Research Article

The Dwarf Saltwort (*Salicornia bigelovii* Torr.): Evaluation of Breeding Populations

Abdullah A. Jaradat[1] and Mohammed Shahid[2]

[1] USDA-ARS Research Lab and Department of Agronomy and Plant Genetics, University of Minnesota,
 803 Iowa Avenue, Morris, MN 56267, USA
[2] Plant Genetic Resources Program, International Center for Biosaline Agriculture, P.O. Box 14660, Dubai, UAE

Correspondence should be addressed to Abdullah A. Jaradat, abdullah.jaradat@ars.usda.gov

Breeding populations of the dwarf saltwort (*Salicornia bigelovii* Torr. (Chenopodiaceae)) have been evaluated under high seawater salinity ($45\,dS\,m^{-1}$) for phenotypic, morphometric, biomass and seed traits to select and develop genotypes for biomass, seed, and vegetable production using sea and brackish water and marginal land resources. The largest variation between populations was found for harvest index and the smallest for number of spikes per plant; however, a more complex structure of variance was found when fixed and random factors were considered. Multivariate relationships between and within architectural and fitness-related traits suggest that biomass and seed yield gains can be achieved by manipulating plant architecture. Discriminant analyses between populations resulted in populations being correctly (mean 83%) classified. Prediction (R^2) and validation (Q^2) coefficients in the partial least squares regression modeling, using three phenotypic markers, 12 morphometric traits, nine populations and 24 families(populations), were 0.86 and 0.78; respectively ($P < 0.002$) for plant dry weight and 0.89 and 0.83, respectively ($P < 0.001$) for seed yield per plant. We developed a procedure to identify populations and families within populations with favorable combinations of phenotypic and morphometric traits that are suitable for the development of Salicornia varieties for biomass, seed, or green vegetable production.

1. Introduction

Natural and man-made selection for salt tolerance has been going on for millennia [1, 2]. Compared to the more resource-demanding and lengthy breeding process of salt-tolerant glycophytes, domestication of a halophyte, or a facultative halophyte [3, 4], such as, *Salicornia bigelovii* is more practical for rapid development of such crops [5]. With the growing scientific and social recognition of the diverse values of *Salicornia bigelovii*, new agricultural production systems based on the use of saline water; including brackish, drainage, and seawater, and marginal land resources have been developed in several parts of the world [6] including the Arabian Peninsula [7]. A pilot-scale *Salicornia* farm, using central-pivot seawater irrigation system, was established in the eastern coastal region of the Arabian Peninsula [8] with biomass and seed yields of about 18–24 and 1.3–$2.0\,Mg\,ha^{-1}$, respectively. Some authors (e.g., [5]) consider that the utilization of halophytes as forage crops is their greatest value as compared to other ecosystem services they may provide [9]. Although halophyte forage and seed products can replace conventional ingredients in animal feed rations, however, there are some restrictions on their use due to high-salt content and antinutritional compounds present in some species [10]. Halophytes have applications in recycling saline agricultural wastewater [9] and reclaiming salt-affected soils in arid-zone irrigated agriculture [5]. More recently, a steadily increasing number of halophytes, including *Salicornia bigelovii,* are being grown as vegetable or horticultural crops, mostly for the export market [6, 11].

Salicornia spp. is a widespread genus of succulent halophytes with compressed leaves that may be transformed into jointed, photosynthetic shoots [12]. The genus belongs to the subfamily Salicornioideae and the family Chenopodiaceae. Salicornioideae contains some of the most salt-tolerant terrestrial plants that thrive well in salt marshes, coastal areas, and among mangroves of different types [3]. There is a wide range of physiological, morphological, and biochemical adaptation mechanisms in these plants, which may result in a wide variation in their level of salt tolerance. Dwarf saltwort (*Salicornia bigelovii* Torr.) inhabits the broadest range of salinity and has very little phenotypic response to salinity gradient [13, 14]; it is a predominantly cross-pollinated annual salt marsh plant with green succulent jointed stems that form terminal fruiting spikes with seeds born in spikelets (i.e., jointed spike segments) [12, 15]. The tetraploid ($2x = 4n = 36$) wild species is a North American and Caribbean element naturally distributed in coastal marshes. Determinacy, a long anthesis (for vegetative, green salad production), and seed shed (for seed production) upon maturity characterize the domestication syndrome of the species [12].

Due to its exceptional salt tolerance and adaptation to marginal lands and hot climates, *Salicornia bigelovii* Torr has great potential as a domesticated biomass, oilseed, and forage crop plant [12, 16, 17]. It has been evaluated as an oilseed and vegetable crop in the desert coastlines of Mexico [18], the Middle East [7, 8], and Africa [6] with encouraging results. The seed germinates directly on seawater [19] and has high oil (30%) and protein (35%) content and low concentration of salt (<3%). The oil yield and quality are comparable to that of major oilseed crops with a high content of polyunsaturated linoleic (75%) and linolenic (omega-3) fatty acids. In addition to its value in human diet, the oil can be used for the production of biodiesel [20, 21].

A few plant breeding and management programs have been initiated in different parts of the world to develop improved germplasm collection appropriate for biomass, oilseed, or vegetable production and develop appropriate management practices for large-scale production [4, 6, 8]. Currently, *S. bigelovii* is commercially cultivated as a minor specialty vegetable crop for the USA and European fresh produce markets [6]. However, its potential as an oilseed, forage, biomass crop and its carbon sequestration still need to be shown [2, 4]. The program initiated by BEHAR (Arabian Saline Water Technology Company Ltd.; Ali Maghboul, personal communication) aimed at selecting genotypes of *S. bigelovii* and developing management practices appropriate for central pivot irrigation system using seawater. A large number of breeding accessions have been acquired by and evaluated at the Genetic Resources Program of ICBA. The objectives of this first part of the study were to (1) characterize and evaluate the germplasm for a number of phenotypic and morphometric plant, spike, and seed traits, (2) study trait relationships and identify sources of variation within the germplasm collection, and (3) develop multivariate models and identify populations, families, or genotypes for high biomass or seed production.

