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

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המכונים למחקר שימושי
אוניברסיטת בן-גוריון בנגב
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Executive Summary

In the Aral Sea Basin, 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. The reclamation of these lands must involve salt-resistant plants of economical value (e.g., fodder crops). Saltgrass (*Distichlis spicata*, Poaceae) is a plant species, native in 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 program are: 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. During the current year, we have terminated stage I of evaluation and selection. Six accessions were identified as having potential for good forage quality. We have also pointed out favorable origin countries for further exploration of better saltgrass strains. An exploration as such will be required, since forage quality does not necessarily correlate with salt resistance or adaptability to the Turkmen deserts, as shown by the Turkmen group. Nevertheless, at least two saltgrass accessions grew reasonably well in Turkmenistan and will be examined in large-scale field experiments in the following years. A Turkmen trainee in Israel is involved in most research activities carried out during years 2002-3 and will continue to the end of the project. Mutual visits of researchers are planned on February and April 2004.

Section I

A. Research Objectives

In the Aral Sea Basin, 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. The reclamation of these lands must involve salt-resistant plants of economical value (e.g., fodder crops). Saltgrass (*Distichlis spicata*, Poaceae) is a plant species, native in 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_r>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 program are:

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.

Research goals in year 2003 were to terminate stage I of the selection of saltgrass accessions according to forage quality parameters, to examine their response to salinity, and to test the adaptability of several accessions to the Turkmen environmental conditions.

B. Research Accomplishments

Forage Quality of Saltgrass Accessions

Mean DM, ash, and NDF contents for all saltgrass accessions examined in the present study were 43.1, 6.83, and 72 (%, respectively) (Table 1). Crude protein content averaged 11.6% and ranged from a low of 6.85% to 18.92%. *In vitro* metabolizable energy yields and organic matter digestibility were higher in sheep than in goats 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 was 50.6±2.64 % for sheep and 47.8±2.55 % for goats. Leafiness averaged at 6.96 with remarkable differences between accessions

within, but especially among groups from distinct origins.

Table 1: 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
		(%)	(% from 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. The group of the GA accessions solely 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).

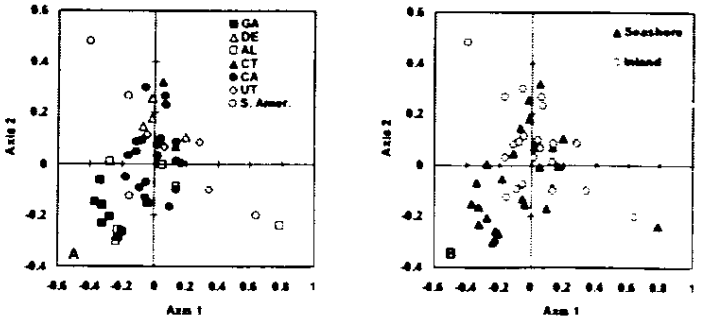

Figure 1: PCA analyses of saltgrass accessions according to country (A) or ecosystem (B) of origin, using all parameters of forage quality that were examined in this study (see text).

In a simpler analysis, where only two quality indices (CPI and MEI) were used (Fig. 2A), most of GA accessions grouped together at the upper left quarter of the array (negative CPI and positive MEI). In the same analysis, 3 out of the 4 South American accession located at the upper right quarter, indicating for positive CPI and MEI. All AL accessions were at the negative half of the CPI axis. The CA accessions that comprised the largest group scattered randomly among all quarters of the array, as well as accessions from the rest countries of origin (Fig. 2A). When analyzed according to inland or seashore origin, about 70% of seashore accessions were at the left, negative side of the CPI while 70% of the inland were at the opposite, positive side (Fig. 2B). On the other hand, the MEI axis did not have any discrimination effect on either of the two groups.

