown with fresh water (1.5 $dS \cdot m^{-1}$) but it declined sharply at 10 $dS \cdot m^{-1}$, and decreased moderately or became stable as salinity was increased up to 50 $dS \cdot m^{-1}$. In a few exceptional accessions from DE and CA, RGR pattern was different, beginning its decline only at 30 $dS \cdot m^{-1}$, yet their initial RGR at fresh water were usually very low.

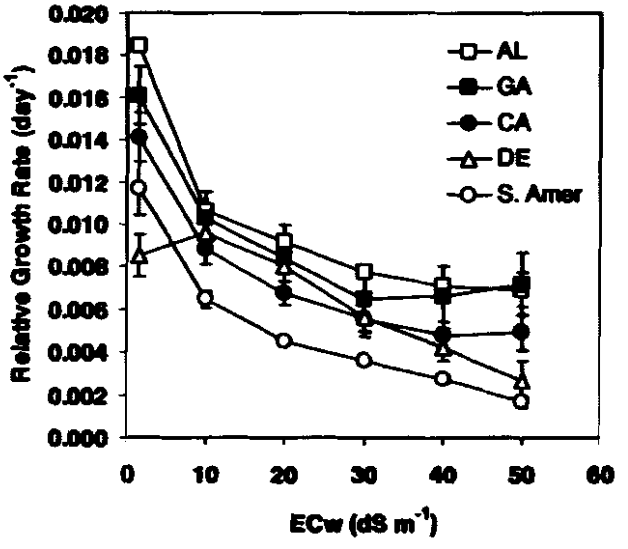

Fig. 3. RGR (relative growth rate) response to water salinity level of saltgrass plants grown hydroponically. Compared are five groups of saltgrass accessions sorted according to country of origin (mean ± SE).

GA and AL accessions had the highest RGR throughout all salinity levels (including fresh water), with a relatively stable performance (>0.008 day^{-1}) between 20 and 50 $dS \cdot m^{-1}$. The S. American accessions displayed the lowest RGR values (<0.004 day^{-1}), indicating low vigor under highly saline environments (>20 $dS \cdot m^{-1}$) (Fig. 3). From the six accessions selected (Table 3),

five were examined for RGR response to salinity (Fig. 4). GA6 showed obvious superior performance at both fresh and saline conditions, even at very high salinity levels. GA2 displayed a similar pattern but at much lower RGR levels. DE3 had substantially low RGR when grown with fresh water, however, it remained stable as salinity was increased to 20 $dS \cdot m^{-1}$ and declined sharply at 30 $dS \cdot m^{-1}$ and above. CA17 and CH1 displayed the poorest performance even at 10 $dS \cdot m^{-1}$.

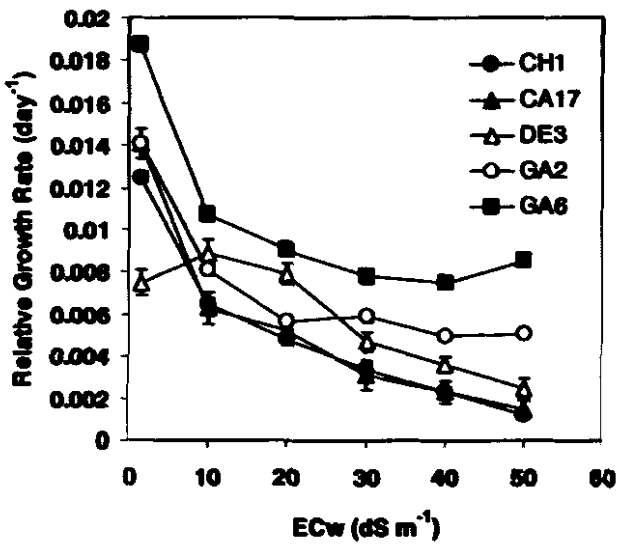

Fig. 4. Comparison of RGR response to water salinity level between five accessions (out of six) that displayed the highest quality index (QI) among our saltgrass collection (see also Tables 2 and 3). Values represent means (±SE) of 6 replications per accession per salinity level.

Accessions GA6 and CH1 were tested in two additional experiments aimed at evaluating their response to fertilizer levels on a background of salinity. In the first experiment, plants were grown under full sunlight in polystyrene boxes (100x40x18 cm each) containing a well-drained perlite-2 medium. Plants were drip-irrigated with fresh (1.5 $dS \cdot m^{-1}$) or saline (7 $dS \cdot m^{-1}$) water and with an on-line liquid fertilizer (6:6:6 (% N:P:K, respectively), Fertilizers & Chemicals Ltd., Israel) supply at 15, 30, 60, or 120 ppm N. Each combination of salinity and fertilization level had four repetitions. Irrigation took place 3 times a day at 150% of daily evaporation (as recorded by a nearby meteorological station). Drainage water was automatically collected and measured daily. Electrical conductivity, pH and N concentration in drainage water were recorded once a week. The growing season began on March and four successive harvests were obtained until November. Yield and quality parameters such as dry matter (DM), ash, and crude protein (CP) contents were recorded yearly. The experiment was conducted twice, in years 2003 and 2004 and the results are summarized in Table 4.

Table 4: Yield and forage quality parameters of two saltgrass accessions (CH1 and GA6) grown with fresh or saline irrigation water at four levels of fertilization in a soilless culture (see also text). Figures are means of 4 replications and 8 harvests during years 2003 and 2004, between March and November.

	Saline (1.1-1.5 dS·m^{-1})				Fresh (6.8-7.2 dS·m^{-1})			
N (ppm)	15	30	60	120	15	30	60	120
Yield (kgFW/m^2)								
CH1	1.2d	1.8c	2.4c	3.3b	1.4d	2.1c	2.9b	3.7a
GA6	0.9d	1.7c	2.6c	3.8b	1.5c	2.2c	3.4b	4.3a
	ns	ns	ns	*	ns	ns	*	*
DM content (%)								
CH1	48.3a	47.9ab	45.2b	44.6b	45.0b	44.2b	42.1c	41.5c
GA6	50.1a	48.7a	45.3b	43.0bc	44.1b	43.7b	43.3bc	42.1c
	*	ns	ns	ns	ns	ns	*	ns
Ash content (% of DM)								
CH1	10.5a	10.4a	10.2a	9.6a	8.3b	7.9bc	7.7bc	7.5c
GA6	9.2a	9.0a	8.8a	8.4ab	7.7b	7.4b	7.1bc	6.8c
	*	*	*	*	*	ns	*	*
CP content (% of DM)								
CH1	6.2e	7.5d	11.2c	12.8bc	10.3c	13.2bc	18.8a	19.1a
GA6	6.1d	8.2c	9.5bc	10.6b	6.6d	8.8c	10.9b	13.1a
	ns	ns	*	*	***	***	***	***

ns, not significant; *, **, and *** indicate for significant differences at P<0.05, 0.01, and 0.001, respectively, within columns in each section. Different letters indicate for significant differences within a row.

Both saltgrass accessions were strongly affected by salt stress, which caused a significant reduction (14 and 21% in CH1 and GA6, respectively) in yield. On the other hand, the response to increased fertilizer level was far more dramatic; yields were 2.6 to 4 times higher at the highest fertilization level (120 ppm N) than at the lowest one (15 ppm N). Furthermore, at the highest fertilization level, the differences in yield between fresh and saline water plants declined to 11% in both accessions (Table 4).