2. Materials and Methods

2.1. Germplasm. Seed of nine breeding populations developed by Dr. Ali Maghboul (Personal communication; Arabian Saline Water Technology Company Ltd.) was introduced to the genebank of the International Center for Biosaline Agriculture (ICBA), Dubai, UAE in 2001 and was used to select, evaluate, and improve germplasm appropriate for biomass, seed, and green vegetable production using seawater and coastal land resources. A large number of plants (913), belonging to 24 families and derived from nine breeding populations were used in this study. The germplasm was characterized by large diversity based on several phenotypic plant and spike markers. The germplasm collection was grown out in a field nursery at ICBA Research Station (25° 05′ 45.98″ N; 55° 23′ 25.29″ E; elevation 30 m) during the growing season of 2001 and 2002.

2.2. Field Nursery and Plant Evaluation. Seed germination and early seedling growth were carried out under greenhouse conditions using freshwater for irrigation. Approximately 5 seeds were planted/pot and thinned to one plant/pot after seedling establishment. Fully developed plants were transplanted to a field nursery on a carbonatic, hyperthermic typic torripsamments soil [22], whereby potted single plants (pot volume 3.1 L, filled with medium-textured sand and a potting mix of 3 : 1 by volume) were placed in furrows arranged in spaced rows to resemble a field situation. Each germplasm entry in the nursery was planted in two rows, four meters long; plants were spaced at 50 cm between rows, and the final stand was adjusted to 100 plants row^{-1} (or approximately 200,000 plants ha^{-1}). Seawater irrigation (25%) commenced one week after transplanting and was increased to 50 and 100% ($45\,\text{dS m}^{-1}$) three and four weeks after transplanting, respectively. An automated drip irrigation system was constructed to deliver 2 L of irrigation seawater containing at time of applying fertilizers the required dose of a 20-20-20 N-P-K plus micronutrients application. The volume of irrigation seawater was adjusted to account for evapotranspiration and leaching requirements. Each germplasm entry was monitored throughout the growing seasons for growth and development, and a number of phenotypic and quantitative (morphometric) traits were scored on 50 random plants per germplasm entry upon maturity and on their yield components after harvest.

2.3. Data Collection. Phenotypic and morphometric data were scored, measured, or calculated on whole plants, spikes, spike segments, and seed. Subsamples of 10 random plants per germplasm entry were scored for color using the Munsell Color Chart [23], and their spikes were scored for surface texture and tip shape. Morphometric traits measured on whole plants were plant height, dry weight, height of lowest spike on the stem, number of spikes plant^{-1}, and seed weight plant^{-1}. Morphometric traits measured on spikes of the same random plants per germplasm entry were spike length, spike weight, number of seed spike^{-1}, and number of seed (spike

segment)$^{-1}$. Finally, number of seeds g^{-1} and spike fertility were calculated for the same subsamples.

2.4. Statistical Analyses. Data collected during the growing seasons were tested for homogeneity of variances before conducting univariate and multivariate statistical analyses. Shapiro-Wilk's test was conducted to test for normality of the distribution of each morphometric variable; there was no need to carry out variable transformation in any of these variables. Descriptive statistics (mean, minimum, maximum, and coefficient of variation) were calculated for each of the 12 morphometric traits. Phenotypic markers were scored for plant color, texture of spike surface, and shape of spike tip. One-way analysis of variance (ANOVA) and pairwise correlation analyses were carried out for morphometric traits using the overall nursery variance as a measure of spatial variation. Canonical discriminant analysis (as a multivariate analysis of variance) was performed on populations using morphometric traits. Percent correct classification was estimated for each population. A variance components analysis, with populations and plant color phenotypes as fixed factors and families (populations) and plant color phenotypes (families) as random factors, was carried out for all 12 morphometric traits. Percent variance explained by random factors was estimated for each morphometric trait. The whole germplasm collection of 24 families in nine populations and their phenotypic markers and morphometric traits were subjected to principal components analyses to identify latent variables accounting for the largest amount of variation in the germplasm collection. Finally, the partial least squares regression option in the nonlinear iterative partial least squares (NIPALS) algorithm was used to develop prediction and validation regression models and estimate plant dry weight and seed yield plant^{-1} as functions of phenotypic markers, morphometric traits, populations, and families in this germplasm collection of *S. bigelovii*. Regression coefficients in the validation models are considered as components of a selection index for large dry matter yield or large seed yield in this germplasm. All statistical analyses procedures were carried out using relevant modules in STATISTICA v.10 software program [24].

3. Results

Environmental conditions, soil characteristics, and chemical composition of irrigation water (Table 1) resemble typical natural settings for the growth and development of *S. bigelovii*. Salinity (45.0 dS m^{-1}) and density (1.031 g cm^{-3}) of the irrigation water were higher than average seawater values (about 35.0 dS m^{-1} and 1.028 g cm^{-3}), respectively, due to the geohydrothermal characteristics of the Gulf near Dubai, UAE.

3.1. Descriptive Statistics. Descriptive statistics (mean, minimum, maximum, and coefficient of variation) for 12 morphometric traits measured on 913 plants of nine breeding populations of *S. bigelovii* Torr. under field conditions during the 2001-2002 growing seasons (Table 2) indicate a

TABLE 1: Irrigation water analysis.

Variable	mg L^{-1}
Sodium (Na$^+$)	13,044
Magnesium (Mg^{+2})	1,500
Calcium (Ca^{+2})	520
Chloride (Cl^{-1})	23,000
Sulfate (SO$_4^{-2}$)	3,100
Bicarbonate (HCO$_3^{-1}$)	171
Carbonate (CO$_3^{-2}$)	24
Organic C	1.94
EC	45 dS m^{-1}
Density	1.031 g cm^{-3}

TABLE 2: Descriptive statistics for 12 morphometric traits measured on 913 plants of nine breeding populations of *S. bigelovii* Torr. under field conditions during the 2001-2002 growing seasons.