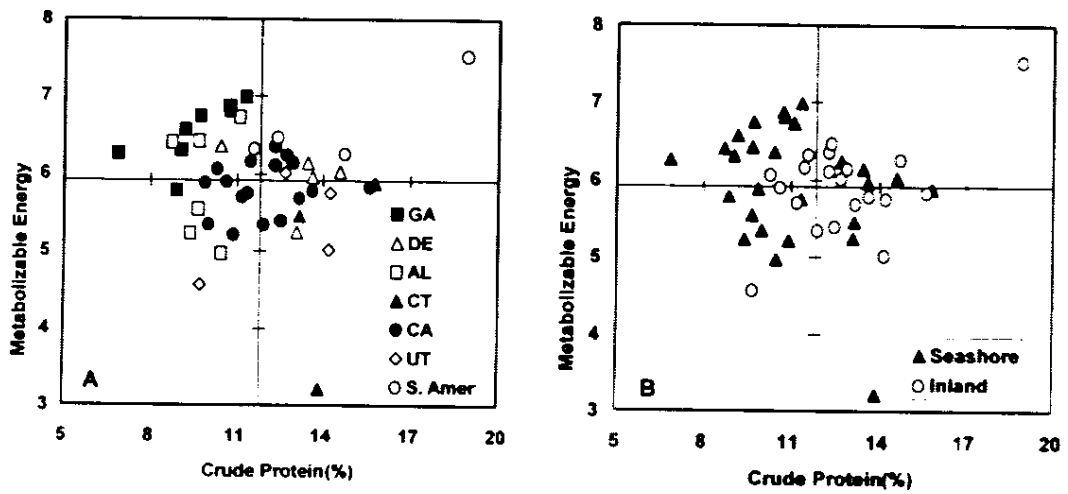

Figure 2: Segregation of saltgrass accessions according to country (A) or ecosystem (B) of origin, using the indices of crude protein content (CPI) and metabolizable energy yield (MEI) as the dissecting factors.

Dissection of all quality criteria according to the country of origin revealed no significant differences for DM, ash, and NDF contents (Table 1). On the other hand, significant differences were found for CP, ME, OMD, and LFS. When ranked by quality index (QI), the GA group was the best, significantly different from all other groups excluding the S. American group (Table 1). While the GA group obtained the highest mean LFS, had reasonably high ME and OMD means but the lowest CP mean, 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 for relatively poor quality, although some had reasonable values for one parameter or another (Table 1). Dissection for inland *vs.*

seashore accessions showed a significant advantage in LFS for seashore ecotypes, higher CP content for inland accessions, and no significant differences for all other quality parameters (Table 1). Ranking all saltgrass accessions according to QI resulted in a list, of which the best six are presented in Table 2.

Table 2: Forage quality properties of the best six saltgrass accessions, ranked according to the quality index (QI).

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

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 sharply declined at 10 $dS \cdot m^{-1}$, and moderately decreased or became stable as salinity was increased up to 50 $dS \cdot m^{-1}$. In 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.

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

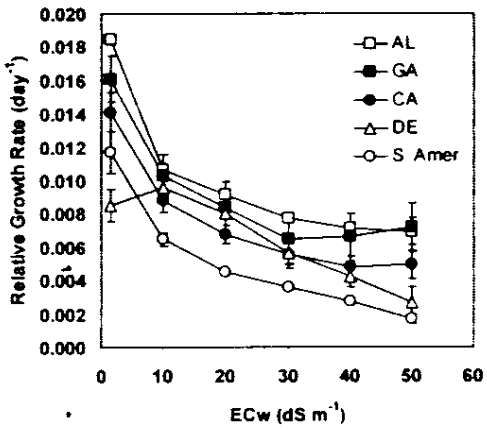

Figure 3: The response of relative growth rate (RGR) of saltgrass accessions to water salinity levels. Plants were grown in water culture. Values present means of results obtained by accessions from the same country of origin.

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 least performance already at 10 $dS \cdot m^{-1}$.

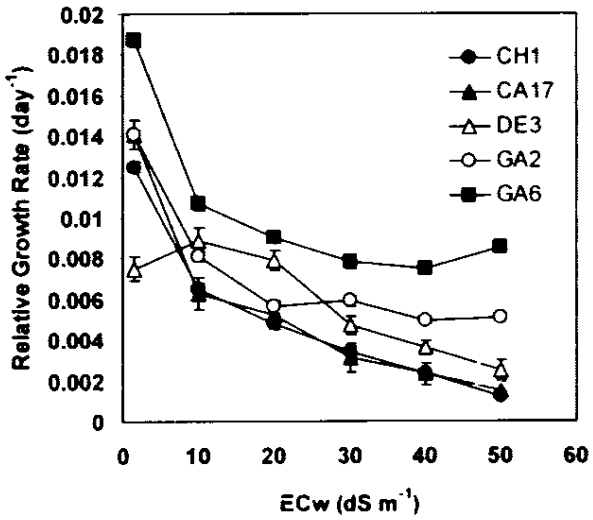

Figure 4: The response of relative growth rate (RGR) to water salinity levels of five saltgrass accessions that were most highly ranked according to forage quality parameters (see also Table 2).