The dry matter content was higher at the saline treatment but tended to decrease with the increasing fertilization levels. As may be expected, the ash content was significantly higher at the saline treatment, however, the absolute levels of ash content at the salinity levels practiced in this experiment were still low enough to be at the acceptable range for fodder. Also in this case, the ash contents tended to decline as the fertilization level increased (Table 4).

Nevertheless, the most profound impact of fertilization was on the content of

crude protein, which was doubled from the lowest to the highest level of fertilizer. Salt stress, on the other hand, considerably reduced the CP content, particularly in accession CH1 (Table 4). The differences between the two accessions were most obvious in the parameters of ash content and CP, and were especially pronounced in the response to fertilization under fresh water irrigation.

The second experiment was conducted on a small plot (about 200 m^2) of sandy soil, in order to test in a small scale an experimental system designed to determine the threshold of salinity level and the consequences of further increased salinity on plant performance under a high and constant fertilization level ((6:6:6 (% N:P:K, respectively), Fertilizers & Chemicals Ltd., Israel, at a constant level of 120ppm N). The same two accessions, CH1 and GA6, were planted in four separate strips, two per accession, across the irrigation lines. Four irrigation lines, with sprinklers hanging 85 cm above soil surface at 2 m distances on-line, were placed to produce an even coverage of irrigation water on soil surface. A gradient of salinity across the plot (Fig. 5) was obtained by the injection of brine to each line at a different rate of pulse.

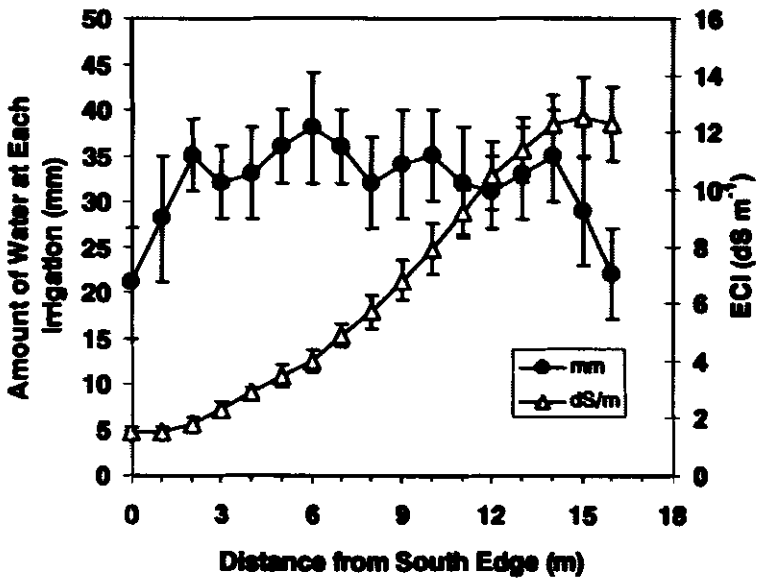

Fig. 5. Average amounts of water per irrigation event and the salinity gradient obtained at the small-scale field experiment in year 2004.

The plot was planted on August 2003 and irrigated with fresh water until the first effective rains in November. The experiment began in May of the subsequent year, when the plants obtained full coverage. The total amount of irrigation water during the growing season was 1832 mm. Three harvests were done until November 2004. Four strips of 1 m width were mowed for each

accession along the salinity gradient and the cumulative yields are presented in Figure 6 on a fresh weight basis.

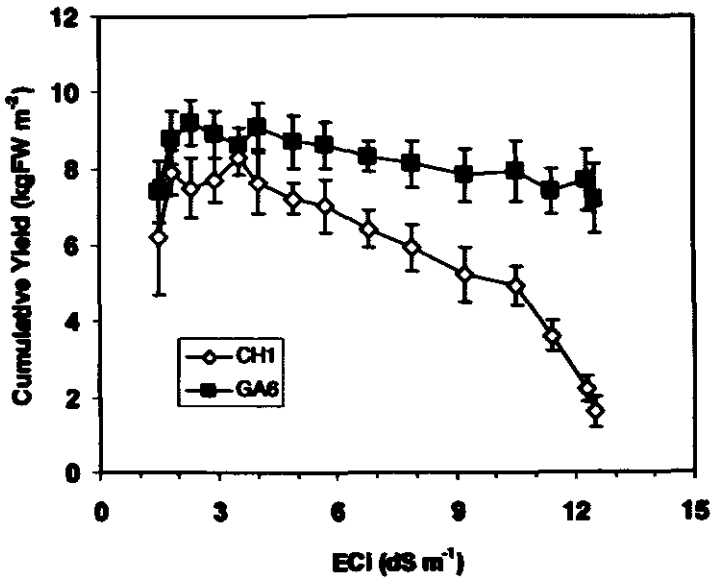

Fig. 6. Effect of salinity gradient on the cumulative fodder yield of two saltgrass accessions under excess amounts of water and at high fertilization level.

At relatively low levels of salinity (up to 6 $dS \cdot m^{-1}$), both accessions obtained high forage yields, ranging between 7 and 10 $kgFW/m^2$. Significant differences in yields occurred when salinity levels increased; while the yield of accession GA6 remained high and stable even at 13 $dS \cdot m^{-1}$, that of CH1 declined steadily to about 5 $kgFW/m^2$ at 11 $dS \cdot m^{-1}$ and then sharply dropped with any further increase in salinity (Fig. 6).

E.5. Phase 4 – Examinations and Implementation in Turkmenistan

Promising saltgrass accessions were brought to Turkmenistan at the beginning of the project, soon after a preliminary evaluation of our collection had been completed. Half of the plants were planted in a field nearby Ashgabat on June 2001 in order to gain biomass during the summer and provide plant material for field experiments of larger scale. The other half was grown in buckets and was planted in a field of the Karakul Experimental station much later, towards the autumn. Although the plants grew to some extent, there was insufficient biomass to conduct the planned large-scale field experiments. Selected accessions were also taken to Turkmenistan in the spring of 2004, however, no information was provided regarding those plants.

F. Discussion, Impact, Relevance, and Technology Transfer

Many trials have been carried out for the purpose of developing halophytes into economically viable crops (Shay 1990). Salt accumulation in the aboveground parts of the plant appeared to be a crucial parameter, which limited further development of apparently promising salt-tolerant fodder plant species. For instance, *Atriplex* species were found to be highly productive with seawater irrigation (Pasternak *et al.* 1993), nevertheless *in vivo* feeding trials showed that *Atriplex* was a poor fodder source due to high ash content, low intake and low nitrogen digestibility (Arieli *et al.* 1989). Saltgrass, on the other hand, was not as tolerant as the *Atriplex* species or as other chenopods but it did not concentrate salts in leaves (Pasternak & Nerd 1995). This was also reported by Marcum (1999), who examined salt-resistant members of the Chloridoideae sub-family of grass and found saltgrass to accumulate the least salt under high salinity levels. In the present study, ash content of saltgrass averaged 6.8% (Table 2) and when grown under high salinity levels, it never exceeded 11% of DW (data not shown), which was considerably less than in *Atriplex* species (which are usually above 20% ash). Therefore, and at least from the viewpoint of salt content in edible plant parts, saltgrass seems potentially suitable as a forage species.