Trait	Mean	Min-Max	C.V. (%)
(1) Plant height (cm)	55.8	38.5–75.4	33
(2) Plant dry weight (g)	52.1	36.1–69.0	13
(3) Height of lowest spike (cm)	20.2	5.0–48.2	37
(4) Spike length (cm)	6.7	3.5–14.5	30
(5) Spike weight (mg)	0.3	0.11–0.78	45
(6) Spikes plant^{-1}	105.0	45.0–200.0	23
(7) Seed weight plant^{-1} (g)	3.1	0.82–6.5	47
(8) Number of seeds (spike segment)$^{-1}$	1.8	0.2–4.4	51
(9) Number of seeds spike^{-1}	28.1	10.0–82.0	49
(10) Number of seeds g^{-1}	1,001.0	653–1,217	17
(11) Spike fertility (%)	31.8	8.0–72.0	47
(12) Harvest index (%)	6.1	1.12–18.5	61

wide range of variation within traits (as indicated by the range) and among traits (as measured by C.V.; 13–61%). Architectural traits (e.g., plant height, spike length, height of lowest spike, and spikes plant^{-1}) were less variable than traits directly related to reproductive effort. The latter group (e.g., spike and seed weight plant^{-1}, number of seed segment^{-1} and spike^{-1}) displayed the largest level of variation (C.V. = 45 to 51%). Spike fertility, as an important reproductive trait, indicated that certain plants were almost barren (8%) while other spikes were highly fertile (72.0%). Nevertheless, the harvest index was the most variable (C.V. = 61%) among all traits and, on average, was very small (6.1%).

One-way ANOVA for 12 morphometric traits measured on 913 plants of nine breeding populations of *S. bigelovii* Torr. under field conditions (Table 3) indicated the presence of large level of variation among populations for all traits. The F values, as ratios between variance due to differences between populations and variance due to within population differences (i.e., error variance), suggested that harvest index exhibited the largest level of variation between populations ($F = 107.2$; $P < 0.0001$), followed, in decreasing order,

TABLE 3: One-way ANOVA for 12 morphometric traits measured on 913 plants in nine breeding populations of *S. bigelovii* Torr. under field conditions in 2001-2002 growing season (all *F* values are significant, $P < 0.05$).

Trait	Variance due to populations	Error variance	*F* value
(1) Plant height (cm)	10,005.9	915.3	10.9
(2) Plant dry weight (g)	418.9	35.2	11.9
(3) Height of lowest spike (cm)	527.6	38.4	13.7
(4) Spike length (cm)	18.4	3.46	5.3
(5) Spike weight (mg)	0.1	0.01	7.7
(6) Spikes plant^{-1}	1,632.7	557.21	2.9
(7) Seed weight plant^{-1} (g)	38.9	0.78	50.1
(8) Number of sees (spike segment)$^{-1}$	7.1	0.66	10.7
(9) Number of seeds spike^{-1}	2,067.6	128.2	16.1
(10) Number of seeds g^{-1}	355,758.2	15,574.5	22.8
(11) Spike fertility (%)	1,674.0	165.2	10.1
(12) Harvest index (%)	326.0	3.04	107.2

by seed weight plant^{-1} ($F = 50.1$; $P < 0.0001$), number of seeds g^{-1} ($F = 22.8$; $P < 0.002$), and number of seeds spike^{-1} ($F = 16.1$; $P < 0.002$). The smallest level of variation between populations was displayed by number of spikes plant^{-1} ($F = 2.9$; $P < 0.05$) followed by spike length ($F = 5.3$); whereas, the remaining traits exhibited intermediate (F values from 7.7 to 13.7) and significant levels of variation between populations.

3.2. Trait Associations. Significant ($P < 0.05$) correlation coefficients between 12 plant, spike, and seed morphometric traits measured on 913 plants of nine *S. bigelovii* populations (Table 4) reflect the level of pairwise (dis)-association among and within architectural and reproductive-effort traits. Out of 66 possible pairwise correlation coefficients, 40 (or 61%) were significant ($P < 0.05$), 24 of which (or 60%) were positive and the remaining 16 (or 40%) were negative. In general, and unless they are competing for resources, the closer the traits to each other either architecturally or ontogennetically, the larger and stronger was the association between them as expressed by the magnitude and sign of the correlation coefficient. The architectural traits (i.e., plant height, plant dry weight, height of lowest spike, spike length, and number of spikes plant^{-1}) had positive and significant but relatively small correlation coefficients among themselves (0.20 to 0.48); whereas, the reproductive-effort traits displayed the largest positive (0.65 to 0.95) correlations coefficients among themselves. As architectural traits, plant height, plant dry weight, spike length, and height of lowest spike, in this order, had relatively small but significant and increasing number of negative correlation coefficients with reproductive-effort traits. Negative correlation coefficients between harvest index and each of plant height (-0.35)

and plant dry weight (-0.37) were the largest to be found between architectural and reproductive-effort traits.

3.3. Variance Components. Variance components analyses based on fixed (populations and plant color phenotypes) and random (families within populations and plant color phenotypes within families) in nine breeding populations of *S. bigelovii* (Table 5) illustrate the complex control of trait variances and the amount of variance explained by sources of variation. At a multivariate level, differences between populations were significant ($P < 0.05$) for eight traits or marginally significant ($P < 0.07$ to < 0.09) for the remaining four. Differences between plant color phenotypes (i.e., red, yellow, and yellowish red) were significant or marginally significant for height of lowest spike, spike weight, and number of seeds g^{-1}. A more complex structure of variance components partitioning is evident when the random factors (i.e., families within populations) and plant color phenotypes within families are considered. The maximum amount of variance accounted for by both random factors (73.7%) was for number of seeds g^{-1} and the smallest (2.8%) was for height of lowest spike.

Differences between families within populations did not account for any variation in three traits, accounted for nonsignificant variance (1.7 to 9.2%) in three traits and for marginally significant ($P < 0.06$ to < 0.09) variances (17.7 to 54.2%) in the remaining six traits. Differences between plant color phenotypes within families accounted for significant (7 traits) or marginally significant (3 traits) amounts of variation in 10 traits; the only nonsignificant variances were those for height of lowest spike (2.8%) and spike length (5.9%). Larger amounts of variance in five traits were accounted for by differences between plant color phenotypes within families as compared to families within populations; the latter accounted for more variances in five traits and did not differ in the remaining two (i.e., height of lowest spike and spike length).

3.4. Discrimination between Populations. Discriminant analyses between *S. bigelovii* populations were based on 12 morphometric traits (Figure 1) and resulted in 50% (populations Sb7 and Sb8) to 100% (population Sb9) correct classification of these populations. The remaining populations were correctly classified with >70% correct classification except Sb3 (65%). The first and second canonical discriminant roots explained 0.58 and 0.25 of total variation, respectively. A third canonical discriminant function accounted for the remaining 17% of variation (not presented). Of the 12 morphometric traits, eight and five had significant standardized coefficients on the first and second canonical discriminant functions, respectively. The standardized coefficients make it easy for direct comparisons of the strength of these traits in discriminating between populations. Seed weight plant^{-1} and plant height, with strong negative (-1.39) and positive (0.95) standardized coefficients, respectively, on the first canonical discriminant function and number of seeds g^{-1}, with a strong negative (-1.33) standardized coefficient on the second discriminant function, largely contributed

TABLE 4: Significant ($P < 0.05$) correlation coefficients between 12 plant, spike and seed morphometric traits measured on 10 random spike each of 913 plants in nine *S. bigelovii* populations.