Saltgrass adaptation to environmental conditions in Turkmenistan

Planting material of saltgrass was introduced to Turkmenistan already in May, 2001 and planted on light grey soil in Ak-Tokay tree-nursery in the foothills of Kopetdag mountains.

Table 3: Persistence rates of saltgrass accession in Turkmenistan (Ak-Tokay tree-nursery in the foothills of Kopetdag) at non-saline conditions.

Accession	Replicates at planting	Persistence (%)	
		Initial	End of first season
GA6	15	80	73
ÁL2	8	50	37
CA1	6	66	50
CA12	12	100	100
Arg1	6	43	40
CH1	4	33	0

Phenological observations during the first year showed that ecotypes differed much in persistence. CA12 displayed the highest persistence – 100%. In the beginning of July the height of spring shoots reached 11-16 cm, many rhizomes developed and rooted and their length reached 45-50 cm. The prosperous rhizome production enabled easy propagation. GA6 had slightly smaller persistence rates – 80%, however, plant performance was good as well; the height of the vegetative shoots was 15-17 cm, rhizomes – 50-70cm. Thus, the gain was even higher than that of CA12. The persistence of the other four accessions was significantly smaller, as shown in Table 3. The performance of these accessions was much worse than that of GA6 and CA12; there were no rhizomes appearing, and the height of vegetative shoots was 5-7cm. The phase of sexual reproduction started in September and was much more intensive in GA6 and CA12 than among other accessions. By the end of the vegetative season (mid-November), the persistence of most accessions declined (Table 3).

In the spring of 2002, the vegetative period began at the middle of March. Accessions GA6 and CA12 grew well, whereas the rest failed to recover from the heavy winter. In the beginning of May 2002, some of the plants were replanted in Kulbukan stationary, which is 22 km to the East of Ashgabat, near Karakum channel. Replanting was carried out in order to test saltgrass at saline conditions. Soils were primarily sandy, sites situated near the channel were saline at levels that depended on the remoteness from the channel and vary according to the season of the year. Also, ground waters rise up to the higher soil layers. CA12 displayed a better persistence rate, 80%, showing even better performance than in non-saline conditions. GA6, on the other hand, seemed to suffer from the saline environment, exhibiting only 56% persistence.

A second replanting session took place on the autumn of 2002, again from the foothills to Kalbukan stationary. That session was less successful than the former one in the spring, probably due to the extreme winter temperatures (-15°C) that did not allow for a full establishment of the plants in the new location. Again, CA12 showed better performance than GA6 (53 vs. 45%, respectively).

Growth and development of saltgrass on the saline soil was slower than on the normal one (Table 4). The table shows the biometric characteristics of the remaining two saltgrass accessions on the different soil types and different salinity levels. At the beginning of the vegetative season, the plants''appearance seemed normal, although development was slower comparing to that of foothills sites. Nevertheless, soil salinity increased rapidly in the summer due to excessive watering of the adjoining sites. In response, shoot growth slowed down, leaf color paled, apices dried and rhizome growth was inhibited. Soil washing, which was repeatedly carried out, brought about significant improvement in plant performance, especially for GA6. In some cases, some aspects of plant performance even improved under moderate salinity levels. However, under severe salinity levels, all plant growth parameters are oppressed (Table 4).

Table 4: Performance of two saltgrass accessions at three salinity levels at different sites in Turkmenistan.

Accession	Soil	Salinity level	Length (cm)			Quantity		
			shoot	rhizomes	leaf	replicates	shoots	Leaves
GA6	Gray	Low	33	85	10	12	10-30	18
	Sand	Moderate	30	42	7	21	20	14
	Sand	Severe	28	-	5	30	15	8
CA12	Gray	Low	31	80	12	15	15-40	20
	Sand	Moderate	30	45	8	62	63	23
	Sand	Severe	15	-	7	98	42	16

Due to water availability and technical constraints, plants grown on saline soils in Kalbukan were irrigated less frequently (once a month) than at the non-saline foothills site (twice a month). Although it seems that saltgrass can grow sufficiently only on moderately salinized soils, its ability to obtain reasonable yield should be reassessed under frequent, controlled irrigation

regimes.

Figure 5: saltgrass (GA6) plots in Kalbukan stationary, two months after transferred from the foothills site.