In addition to the desirable low salt accumulation, protein content and metabolizable energy yield are of primary importance when the feed-value of potential fodder is determined (Minson 1981). In wild saline environments, saltgrass is grazed readily by livestock (Ginzo *et al.* 1986; Brinzela *et al.* 1990) and wildlife species (Powell 1993; Weller 1994). From the limited data available, the contribution of saltgrass to livestock diet is low (Brinzela *et al.* 1990). The mean values of CP, OMD, and IVME (Table 2) put saltgrass at an intermediate range of fodder quality - above straw but below customary forage crops such as alfalfa. Nevertheless, the variability among accessions was considerably high, and displayed many positive traits that could bring them to a high quality range. For instance, 14 accessions (out of 48) exhibited CP values higher than 13%, 17 had IVME yield (for sheep) above 6.5 kJ/g DM, and 20 accessions had an OMD above 52%.

The high variability found here is not surprising, since saltgrass is a wild plant species still in the earliest steps – collection and evaluation - of the seven-

phase-path of domestication determined by Vogel *et al.* (1989). Intra-specific variation among saltgrass ecotypes has been reported in the ecological literature for a number of parameters that included growth rate, rhizome morphology, and response to disturbance (Brewer & Bertness 1996) as well as in response to chloride and sulfate salinity and to selenium soil contamination (Enberg & Wu 1995; Wu *et al.* 1997). Analyses of genetic and morphologic diversity in our broad collection of saltgrass accessions revealed that in spite of the dominance of clonal reproduction, genetic variability within a population was larger than among populations (Ram *et al.* 2004). We used similar statistical techniques in the present study to relate between fodder quality traits and country or ecosystem of origin (Figs. 2 and 3) and demonstrated again that the variability within a group of origin is high enough to find excellent as well as poor genotypes side by side. These results strengthen a former conclusion that the initial selection of saltgrass should rely mostly on the performance of individual accessions (Ram *et al.* 2004).

Still, some level of discrimination according to the origin of accessions could be made; the GA accessions had a significantly higher quality index (QI) than that of the other groups (Table 2), mainly due to high LFS and ME (in spite of relatively low CP content). In addition, CP and LFS were the parameters in which inland and seashore accessions differed significantly (Table 2 and Fig. 3B). The emergence of LFS as a valuable determinant in saltgrass selection may be associated with a previous suggestion to use 'leafiness' (in its conservative meaning - leaves/whole canopy ratio) as an indicator of fiber content and, consequently, as an instant tool to evaluate fodder quality (Hacker & Minson 1981; Christiensen *et al.* 1984). Indeed, a high fiber content was the major drawback of a single saltgrass accession (Seabrook, DE) examined by Pasternak *et al.* (1993). High fiber contents (NDF ranging between 60 and 83%) were found for saltgrass in the present study (Table 2), however, in contrast to expectations it did not correlate with 'leafiness'(R^2 = 0.13) nor with 'LFS' (R^2=0.25). This may be explained by the fact that some accessions had relatively large but stiff and spiky leaves while others, with small leaves, where much softer. Therefore in saltgrass (at least), fiber content should be directly determined. LFS, although somewhat subjective, provides a useful discrimination tool.

When grown with fresh water, vigor may provide a good indication for the productive potential of saltgrass as a crop. The relative growth rate (RGR) is a sensitive physiological parameter for vigor, as it ignores initial differences in plant size on one hand, while a small difference in RGR builds a large difference in biomass over a relatively short period, on the other hand. Difficulties to obtain sufficient amounts of plants restricted those experiments to fewer accessions. Even though, the RGR measurements provided significant differences between accessions on the basis of country of origin (Fig. 3); accessions originating from the south-Atlantic coast of USA exhibited higher RGR values than other accessions under fresh water, which is in agreement with the LFS parameter. Nevertheless, if designated to saline environments, selected accessions should also exhibit high and stable performance at various salinity levels rather than just survive. In spite of a general sharp decline in RGR levels with the increase in salinity levels (Fig. 3), the AL and GA (to a lesser extent) accessions, again, demonstrated superior RGR values under highly saline conditions, compared with the other groups. It is noteworthy, that the performance of the DE accessions tended to improve under mild salinity levels but decreased sharply when salinity was high. This is in agreement with previous findings of Gallagher (1985), though it appears to be an exception to the general pattern of saltgrass growth-response to salinity.

Using a ranking according to QI, we chose six saltgrass accessions (Table 3) that seemed promising for further examinations. Yet, several notes must be made. The quality index used in this report does not employ any priority scale between the parameters examined. One may legitimately suggest different weighing of the parameters according to specific selection goals, which may change the ranking results considerably. Three out of the six accessions originated from Georgia, USA, in accordance with the general pattern obtained by that group. However, the protein contents that characterize that group must be improved, perhaps through enhanced fertilization, as shown in our latter experiments (Table 4). On the other hand, the relatively poor performance of the South American accession under saline conditions (Figs. 4 and 6) does not disqualify the whole group; on the contrary, much effort should be made to increase the collection of South-American saltgrass.

We have shown that the selected saltgrass accessions are positively and

highly responsive to considerable levels of fertilization, which can also compensate for reduction in productivity due to adverse effects of salinity (Table 4). Although not examined directly, productive saltgrass fodder can be obtained using large amounts of water. In nature, saltgrass can survive very long periods of drought, displaying an underground phase of dormancy. Nevertheless, in all habitats, the growing season of saltgrass in strongly related to unlimited supply of water (e.g., inundation). This may be in accordance with the requirement to apply a large quantity of water to leach the salt in the soil reclamation process.

Being a pioneer plant, saltgrass can be useful in the initial steps of the reclamation process of salinized former farmland (Gallagher 1985; Pasternak & Nerd 1995). Due to its weak ability to compete with other plant species when salinity declines (Warren & Niering 1993; Shumway 1995), saltgrass can be easily replaced with more valuable crops. These features strongly support earlier opinions (Yensen & Bedell 1993) that saltgrass is suitable for domestication as a forage crop for saline environments. However, the efforts to identify and characterize new saltgrass ecotypes should be continued, as the results of the present study indicate that the likelihood to succeed is considerably high.

The introduction of foreign plant species to a region may hold a great agricultural potential, as have been proven many times in the history. Alternatively, it might fail or even worse – to endanger the local plant community. Unfortunately, we were not able to evaluate this problem in the present project. This may be due to the following reasons:

1. Plant propagation. Saltgrass reproduction is mainly clonal and seed production is usually poor and sporadic. Having some previous experience with a single accession (Pasternak and Nerd 1995), we thought that the clonal propagation would be rapid and easy. However, most accessions were not as fast in rooting, a fact that delayed (in some cases, even prevented) the implementation of large-scale field trials in Israel and had negative influence on the practical work in Turkmenistan.
2. Harsh climate and water requirements. We have known that saltgrass water consumption is high and this information was transferred to the

Turkmen team. However, the water availability did not meet the water needs of saltgrass during the long, extremely warm and dry summer in Turkmenistan. Transportation to the experimental sites was also problematic. In addition, the extremely cold winter was not in favor of several accessions that did not recover in the following season.