Trait	1	2	3	4	5	6	7	8	9	10	11
(1) Plant height (cm)	1										
(2) Plant dry weight (g)	0.34	1									
(3) Height of lowest spike (cm)		0.20	1								
(4) Spike length (cm)		0.30		1							
(5) Spike weight (mg)	0.20	0.31		0.79	1						
(6) Spikes plant⁻¹	0.48		−0.16			1					
(7) Seed weight plant⁻¹ (g)		−0.14	−0.13	0.19	0.31	0.20	1				
(8) Number of seeds (spike segment)⁻¹			−0.14	−0.23	−0.16		0.56	1			
(9) Number of seeds spike⁻¹				0.32	0.27		0.69	0.79	1		
(10) Number of seeds g⁻¹			−0.23		−0.24		−0.53		−0.22	1	
(11) Spike fertility (%)				−0.24	−0.16		0.55	0.95	0.78		1
(12) Harvest index (%)	−0.35	−0.37	−0.18	0.14	0.16		0.88	0.52	0.65	−0.43	0.51

TABLE 5: Variance components analyses based on fixed (populations and plant color phenotypes) and random (families within populations and plant color phenotypes within families) in nine breeding populations of *S. bigelovii*.

Trait	Probability of *F* Fixed factors		Probability of *z* (% variance) Random factors	
	Populations	Plant color phenotypes	Families (populations)	Plant color phenotypes (families)
(1) Plant height (cm)	0.05	0.75	0.12 (8.5)	0.05 (21.5)
(2) Plant dry weight (g)	0.08	0.89	0.32 (9.2)	0.06 (15.9)
(3) Height of lowest spike (cm)	0.02	0.02	0.0 (0.0)	0.30 (2.8)
(4) Spike length (cm)	0.03	0.20	0.0 (0.0)	0.19 (5.9)
(5) Spike weight (mg)	0.03	0.07	0.47 (1.4)	0.03 (27.5)
(6) Spikes plant⁻¹	0.05	0.88	0.0 (0.0)	0.05 (19.5)
(7) Seed weight plant⁻¹ (g)	0.03	0.51	0.06 (38.4)	0.02 (24.2)
(8) Number of sees (spike segment)⁻¹	0.08	0.31	0.07 (46.0)	0.05 (7.5)
(9) Number of seeds spike⁻¹	0.07	0.42	0.09 (22.3)	0.08 (10.7)
(10) Number of seeds g⁻¹	0.05	0.06	0.07 (54.2)	0.04 (19.5)
(11) Spike fertility (%)	0.09	0.36	0.09 (45.0)	0.09 (8.5)
(12) Harvest index (%)	0.05	0.40	0.09 (17.7)	0.01 (39.7)

to these levels of discrimination between populations. Two populations (Sb1 and Sb9) were largely separated from the remaining populations on the first and second canonical discriminant functions, respectively. One population (Sb4) seemed to be composed of two distinct subpopulations, with one of them straddling negative and positive sides of both canonical discriminant functions; whereas, most samples of each of the remaining populations were confined to one half of the two-dimensional plot.

3.5. Multivariate Relationships. Coefficients of determination (i.e., Q^2 values of the validation model) of the first and second principal components (PC1 and PC2, resp.), a scatter plot of 24 families derived from nine *S. bigelovii* populations and their phenotypic markers and morphometric traits are presented in Figure 2. Both PCs explained a total of 0.59 of total variation, and it was necessary to extract four more PCs to explain the remaining 0.41 of total variation (data not presented). Populations, families (populations), phenotypic, and morphometric traits differed as to their loadings (i.e.,

correlation coefficients) on PC1 and PC2. The nine populations and the 24 families were separated by both PCs into four groups, each was associated (positively or negatively) with a number of phenotypic and morphometric traits. With a few exceptions, most populations and families displayed large levels of dispersion within the two dimensional plot.

The relatively stronger level of separation across PC1, with $Q^2 = 0.37$, as compared to PC2, with $Q^2 = 0.22$, suggested that populations in quadrate I and IV (Sb1 and Sb5) are associated, in a decreasing order, with larger harvest index, seed weight plant⁻¹, seeds spike⁻¹, spike fertility, and seeds (spike segment)⁻¹; whereas populations in quadrates II and III are associated with smaller values of these traits, but larger values of seeds g⁻¹ (i.e., lighter seed), plant height, and plant dry weight. Differences between plant color phenotypes (i.e., red, yellow and yellowish red) across both PCs were larger than differences between spike phenotypic traits. Combinations of plant and spike phenotypic variants and morphometric traits comprise a visual selection index to identify populations or families for specific objectives.
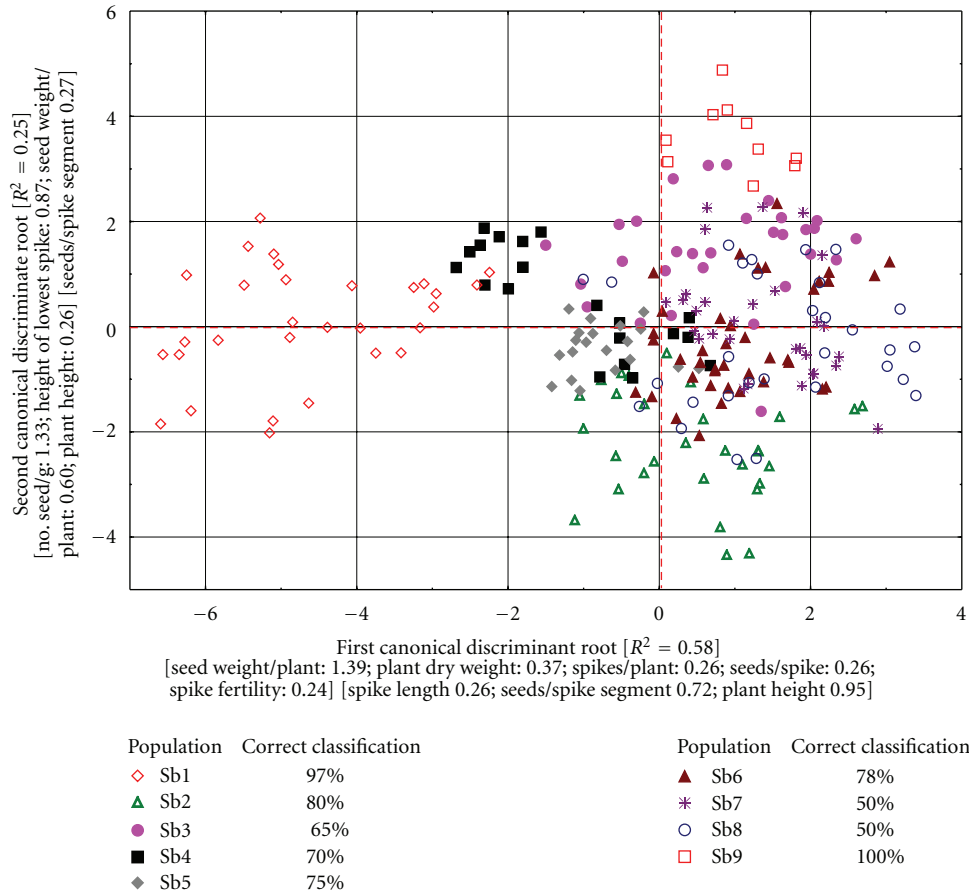
FIGURE 1: Discriminant analyses, variance accounted for by the first and second canonical discriminant functions, standardized loadings of morphometric traits, and percent correct classification of nine *Salicornia bigelovii* populations evaluated for phenotypic markers and morphometric traits under seawater irrigation.