Conclusions

In Israel, we have identified six saltgrass accessions with promising quality traits. However, most of them appeared salt-sensitive (relative to average saltgrass performance). One of those accessions (GA6) was found to succeed, but another one (CH1) completely failed under field conditions in Turkmenistan. In addition, Georgia (USA) and Chile (S. America) originated the best accessions, and therefore should be targeted for future missions of improving the collection of saltgrass germplasm designated to forage. In the frame of the present project, agricultural methods (irrigation and fertilization regimes) should be developed for saltgrass cultivation in Turkmenistan. All selected accessions will be introduced (or re-introduced) to Turkmenistan and examined under carefully controlled salinity and watering levels. Future project should be focused on breeding between GA and Chilean accessions to produce vigorous, high quality, highly salt-tolerant saltgrass clones.

C. Scientific Impact of Collaboration

The major scientific impact of collaboration this year was the training of Ms. Irina Pirogova in various methods of forage quality analyses. We are sure that when she returns to Turkmenistan, she would be able to establish an independently working laboratory for forage quality

assessments. Unfortunately, and mainly due to security problems in Israel on one hand, and bureaucracy in relevant periods in Turkmenistan, mutual visits of investigators did not take place. However, we intend to improve that part during the next coming months and at that opportunity, to discuss ways to improve saltgrass performance in Turkmenistan.

D. Description of Project Impact

The present results of the project are yet premature to implement. Nevertheless, much progress has been done in assessing the potential of saltgrass to become a legitimate forage crop, and in identifying specific accessions and saltgrass origin countries with much potential for further examination. We believe that about a year after the end of the present project, a considerable field of high quality saltgrass will exist and provide fodder for animal feed trials. Within another two years, and if financial support lasts, there will be several high-quality, vigorous, salt-tolerant clones of saltgrass available for examination in field conditions and for animal feed experiments in Turkmenistan At that time, the agricultural technologies required for successful cultivation of saltgrass will be in hands.

E. Strengthening of Developing Country Institutions

At the present stage of the project, the major strength contributed to the institute in Ashgabat is the skills and experience gained by the trainee. Scientific equipment required to establish a forage quality laboratory will be transferred to Turkmenistan at the end of the project, as mentioned in the program.

F. Future Work

In Israel, two experiments are being carried out now, one in soilless culture and the other in the field, that are aimed to test two saltgrass accessions (GA6 and Arg2) for their response to nitrogen fertilization under saline and non-saline conditions. As saltgrass propagation is considerably slower than estimated at the beginning, and due to the lack of available area suitable for large-scale field experiment, we decided that such experiments will be carried out solely in Turkmenistan. Towards the next coming spring, the Israeli and the Turkmen investigators will transport plant material to Turkmenistan to establish those trials. These large-scale field experiments will provide hopefully material for the controlled animal-feed trials planned in the

research program. Regarding that issue, we are behind schedule hence we ask to extend the timeframe of the project to the end of year 2005, without any increase of the budget.

Section II

A. Managerial Issues

During 2003, there were "connection problems" between the Israeli and Turkmen sides. These problems were due to security problems in Israel that prevented a planned visit of Dr Durikov in Israel. On the other hand, bureaucracy constraints in getting Visa to Turkmenistan at the appropriate time made our visit impossible. In addition, the e-mail connection was not steady enough to exchange information and to have sufficient control on the experimental work in Turkmenistan. Those were the reasons why we were unable to submit the half-year managerial report on June 2003, so the present report covers the whole year. We hope that following the reconstruction of the bilateral work recently (which made the submission of the present report possible), we shall be able to fulfill the objectives of the project almost as outlined in the program.

B. Budget

Until now, there were no special budget problems. We extended the stay of the Ms. Pirogova, the Turkmen trainee, in Israel and used some of salary budget in Israel to cover her expenses. As she intends to stay in Israel until the end of the project, we plan some budget changes in the next coming months. Whether our request to extend the project timeframe is accepted, further budget modifications are expected.

C. Special Concerns: none.

D. Collaboration, Travel, Training and Publications

As already mentioned, collaboration during year 2003 was mainly at the level of training. There were no travels, as explained above. We are at the final stems towards submission of an article, which will be based on data appearing above, in the scientific report.

E. Request for American Embassy Tel Aviv or A.I.D. Actions

Examining *in vivo* feed value of selected saltgrass accessions requires large amounts of plant

material that will not be available within the timeframe of the project, we request the AID authorities to extend the project until December 2005. We believe that if accepted, most research objectives will be achieved and the results will be implemented in the Aral Sea basin, thus contribute to food security and livestock husbandry.