3. Technical capacity. In Turkmenistan, there was an absence of infrastructure such as vehicles, modern water and electricity supply in the field.
4. Cooperation between the two teams. The flow of information between the teams via e-mail was cut frequently due to local problems in Turkmenistan, resulting in long of non-communication. Visits in Turkmenistan were not easy to arrange; beyond the war in Afghanistan and afterwards in Iraq and the consequent security problems, there were many bureaucratic obstacles (Turkmen institute administration, visa, etc.) to arrange visits at the right and relevant time. These problems made our (Israeli) ability very poor to know, control, and furthermore, to repair scientific and practical issues in the project implementation in Turkmenistan.

It is difficult to assess now whether the findings of the study will be useful to the Turkmen agriculture immediately, although it is hoped that the it will be so in the future. Main impact on the Turkmen side thus far was the expertise acquired by the Turkmen trainee in laboratory and field work in Israel, which will hopefully be transferred to her country.

G. Project Activities/outputs

1. Meetings. PI visit to Turkmenistan, May 2001.

Co-PI visit to Israel, April 2004.

Co-CI (Dr Mendlinger) visit to Turkmenistan, September 2004.

Our requests to arrange a visit of the Israeli team to Turkmenistan during the 2005 spring in order to conclude the project was cancelled due to bureaucratic problems.

2. Training. Ms Irina Pirogova was sent by the Turkmen team already in 2001 for laboratory and field work training. She spent 4 years in Israel,

actively involved in all aspects of the scientific work in the project and acquired various useful skills.

3. Publication

Bustan A., D. Pasternak, **I. Pirogova, M. Durikov**, T. T. deVries, S. Meccawi, and **A. A. Degen**. 2005. Evaluation of saltgrass as a fodder crop for saline environments. J. Science Food & Agriculture 85: 2077-2084.

H. Project Productivity

The project accomplished most of its objectives in Israel but less so in Turkmenistan. The reasons were detailed in pages 21 and 22 of this report.

I. Future Work

It has been shown that saltgrass hass the potential to be used as a fodder crop for raising livestock in high salinity areas. Large feeding trials, both in the laboratory and field are needed to further assess the nutritive value of this grass.

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Evaluation of saltgrass as a fodder crop for livestock

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Abstract: Farmland salinization due to unsustainable agricultural practices has become a worldwide problem. Salt-resistant forage crops, introduced at the primary stages of land reclamation, can provide fodder for livestock, thus adding economic benefit to the process. Saltgrass (*Distichlis spicata*), a wild halophytic grass species distributed in salt marshes in America, is occasionally grazed by livestock and wild animals. In attempts to domesticate this species, we evaluated and ranked the fodder potential of groups of accessions from several sites in North and South America. Ash content never exceeded 110 g kg^{-1}, even when plants were grown with salty water. Crude protein content was variable and averaged 116 g kg^{-1} of DM. Mean yield of metabolizable energy was 6.30 and 5.61 MJ g^{-1} DM for sheep and goats, respectively. Organic matter digestibility (*in vitro*) was higher in sheep than in goats (506 g kg^{-1} and 478 g kg^{-1}, respectively) for all saltgrass accessions. Differences in quality parameters were usually larger within than among groups of accession when sorted according to country of origin or ecosystem. Accessions from the South Atlantic coast of North America and from South America were superior in several parameters. South Atlantic coast accessions were relatively vigorous and were productive under saline conditions, as indicated by their relative growth rate (RGR) in small-scale experiments. Six outstanding saltgrass accessions were chosen for further examination. The results of the present study indicate that saltgrass holds considerable promise for selection and suggest that efforts should continue to identify and characterize additional saltgrass ecotypes.
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Keywords: saltgrass; *Distichlis spicata*; accessions; fodder; leafiness; digestible organic matter; metabolizable energy yield; saline

INTRODUCTION

Almost 1 billion hectares of land, which accounts for 10% of the earth's surface, are affected by salinity and over 50% of all irrigated lands have a salinity problem.[1] Most crop plants are glycophytes or non-halophytes that do not express their full genetic potential for growth, development and productivity under salt stress.[2,3] Primary salinization, that is salinization resulting from natural causes, contributes significantly to the global process. However, salt-affected soils are most often the result of human activity, mainly through mismanagement of irrigation systems.[4] In irrigated lands, salinization of the soil occurs where evapo-transpiration rates exceed irrigation rates or where drainage capacity of the soil is low. In most arid and semiarid lands, irrigation that is less in volume than evapo-transpiration is often practiced because of water scarcity. The diminishing supply of good-quality water for irrigation often results in the use of low quality water, high in salinity.[5,6] In addition, low awareness of these problems leads to poor drainage infrastructure, which in turn accelerates soil salinization processes.[4]

The environmental problems of the Aral Sea basin has demonstrated how salinity can be the cause of a food security problem.[7] Livestock production, mainly Karakul sheep, goats and Asian camels, is of major economical importance in the Aral Sea basin. Based solely on grazing, animal performance can be low due to low crude protein and low metabolizable energy intake from the local herbage and shrubs.[8]

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Salt-resistant forage crops can be introduced to salinized lands to serve as a primary stage of the reclamation of former agricultural lands and also provide alternative forage for livestock.

Saltgrass (*Distichlis spicata* L, Poaceae) has potential for being a salinity-tolerant forage crop.[9–11] However, domestication of saltgrass requires a long procedure of selection and breeding, as it is still a wild plant species. In this study, we evaluated a broad collection of saltgrass accessions as fodder crops to select candidate accessions for future breeding programs. Fodder quality of a crop is complex to determine because it involves parameters such as yield, chemical composition, digestibility and palatability. Desirable traits, namely high contents of crude protein (CP), metabolizable energy (ME), organic matter digestibility (OMD) and dry matter (DM), low contents of ash and neutral-detergent fiber (NDF), and a high level of leafiness (LFS) were used to define a quality index (QI) in order to assess the potential of a saltgrass accession to become a forage crop. We also sought possible relationships between geographical origin and quality traits that might guide collection missions aimed at expanding the available saltgrass germplasm. In additional, growth response under a range of salinity levels was used as an indication for the potential performance of selected saltgrass accessions in saline environments.

MATERIALS AND METHODS

Plant material

Plants were collected from several locations in the USA and South America (Table 1). Most of the plants were collected from the wild on May and June 1998. In the USA, plants were collected from a few widely dispersed sites (distances between sites ranged from 10^2 to 10^4 km). In California, several ecotypes were contributed from a collection of saltgrass from various known sites in the Central Valley (Dr Dyer, USDA, Soil Conservation Service, Lockeford, CA). Several ecotypes that originated in other parts of the USA were obtained from a collection in Delaware (Professor Gallagher, College of Marine Studies, University of Delaware, Lewes, DE). For South American ecotypes, each plant was the only representative of a region.