3.6. Prediction and Validation Models. The first partial least squares regression component (PLSC1) was used to predict and validate dry weight (Figure 3(a)) and seed yield (Figure 3(b)) plant^{-1} in *S. bigelovii* as functions of phenotypic markers and morphometric traits. Only 0.69 of the variation in all variables that entered the model (R^2X) was needed to extract the first PLSC which explained 0.86 and 0.78 of variation in plant dry weight at the prediction (R^2Y) and validation (Q^2Y) stages of model building, respectively (Figure 3(a)). Trait loadings (i.e., correlation coefficients between a trait and the PLSC) differed between and within phenotypic and morphometric traits, and there were major differences between populations and families (populations). Traits with large positive loadings (e.g., plant height, spike weight, and spike length) contributed to producing large plant dry weight; whereas, traits with negative loadings (e.g., harvest index, spike fertility) contributed to producing lower plant dry weight. Two populations (Sb2 and Sb8) had larger plant dry weights than Sb3 and Sb6.

The prediction and validation model for seed yield plant^{-1} (Figure 3(b)) was as precise when compared with the prediction and validation of dry plant weight. Only 0.45 of the variation in all phenotypic and morphometric

variables (R^2X) was needed to extract the first PLSC which explained 0.89 and 0.83 of variation in seed yield plant^{-1} at the prediction (R^2Y) and validation (Q^2Y) stages of model building, respectively. Interestingly, plant height accounted for negligible amount of variation to the model building. Spike length and spike weight had intermediate and positive loadings; whereas, seeds (spike segment)$^{-1}$ and spike fertility had the largest loadings on PLSC1. Loadings of all phenotypic traits were relatively small (approximately ± 2.0). A smaller level of variation in seed yield plant^{-1} was accounted for by differences between populations and between families within populations. Noticeably, Sb1 was the only population to contribute positively and more than other populations to PLCS1.

4. Discussion

If we were to live in a "salinized" world [1], then domestication of wild halophytes, such as, *Salicornia bigelovii*, is a necessary step towards the development of commercially viable salt-tolerant crops. Such crops must have high yield potential for biomass, seed, and by-products. In view of global climate change, domesticated halophytes should be able to
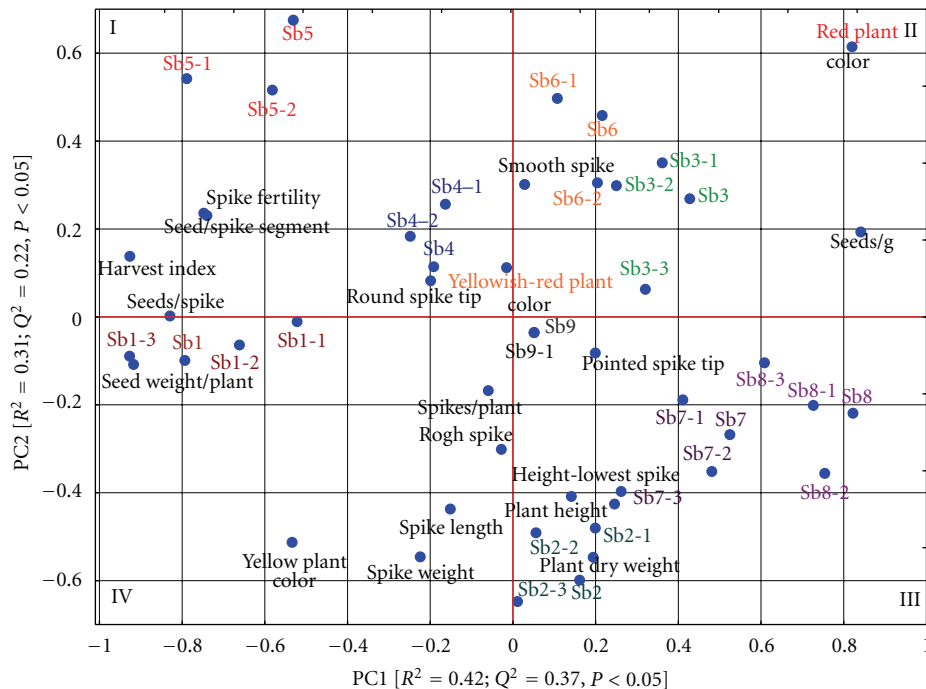
FIGURE 2: Coefficients of determination for the prediction (R^2) and validation (Q^2) models of the first and second principal components (PC1 and PC2, resp.) and a scatter plot of 24 families derived from 9 *S. bigelovii* populations and their phenotypic markers and morphometric traits evaluated under seawater irrigation.

provide additional ecosystem services, such as, greenhouse gas emission reduction and carbon sequestration either in soils, plant biomass, or both. A modern-day domestication process starts with screening and evaluating large germplasm collections and selecting the most adapted and productive genotypes for varietal development. Results of the current evaluation study indicated the presence of large differences, at univariate and multivariate levels, between populations and between families within populations and that these populations have diverged genetically to allow for effective selection of diverse genotypes [25]. The continuous variation, and the level of variation (as measured by C.V.) and covariation (as deduced from correlation coefficients) in the morphometric traits, indicated that these are highly variable populations, presumably due to the outcrossing nature of the breeding system [26]. A strong covariation among morphometric traits suggests the presence of constraints on their independent evolution due to pleiotropy, linkage, or selective forces that maintain particular trait combinations [27].

The wide range of phenotypes identified in this germplasm is obviously determined by a complex set of factors. The role of this phenotypic plasticity has been shown clearly in several populations (e.g., Sb7 and Sb8) and even families within populations (e.g., families within Sb1 and Sb8 along PC1; Figure 2). Environmental factors (temperature, salinity, soil factors) and, probably, plant density may have an impact on phenotypic diversity in *Salicornia*. Moreover, outcrossing and a long anthesis period may have caused large levels of phenotypic variation. Nevertheless, the degree of underlying genotypic variation has yet to be elucidated.

Although we did not directly evaluate plant architecture (which is determined by a simple morphology [11]), and regardless of the large plasticity (e.g., in plant height and spikes plant^{-1}), the growth of *Salicornia bigelovii* appeared to be determinated, in spite of the relatively long anthesis phase, [28] producing, on average, 15–20 g of seed m^{-2}. Under a subtropical environment, which is the most conducive for its growth and production, *Salicornia* can reach >50 cm in height, produce 40–50 g of seed plant^{-1}, with most spikes (and seed yield) being born on the upper third of the plant [15]. *Salicornia* germplasm used in this study, being comprised of ecotypes of different origins and no selected "varieties", displayed a wide range of both plant height (38.5–75.4 cm; C.V. = 33%) and position of the lowest first fruiting spike (5.0–48.2 cm; C.V. 37%); however, these two traits were not significantly correlated (Table 2). The position of the lowest spike (as an indicator of the onset of reproductive phase during the ontogeny of the plant) may have implications for length of the vegetative growth phase, seed maturity, and uniform harvest of seed yield [13]. A late onset of the reproductive phase is required for a large yield of fresh fiberless vegetative shoots with market value. Terminal fruiting spikes in *Salicornia* are produced at the shoot tips, and vegetative development is retarded thus impacting fresh yield [11].

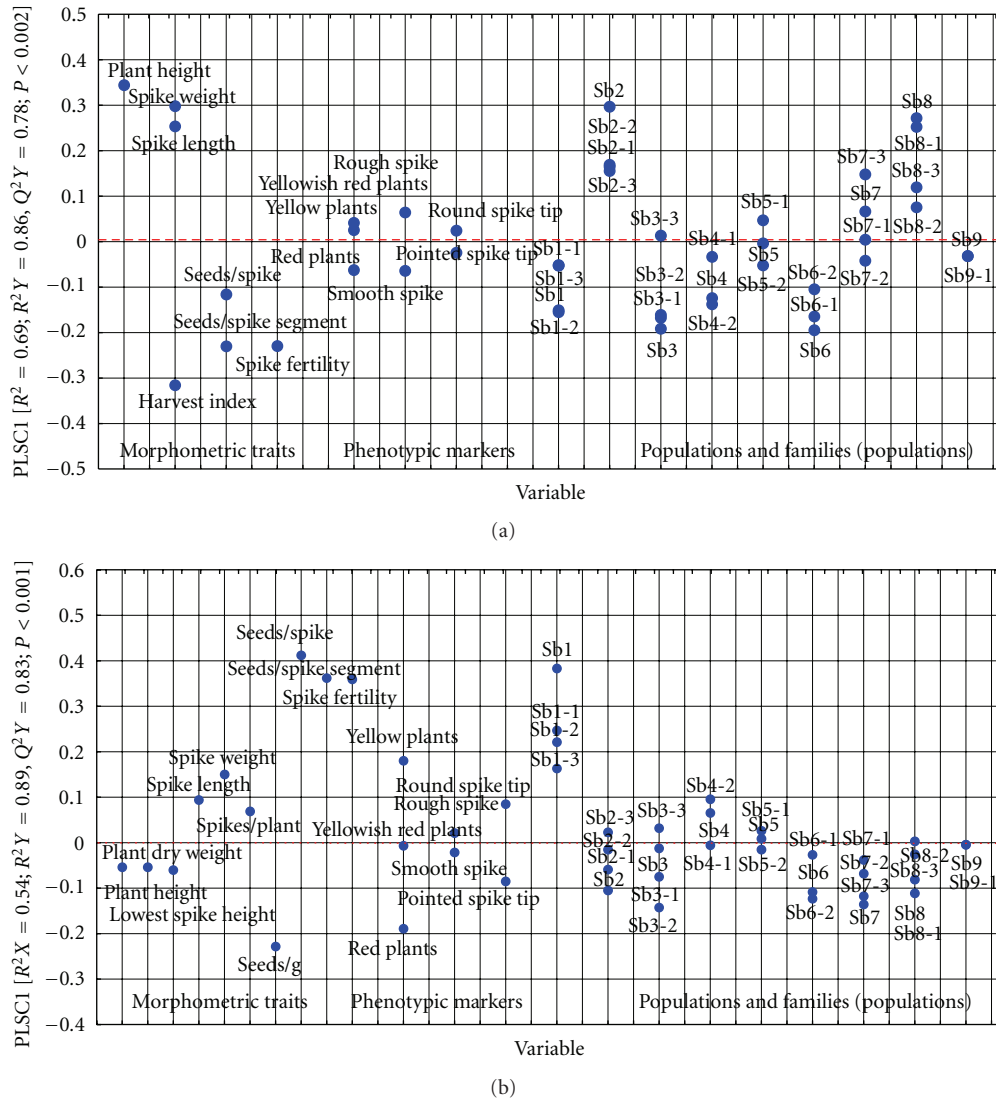A general indicator of germplasm productivity can be deduced from harvest index estimates (1.12–18.5%) and

(a)

(b)

FIGURE 3: The first Partial least squares regression component (PLSC1) to predict and validate plant dry weight (a) and seed yield plant^{-1} (b) in *S. bigelovii* as functions of morphometric traits, phenotypic markers, populations, and families within populations evaluated under seawater irrigation.

its relationship with other yield components (Table 4); earlier harvest index estimates under open field, but not greenhouse, conditions were highly variable as well (e.g., 9.5–15.0%) [15]. Population with lower harvest indices had larger plant weight ($r = -0.35$; $P < 0.05$); however this was not an indication of lower spike fertility as it was associated with seeds spike^{-1} and, as expected, with seeds (spike segment)$^{-1}$; spike fertility was decoupled from plant dry weight (Table 4) and contributed positively to seed yield plant^{-1}, with large loading (0.5) on PLSC1 (Figure 3(a)).