At each site, several (2–5) live rhizomes were dug out, washed from soil and organic matter, wrapped with wet paper and kept in plastic bags in a cool-box (5–15 °C) for 2–5 weeks before being planted in pots containing perlite and peat (1:1). Plants were kept in quarantine for 5 months before being transferred to a greenhouse at Ben-Gurion University of the Negev (Beer Sheva, Israel). When plants were established, one rhizome from each bucket representing a different location was cut and replanted individually in a 1-liter pot and was considered as an accession, as confirmed by

Table 1. Sampling sites of saltgrass ecotypes[a]

Ecotype	Country/state	County/location	I/S	Ecosystem
CT1,CT3	Connecticut	New London/Barn Island	S	TMS
CT2	Connecticut	New London/near Mystic	S	TMS
DE1,2,4,5	Delaware	Sussex/near Lewes	S	TMS
DE3	Delaware	Lewes, UDE collection	S	UO
GA1-8	Georgia	McIntosh/Sapelo Island	S	TMS
AL1	Alabama	Mobile/Deer River	S	TE
AL2	Alabama	Mobile/Dauphin Island	S	TMS
AL3-6	Alabama	Mobile/Heron Bay	S	TMS
UT1	Utah	Goshen	I	ISM
UT2	Utah	Big Salt Lake	I	ISM
UT3	Utah	Vernon	I	RB
UT4	Utah	Jordan River	I	RB
CA1	California	Mendota	I	UO
CA2	California	Riverside/Salton Sea	I	ISM
CA3,13,16	California	Kings/Tulare Lake-bed	I	RB
CA4,14	California	S. Luis Obispo/Soda Lake	I	ISM
CA5,17	California	Fresno/Tranquillity	I	RB
CA6	California	S. Luis Obispo/Morro Bay	S	TE
CA7	California	Merced/Hartley Slough	I	ISM
CA8,15	California	San Joaquin	I	RB
CA9	California	San Diego/Mission bank	S	RB
CA10	California	Tulare/Alpaugh	I	RB
CA11	California	Monterey/Elkhorn Slough	S	TE
CA12,18	California	Kings/Kettleman city	I	RB
CH1	Chile	Tamarugal	I	ISM
CH2	Chile	Juta Valley	I	ISM
Arg1	Argentina	Trelew Chubut	I	RB, TE
Arg2	Argentina	Chinchina La Rioja	I	ISM

[a] I — Inland ecotypes; S — seashore ecotypes; TMS — tidal marsh on seashore; UO — uncertain origin; TE — tidal estuary; ISM — inland saline marsh or lake, seasonally inundated; RB – river or ditch bank, seasonally inundated.

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random amplification of polymorphic DNA (RAPD) analysis.[12]

Accessions of three replicates were randomly placed and grown outdoors in pots containing perlite and peat (1:1), drip-irrigated with fresh water (1.5 dS m^{-1}) and on-line liquid fertilizer (Sheffer 5:3:8 (N:P:K, respectively), Fertilizers & Chemicals Ltd, Haifa, Israel) supplied at 50 ppm N. The above-ground portion of the plants was harvested on July 2002, before the occurrence of any signs of bloom, ie of head emergence. Growth was for three months starting April 2002.

Measurements

Seeking tools to discriminate between saltgrass accessions and evaluate their potential for herbivore use, we defined leafiness as 'LFS', which included additional descriptive parameters. Shortly before harvest, leaf/whole-canopy ratio of each accession, shoot softness, vitality and lushness were determined by visual estimation and each parameter was ranked from 1 (low) to 10 (high). The mean of these estimates for each succession was used as a measure of LFS. These evaluations were made among the saltgrass accessions, and not against other grass species.

Harvested plant material was dried in an oven at 50 °C to constant mass to determine dry matter content. Dry matter was ground to fine powder, which was used for further analyses. Samples were analyzed for nitrogen (N) content by the Kjeldahl method[13] and for ash by burning at 550 °C.[14] Crude protein was calculated as 6.25 × N. Neutral detergent fiber (NDF) content was determined as described by Van Soest *et al*,[15] applying a Fiertec System M6 (Tecator, Haganas, Sweden). All measurements were made in triplicate.

Organic matter digestibility and metabolizable energy yield of the saltgrass samples were estimated *in vitro* using the Hohenheimer Gas Method.[16,17] In this method, the gas produced in anaerobic fermentation of substrate is used to predict the nutritive value. Rumen liquor and particulate matter were collected before morning feeding via a rumen fistula from two sheep and two goats fed on a roughage diet of mainly poor quality wheat straw and some lucerne hay; the liquor was homogenized, strained and filtered through glass wool. Incubation media was prepared as described by Menke *et al.*[16] Samples, each of 200 mg DM, were incubated in triplicate in 100-ml calibrated glass syringes in which 30 ml of the incubation media was added. The glassware was kept at 39 °C and flushed with CO_2 before use and the mixture was kept stirred under CO_2 at 39 °C. Gas production, as determined by piston movement, was measured over 24 h after correcting for gas production due to rumen fluid alone. Gas production (GP, ml per 200 mg DM) and crude protein (CP, g kg^{-1} DM) were used to estimate organic matter digestibility (OMD) and *in vitro* metabolizable energy (IVME) yield as follows:[16,17]

$$\text{OMD (\%)} = 24.91 + 0.722\,\text{GP} + 0.0815\,\text{CP}$$

and,

$$\text{IVME (kJ g}^{-1}) = 2.20 + 0.136\,\text{GP} + 0.0057\,\text{CP}$$

Calculations

To rank the saltgrass accessions, a modified version of the method described by Conklin *et al*[18] was adopted. The mean of each quality parameter (DM, ash, CP, NDF, ME and LFS) was subtracted from the value obtained by each accession and the result was divided by the SD of the mean to produce a specific parameter index. For example, the equation of the IVME index took the form:

$$\text{IVMEI}_x = (\text{accession IVME} - \text{mean IVME})/\text{SD}$$

In this case, calculations were made for sheep and goats separately, but the average of IVMEI_x for sheep and goats was calculated (MEI_x) and used for further determination of the quality index (QI). QI was calculated for each saltgrass accession as follows:

$$\text{QI} = \text{DMI}_x - \text{AshI}_x + \text{CPI}_x - \text{NDFI}_x + \text{MEI}_x + \text{LFSI}_x + 10$$

Growth response to salinity

Ramets of each accession were cut and rooted in water culture. Accessions that displayed high rates of rooting and of vegetative growth resumption were further examined for their growth response to salinity. Uniform plants ($n = 36$) of each selected accession were transferred to a glasshouse under full sunlight to grow in water culture in 1-liter containers. Salinity level was gradually increased to produce six salinity treatments (1.5, 10, 20, 30, 40, 50 dS m^{-1}) obtained by $NaCl:CaCl_2$ solution at 3:1 ratio, respectively. The experimental design included six blocks for each selected accession. The growth medium containing fertilizers (N:P:K, 5:3:8, respectively, at 50 ppm N) was maintained at a constant volume by adding fresh water when necessary, and was replaced weekly. Aeration with a bubbling system did not have a noticeable influence on growth and, therefore, was discontinued. Plant growth (whole plant fresh weight) was recorded weekly during 10 weeks in summer. Relative growth rate (RGR) became stable at week 6 and thereafter. Therefore, the average RGR of weeks 6–8 was chosen as an indicator of growth response to salinity. Ash content was determined at the end of the experiment (10 weeks), as described above.

Statistical analyses

Statistical differences between groups of saltgrass accessions (country of origin) or between ecosystems of origin (inland *vs.* seashore) were determined using a one-factorial Analysis of Variance (ANOVA) followed by a Fischer's PLSD test. PCA was exercised on the covariance matrix of all quality parameters or on the most important two of them (CP and ME) with the

MVSP package[19] in order to obtain a two-dimensional ordination pattern of saltgrass accessions using various geographical or eco-geographical parameters for sorting.