The discriminating power of morphometric traits between populations of *Salicornia bigelovii* was strong (70–100%) for most, but not all, populations (e.g., Sb7 and Sb8, with 50% correct classification each) (Figure 1). The average level of discrimination (83.0%) was as high as the one reported recently between two *Salicornia* populations and

was based on molecular markers [29]. In this analysis, seed yield plant^{-1} and plant height were the largest contributors to discrimination between populations, followed, in decreasing order, by number of seeds g^{-1} and seeds (spike segment)$^{-1}$. Traits within each of these pairs were decoupled (Table 4), had opposite loadings on both PCs (Figure 2), and therefore can be easily manipulated in a breeding and selection program [28, 30].

Dry matter and seed yield ha^{-1}, when based on a density of 250 plants m^{-2}, plant dry weight (mean = 52.1; range 36.0–69.0 g plant^{-1}) and seed yield (mean = 3.1; range 0.82–6.5 g plant^{-1}) are equal to, if not larger than, conventional crop yields under comparable rainfed or freshwater-irrigated conditions [31] but lower than yield recorded under greenhouse conditions [6]. However, survival rate and plant architecture, especially branching and number

of spikes plant^{-1}, are density dependent [32]. Nevertheless, *Salicornia* plants produced twice as much fresh and dry matter when irrigation water has optimal (seawater) as compared with suboptimal ($\sim$10 dS m^{-1}) or supraoptimal (>50 dS m^{-1}) salinity [31].

The PLS regression models are appropriate statistical tools to identify populations, families (populations), and trait associations to carry out selection and identify appropriate germplasm for specific purposes. Selections from this *Salicornia* germplasm may attract considerable interest of the private sector to develop varieties appropriate for seawater-based production of biomass for bioenergy, oilseed for biodiesel [21], forage for animal feed [33], and vegetable and polyunsaturated fatty acids for human consumption [11], while conserving freshwater resources.

5. Conclusions

Selections were made from natural populations to develop salt-tolerant varieties of *Salicornia bigelovii* that can be irrigated with seawater. We carried out detailed evaluation of about 1,000 selected plants for 15 plant and seed traits and identified plants appropriate for large seed or large biomass production using seawater for irrigation. We identified populations and families within populations with favorable combinations of traits that are suitable for the development of *Salicornia* varieties for biomass, seed, or green vegetable production. Potentially, these can be used by farmers in small-scale vegetable production, in large-scale biomass and oilseed renewable bioenergy production, or for reclamation of saline lands.
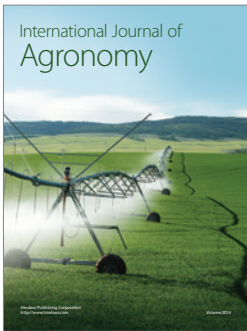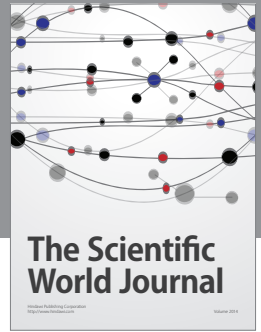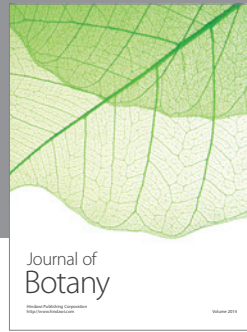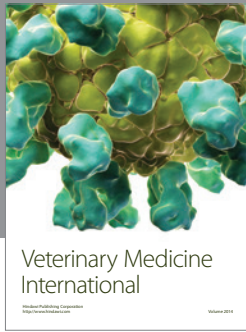
Acknowledgments

References

[1] J. Rozema and T. Flowers, "Ecology: crops for a salinized world," *Science*, vol. 322, no. 5907, pp. 1478–1480, 2008.

[2] A. Galvani, "The challenge of the food sufficiency through salt tolerant crops," *Reviews in Environmental Science and Biotechnology*, vol. 6, no. 1–3, pp. 3–16, 2007.

[3] E. O. Rueda-Puente, J. L. García-Hernández, P. Preciado-Rangel et al., "Germination of *Salicornia bigelovii* ecotypes under stressing conditions of temperature and salinity and ameliorative effects of plant growth-promoting bacteria," *Journal of Agronomy and Crop Science*, vol. 193, no. 3, pp. 167–176, 2007.

[4] T. J. Flowers and A. R. Yeo, "Breeding for salinity resistance in crop plants: where next?" *Australian Journal of Plant Physiology*, vol. 22, no. 6, pp. 875–884, 1995.

[5] M. Ajmal Khan, R. Ansari, B. Gul, and M. Qadri, "Crop diversification through halophyte production on salt-prone land resources," *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, vol. 48, pp. 1–9, 2006.

[6] D. B. Zerai, E. P. Glenn, R. Chatervedi et al., "Potential for the improvement of *Salicornia bigelovii* through selective breeding," *Ecological Engineering*, vol. 36, no. 5, pp. 730–739, 2010.

[7] A. A. Jaradat, "Saline agriculture in the Arabian Peninsula: management of marginal lands and saline water resources," *Journal of Food, Agriculture & Environment*, vol. 2, pp. 302–306, 2005.

[8] M. S. Abdal, "*Salicornia* production in Kuwait," *World Applied Science Journal*, vol. 6, pp. 1033–1038, 2009.