RESULTS

Forage quality of saltgrass accessions

Mean DM, ash, and NDF contents for all saltgrass accessions examined in the present study were 431, 68.3 and 720 g kg^{-1}, respectively (Table 2). Crude protein content averaged 116 g kg^{-1} and ranged from a low of 69 g kg^{-1} to a high of 189 g kg^{-1}. *In vitro* metabolizable energy yields and organic matter digestibility were higher in sheep rumen fluid than in goat rumen fluid for all saltgrass accessions. Mean metabolizable energy yield for sheep was 6.30 ± 0.66 kJ g^{-1}DM, ranging between 4.71 and 8.63 kJ g^{-1} DM, whereas in goats, the mean was 5.63 ± 0.75 kJ g^{-1} DM, ranging from 3.21 to 6.79 kJ g^{-1} DM. Mean organic matter digestibility (OMD) was 506 ± 26.4 g kg^{-1} for sheep and 478 ± 25.5 g kg^{-1} for goats. LFS averaged 6.96 with large differences between accessions within and, especially, among groups from distinct origins.

PCA analyses (that included all quality parameters) of all saltgrass accessions were carried out to reveal possible clustering patterns according to countries or ecosystem (seashore or inland) of origin. GA accessions clustered separately whereas all other accessions were scattered randomly (Fig 1A). No clustering of inland or seashore accession was observed, despite the segregation of the seashore GA group from the generally mixed scattering (Fig 1B).

In a simpler analysis, where only two quality parameters (CP and ME) were used (Fig 2A), most GA accessions grouped together at the upper left quarter of the array (negative CPI_x and positive MEI_x). In the same analysis, three of four South American accessions were located at the upper right quarter, indicating positive CPI_x and MEI_x. All AL accessions were at the left half of the CP axis (negative CPI_x). The CA accessions, which were the largest group, were scattered throughout all quarters similar to the other accessions (Fig 2A). When analyzed according to inland or seashore origin, about 70% of seashore accessions were left of the CP axis (negative CPI_x), while 70% of the inland accessions were on the right side of the CP axis (Fig 2B). However, the ME axis did not have any discriminating effect on either of the two groups.

Dissection of all quality criteria according to the country of origin revealed no significant differences for DM, ash and NDF contents (Table 2). However, significant differences were found for CP, ME, OMD and LFS. When ranked by quality index (QI), the GA group was significantly better than all groups except for the South American group (Table 2). The GA group obtained the highest LFS, had reasonably high ME and OMD but the lowest CP mean, while the South American group had the highest CP, ME and OMD but a relatively low LFS. All other groups had QI values lower than 10, indicating relatively poorer quality (Table 2); however, individual accessions exhibited relatively high values for one or more parameters. Comparison of inland versus seashore accessions showed a significant advantage in LFS for seashore ecotypes, higher CP content for inland accessions, and no significant difference for all other quality parameters (Table 2). The top six ranked saltgrass accessions, according to QI, are presented in Table 3.

Growth response to salinity

Most saltgrass accessions that were examined under six different levels of water salinity displayed a

Table 2. Dry matter (DM), ash, crude protein (CP) and neutral detergent fiber (NDF) contents, leafiness (LFS), and *in vitro* metabolizable energy (ME) and organic matter digestibility (OMD) yields (using sheep and goat rumen fluids) of 48 saltgrass accessions originating from various regions (states; *df* = 6) or ecosystems (seashore versus inland; *df* = 1) of the American continent

		DM	Ash	CP	NDF	ME sheep	ME goats		OMD		
Origin	*n*	(g kg^{-1})				(kJ g^{-1} DM)		*ME average*	(g kg^{-1})	*LFS*	*QI*
GA	8	439	61.0	95.6	736	6.65	6.44	6.55	505	8.38	11.6
S America	4	405	80.6	143.9	728	7.03	6.52	6.64	538	7.25	10.4
DE	5	432	71.5	130.3	712	6.44	5.45	5.95	493	5.60	9.4
AL	6	454	67.3	98.4	777	6.22	5.90	5.88	466	8.33	9.8
CT	3	437	72.4	142.7	760	5.98	5.36	5.67	445	7.67	9.1
CA	18	428	67.8	119.6	693	6.22	5.37	5.79	480	6.33	9.9
UT	4	411	67.3	127.0	700	5.47	5.20	5.34	462	5.75	8.6
		NS	NS	***	NS	*	***	**	**	***	*
Seashore	27	437	65.6	109.6	728	6.35	5.82	6.09	490	7.32	10.2
Inland	21	424	71.0	127.0	705	6.23	5.52	5.88	488	6.40	9.8
		NS	NS	**	NS	NS	NS	NS	NS	*	NS
Total mean		431	68.3	115.7	720	6.30	5.63	5.92	485	6.96	10.0
SD		34.7	10.6	22.2	529	0.66	0.75	0.71	38.1	1.30	2.04

NS, not significant.

*, **, and *** indicate for significant differences at $p < 0.05$, 0.01, and 0.001, respectively.

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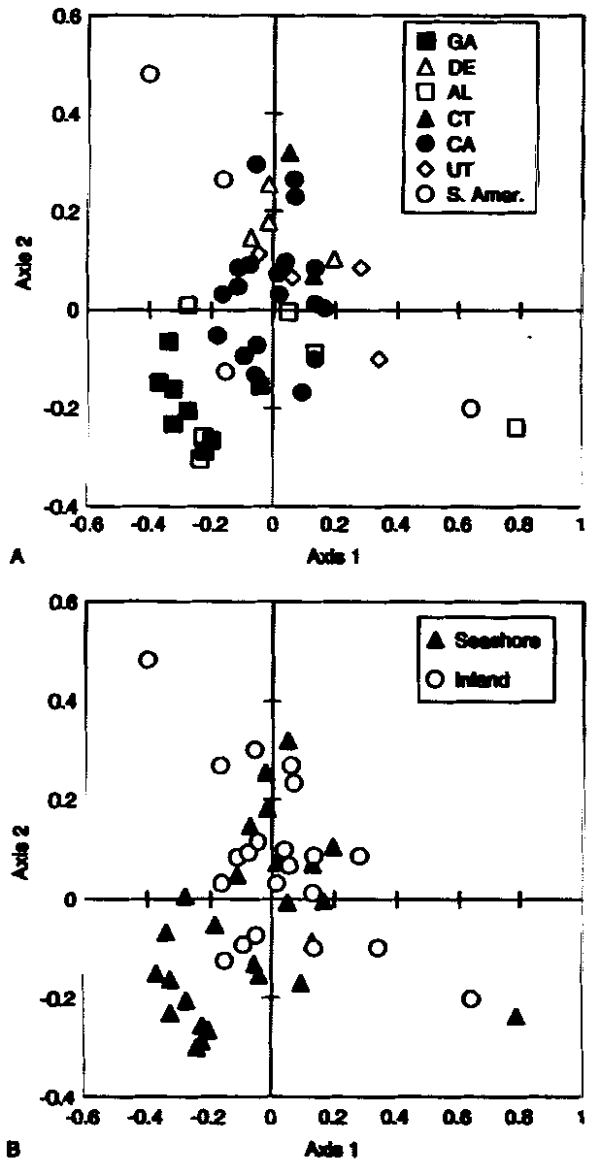

Figure 1. PCA analyses of fodder quality results (all quality parameters included) of 48 saltgrass accessions. A: Accessions are sorted according to country of origin. B: Accessions are labeled according to their ecosystem of origin.