[9] H.-W. Koyro, M. Ajmal Khan, and H. Lieth, "Halophytic crops: a resource for the future to reduce the water crises," *Emirates Journal of Food and Agriculture*, vol. 23, pp. 1–16, 2011.

[10] E. P. Glenn, C. N. Hodges, H. Lieth, R. Pitelka, and L. Pitelka, "Growing halophytes to remove carbon from the atmosphere," *Environment*, vol. 34, pp. 40–43, 1992.

[11] Y. Ventura, W. A. Wuddineh, M. Myrzabayeva et al., "Effect of seawater concentration on the productivity and nutritional value of annual *Salicornia* and perennial *Sarcocornia* halophytes as leafy vegetable crops," *Scientia Horticulturae*, vol. 128, no. 3, pp. 189–196, 2011.

[12] G. Kadereit, P. Ball, S. Beer et al., "A taxonomic nightmare comes true: phylogeny and biogeography of glassworts (*Salicornia* L., Chenopodiaceae)," *Taxon*, vol. 56, no. 4, pp. 1143–1170, 2007.

[13] E. Troyo-Dieguez, A. Ortega-Rubio, Y. Maya, and J. L. Leon, "The effect of environmental conditions on the growth and development of the oilseed halophyte *Salicornia bigelovii* Torr. in arid Baja California Sur, Mexico," *Journal of Arid Environments*, vol. 28, no. 3, pp. 207–213, 1994.

[14] J. C. Dagar, "Ecology, management and utilization of halophytes," *Bulletin of the National Institute of Ecology*, vol. 15, pp. 81–97, 2005.

[15] E. P. Glenn, J. W. O'Leary, M. C. Watson, T. L. Thompson, and R. O. Kuehl, "*Salicornia bigelovii* Torr.: an oilseed halophyte for seawater irrigation," *Science*, vol. 251, no. 4997, pp. 1065–1067, 1991.

[16] Y. Bashan, M. Moreno, and E. Troyo, "Growth promotion of the seawater-irrigated oilseed halophyte *Salicornia bigelovii* inoculated with mangrove rhizosphere bacteria and halotolerant *Azospirillum* spp.," *Biology and Fertility of Soils*, vol. 32, no. 4, pp. 265–272, 2000.

[17] D. G. Masters, S. E. Benes, and H. C. Norman, "Biosaline agriculture for forage and livestock production," *Agriculture, Ecosystems and Environment*, vol. 119, no. 3-4, pp. 234–248, 2007.

[18] S. R. Grattan, S. E. Benes, D. W. Peters, and F. Diaz, "Feasibility of irrigating pickleweed (*Salicornia bigelovii*. Torr) with hypersaline drainage water," *Journal of Environmental Quality*, vol. 37, no. 5, pp. S149–S156, 2008.

[19] E. P. Glenn, J. J. Brown, and E. Blumwald, "Salt tolerance and crop potential of halophytes," *Critical Reviews in Plant Sciences*, vol. 18, no. 2, pp. 227–255, 1999.

[20] F. Anwar, M. I. Bhanger, M. K. A. Nasir, and S. Ismail, "Analytical characterization of *Salicornia bigelovii* seed oil cultivated in Pakistan," *Journal of Agricultural and Food Chemistry*, vol. 50, no. 15, pp. 4210–4214, 2002.

[21] R. C. Hendricks, D. M. Bushnell, and D. T. Shouse, "Aviation fueling: a cleaner, greener approach," *International Journal of Rotating Machinery*, pp. 1–13, 2011.

[22] M. A. Abdelfattah and S. A. Shahid, "A comparative characterization and classification of soils in Abu Dhabi coastal area in relation to arid and semi-arid conditions using USDA and FAO soil classification systems," *Arid Land Research and Management*, vol. 21, no. 3, pp. 245–271, 2007.

[23] D. Nickerson, "History of the Munsell color system, company, and foundation," *Color Research and Application*, vol. 1, pp. 7–10, 1976.

[24] StatSoft Inc, STATISTICA (data analysis software system). Version 10, 2012, http://www.statsoft.com.

[25] S. J. Mazer and V. A. Delesalle, "Floral trait variation in *Spergularia marina* (Caryophyllaceae): ontogenetic, maternal family, and population effects," *Heredity*, vol. 77, no. 3, pp. 269–281, 1995.

[26] D. E. Howland, R. P. Oliver, and A. J. Davy, "Morphological and molecular variation in natural populations of Betula," *New Phytologist*, vol. 130, no. 1, pp. 117–124, 1995.

[27] S. J. Mazer and V. A. Delesalle, "Covariation among floral traits in *Spergularia marina* (Caryophyllaceae): geographic and temporal variation in phenotypic and among-family correlations," *Journal of Evolutionary Biology*, vol. 9, no. 6, pp. 993–1015, 1996.

[28] R. L. Jefferies, A. J. Davy, and T. Rudmik, "Population biology of the salt marsh annual *Salicornia* europaea Agg," *Journal of Ecology*, vol. 69, pp. 17–31, 1981.

[29] Z. L. Xu, Z. Ali, J. X. Yi et al., "Expressed sequence tag-simple sequence repeat-based molecular variance in two *Salicornia* (Amaranthaceae) populations," *Genetic and Molecular Research*, vol. 10, pp. 1262–1276, 2011.

[30] J.-C. Lefeuvre, V. Bouchard, E. Feunteun, S. Grare, P. Laffaille, and A. Radureau, "European salt marshes diversity and functioning: the case study of the Mont Saint-Michel bay, France," *Wetlands Ecology and Management*, vol. 8, no. 2-3, pp. 147–161, 2000.

[31] F. Ayala and J. W. O'Leary, "Growth and physiology of *Salicornia bigelovii* Torr. at suboptimal salinity," *International Journal of Plant Sciences*, vol. 156, no. 2, pp. 197–205, 1995.

[32] M. Engler and J. M. Hartman, "Density effects on growth and survival of *Salicornia bigelovii* and S. europaea," *Biological Bulletin*, vol. 163, pp. 365–366, 1982.

[33] M. S. Kraidees, M. A. Abouheif, M. Y. Al-Saiady, A. Tag-Eldin, and H. Metwally, "The effect of dietary inclusion of halophyte *Salicornia bigelovii* Torr on growth performance and carcass characteristics of lambs," *Animal Feed Science and Technology*, vol. 76, no. 1-2, pp. 149–159, 1998.