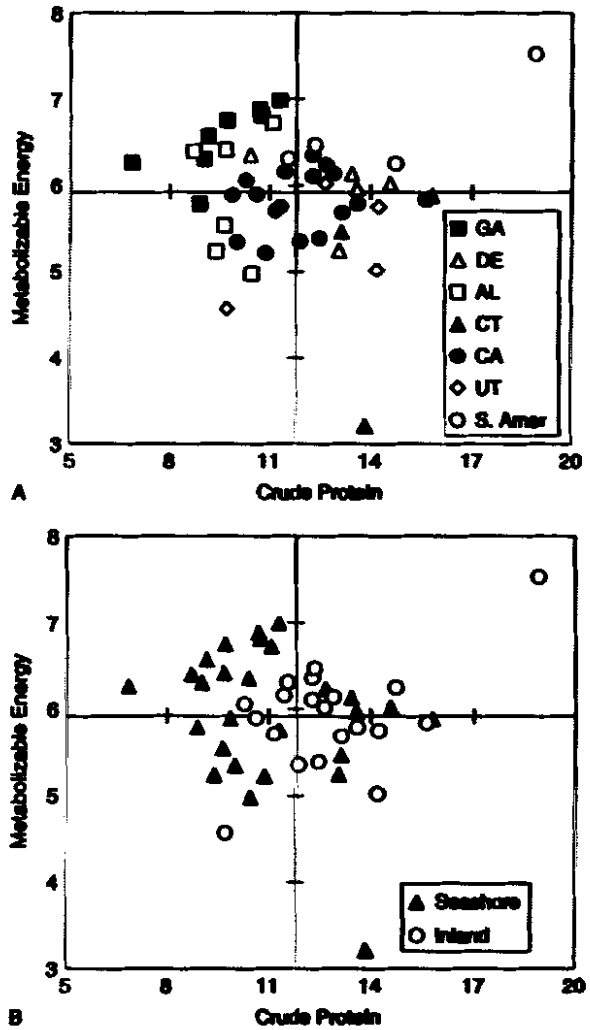

Figure 2. Cluster analyses of 48 saltgrass accessions according to crude protein versus metabolizable energy contents. Accessions are labeled according to country of origin (A) or ecosystem (B).

similar pattern of RGR response to salinity (Fig 3). The highest RGR was obtained when grown with fresh water (1.5 dS m^{-1}) but it declined sharply at 10 dS m^{-1}, and decreased moderately or became stable as salinity was increased up to 50 dS m^{-1}. In a few exceptional accessions from DE and CA, the RGR pattern was different, beginning its decline only at 30 dS m^{-1}, yet their initial RGR at fresh water were usually very low.

GA and AL accessions had the highest RGR throughout all salinity levels (including fresh water), with a relatively stable performance (>0.008 day^{-1}) between 20 and 50 dS m^{-1}. The South American accessions displayed the lowest RGR values (<0.004 day^{-1}), indicating low vigor under highly saline environments (>20 dS m^{-1}). From the six accessions selected (Table 3), five were examined for RGR

Table 3. Forage quality properties of the best six saltgrass accessions (Acc), ranked according to the quality index (QI)

Acc	Origin	DM (g kg^{-1})	Ash (g kg^{-1} DM)	CP (g kg^{-1} DM)	NDF (g kg^{-1} DM)	ME (kJ g^{-1} DM)	OMD (g kg^{-1})	LFS	QI
CH1	Tamarugal, Chile, S America	419	77	189	706	7.53	584	7	14.65
GA2	Sapelo Island, Georgia	436	60	113	679	6.99	536	9	14.59
GA6	Sapelo Island, Georgia	449	70	107	667	6.88	528	9	13.85
GA7	Sapelo Island, Georgia	436	61	92	701	6.57	504	9	12.53
DE3	Lewes, Delaware	501	72	146	672	6.02	505	5	12.50
CA17	Tranquility, California	437	70	136	648	5.78	488	7	12.03

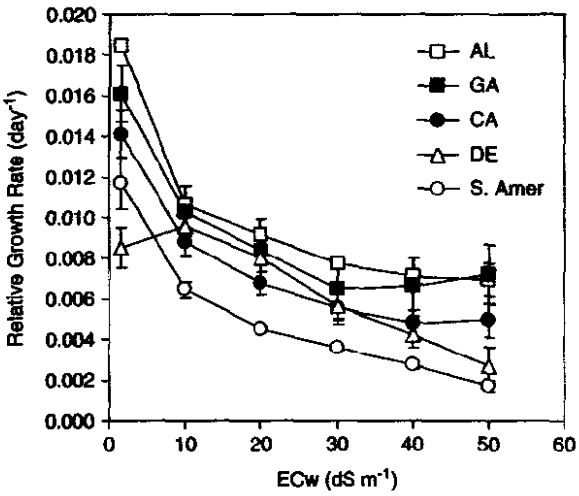

Figure 3. RGR (*relative growth rate*) response to water salinity level of saltgrass plants with five different origins. Bars above and below the symbols indicate ±1 SE.

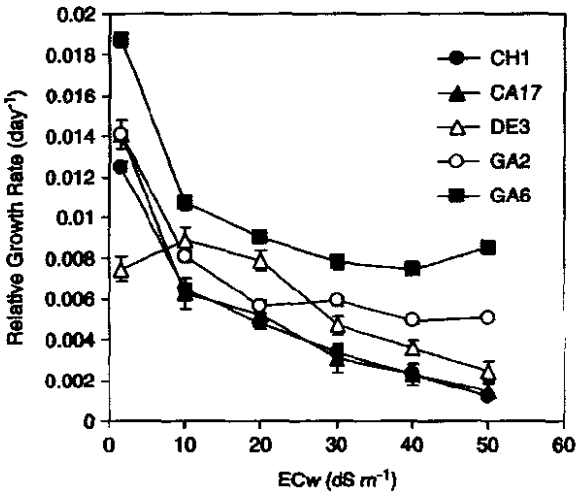

Figure 4. Comparison of RGR response to water salinity level between five accessions (out of six) that displayed the highest quality index (QI) among our saltgrass collection (see also Tables 2 and 3). Values represent means (±SE) of six replications per accession per salinity level.

response to salinity (Fig 4). GA6 showed obvious superior performance at both fresh and saline conditions, even at very high salinity levels. GA2 displayed a similar pattern but at much lower RGR levels. DE3 had substantially low RGR when grown with fresh water, however, it remained stable as salinity was increased to 20 dS m^{-1} and declined sharply at 30 dS m^{-1} and above. CA17 and CH1 displayed the poorest performance even at 10 dS m^{-1}.

DISCUSSION

Many trials have been carried out for the purpose of developing halophytes into economically viable crops[20] (see also www.biosalinity.org). Salt accumulation in the aboveground parts of the plant appears to be a crucial parameter, for it limits further development of apparently promising salt-tolerant fodder plant species. For instance, *Atriplex* species were found

Table 4. Ash content (g kg^{-1} DM) of selected saltgrass accessions grown during 10 weeks in water culture at four salinity levels[a]

	Salinity level (dS m^{-1})			
Accession	1.5	10	30	50
AL1	65 ± 2.2	78 ± 4.1	90 ± 2.9	98 ± 3.1
AL3	63 ± 2.8	79 ± 5.0	92 ± 4.3	94 ± 4.9
Arg1	84 ± 3.1	99 ± 4.4	102 ± 3.7	107 ± 3.1
Arg2	86 ± 3.6	94 ± 3.5	96 ± 3.2	102 ± 3.8
CA1	72 ± 2.4	88 ± 2.9	103 ± 3.5	105 ± 3.7
CA4	66 ± 2.5	84 ± 4.2	89 ± 4.1	89 ± 4.7
CA13	70 ± 1.9	90 ± 3.3	97 ± 3.6	96 ± 4.0
CA17	68 ± 2.6	85 ± 4.4	94 ± 4.0	94 ± 3.8
CH1	79 ± 3.4	94 ± 3.6	103 ± 4.4	106 ± 5.6
CH2	75 ± 3.1	95 ± 4.8	100 ± 5.9	99 ± 4.1
CT2	71 ± 2.9	84 ± 3.1	90 ± 3.7	92 ± 4.2
DE1	75 ± 1.8	88 ± 2.9	96 ± 3.5	99 ± 3.3
DE3	74 ± 2.5	82 ± 2.6	90 ± 3.0	91 ± 3.4
GA2	56 ± 1.4	77 ± 1.9	86 ± 2.2	88 ± 2.9
GA3	62 ± 1.6	79 ± 2.4	89 ± 2.8	90 ± 3.2
GA6	66 ± 1.9	76 ± 1.8	81 ± 2.7	82 ± 3.5

[a] Values are means ± SE of six replicates.

to be highly productive with seawater irrigation,[11] nevertheless *in vivo* feeding trials showed that *Atriplex* was a poor fodder source due to high ash content, low intake by livestock and low nitrogen digestibility.[21] Saltgrass was not as tolerant as the *Atriplex* species or as other chenopods but it did not concentrate salts in leaves.[22] This was confirmed by Marcum,[23] who examined salt-resistant members of the Chloridoideae sub-family of grass and found saltgrass as the lowest salt-accumulator under high salinity levels. In the present study, ash content of saltgrass averaged 68 g kg^{-1} (Table 2) and, when grown under high salinity levels, it never exceeded 11 g kg^{-1} (Table 4), which was considerably less than in *Atriplex* species (which are usually above 200 g kg^{-1} ash). Therefore, and at least based on the criterion of salt content in edible plant parts, saltgrass seems potentially suitable as a forage species.

Besides low salt accumulation, protein content and metabolizable energy yield are of primary importance when the feed-value of potential fodder is determined.[24] In wild saline environments, saltgrass is grazed readily by livestock[9,10] and wildlife species.[25,26] However, apparently, the contribution of saltgrass to livestock diet is low.[10] In the present study, the mean values of CP, OMD, and IVME (Table 2) put saltgrass at an intermediate range of fodder quality: above straw but below customary forage crops such as alfalfa. Nevertheless, the variability among accessions was considerable, and many accessions displayed traits in the high quality range. For example, 14 accessions (out of 48) exhibited CP values higher than 13 g kg^{-1}, 17 had IVME yield (for sheep) above 6.5 kJ g^{-1} DM, and 20 accessions contained more than 520 g kg^{-1} OMD.

This high variability is not surprising because saltgrass is a wild species at the beginning phase of domestication, which typically involves seven

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phases.[27] Intra-specific variation among saltgrass ecotypes has been reported in the ecological literature for a number of parameters that included growth rate, rhizome morphology and response to disturbance[28] as well as in response to chloride and sulfate salinity and to selenium soil contamination.[29,30] Analyses of genetic and morphologic diversity in our broad collection of saltgrass accessions revealed that in spite of the dominance of clonal reproduction, genetic variability within a population was larger than among populations.[12] We used similar statistical techniques in the present study to evaluate associations between fodder quality traits and country or ecosystem of origin (Figs 2 and 3). Our results demonstrated that variability within a group of origin was high, which supports the conclusions of Ram *et al*[12] that initial selection of saltgrass should rely mostly on performance of individual accessions.

Origin of accessions was able to discriminate among groups of accessions with GA accessions having a significantly higher quality index (QI) than the other groups (Table 2). This discrimination was mainly due to high LFS and ME despite low CP content. In addition, CP and LFS were the parameters in which inland and seashore accessions differed significantly (Table 2 and Fig 2B). Using LFS as an important characteristic in saltgrass supports previous work that 'leafiness' (meaning the ratio of leaves to the entire canopy ratio) can be used as an indicator of fiber content and, consequently, as an instant tool to evaluate fodder quality.[31,32] Indeed, a high fiber content was the major drawback of a single saltgrass accession (Seabrook, DE) examined by Pasternak *et al*.[11] High fiber contents (NDF ranging between 60 and 83%) were found for saltgrass in the present study (Table 2); however, in contrast to expectations, it neither correlated with 'leafiness' ($R^2 = 0.13$) nor with 'LFS' ($R^2 = 0.25$). This may be due to some accessions having relatively large but stiff and spiky leaves while others, with small leaves, were much softer. Therefore, in saltgrass at least, fiber content should be determined directly. Although LFS is subjective, it provides a useful discrimination tool. However, the exact relationship of LFS to herbivore preference needs to be investigated further.

Vigor may be a good indicator of the productive potential of saltgrass as a crop in fresh water. Relative growth rate (RGR) is a sensitive physiological parameter for vigor because it ignores initial differences in plant size, on one hand, while a small difference in RGR builds a large difference in biomass over a relatively short period, on the other hand. Difficulties in obtaining sufficient numbers of plants restricted experiments to fewer accessions. However, differences in RGR were observed between accessions on the basis of country of origin (Fig 3). With fresh water, accessions originating from the South Atlantic coast of USA exhibited higher RGR values than other accessions, which agrees with the results for LFS. Nevertheless, if grown in saline environments, selected accessions should exhibit high, stable performance at various salinity levels rather than just survive. Despite a general sharp decline in RGR with increased salinity (Fig 3), the AL and, to a lesser extent, GA accessions again demonstrated superior RGR values under highly saline conditions compared with other groups. It is noteworthy that the performance of the DE accessions tended to improve under mild salinity levels but decreased sharply when salinity was high. This is in agreement with previous findings of Gallagher,[33] though it appears to be an exception to the general pattern of saltgrass growth-response to salinity.

Six saltgrass accessions with the highest QI values were selected for further studies (Table 3). The QI as calculated in our study does not use a weighted scale for parameters. With different selection goals, parameters could be weighted differently, which may change the ranking results. Three out of the six accessions originated from Georgia, USA. However, the protein contents in the GA group must be improved, perhaps through enhanced fertilization, which is under current investigation. Furthermore, the relatively poor performance of the one accession from South America under saline conditions (Fig 4) does not mean that all accessions from South America are poor performers. Additional collections of saltgrass from South America are needed to more accurately evaluate their potential as a forage crop.

Being a pioneer plant, saltgrass can be useful in the initial steps of reclaiming salinized former farmland.[22,33] Because of its poor ability to compete with other plant species when salinity declines,[34,35] saltgrass can be easily replaced with other more valuable crops. These features strongly support earlier opinions[36] that saltgrass is suitable for domestication as a forage crop for saline environments. Our selected saltgrass accessions are currently being examined in small field trials in Turkmenistan and in Israel. Additional efforts to identify and characterize new saltgrass ecotypes should be continued.

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