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# Evaluating the growth performance of eleven *Salicornia bigelovii* populations under full strength seawater irrigation using multivariate analyses

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## Abstract

Halophytes constitute an indispensable part of seawater farming systems implemented in coastal areas of desert lands where they do not compete with good quality land and water resources. *Salicornia bigelovii* is considered one of the most promising halophytic species that will grow in a biosaline context. However, suitable germplasm needs to be identified, domesticated and improved in order to achieve high enough yields to compete economically to conventional crops. In the current study, eleven *S. bigelovii* Torr. populations were evaluated for 22 growth parameters and irrigated with full-strength seawater (SW≈55 dS/m).The study assessed the adaptability of such plant material under the hot and dry climatic conditions that prevail in the Arabian Peninsula. High variation was observed among *S. bigelovii* populations for most of the characteristics under study. *S. bigelovii* genotypes were categorized in four groups based on their growth performance and use: for seed production, green biomass as vegetables for human consumption, and biomass as livestock feed; the fourth category was comprised of those halophytic genotypes that demonstrated poor overall performance. Pearson correlation analysis highlighted positive and negative associations among the growth parameters. Path analysis unveiled some interesting interrelationships among growth parameters that could be used for further selection to increase seed production such as number of seeds per spike, number of branches, days to flowering and duration of seed maturity. Plant height and growth cycle could be selected for biomass improvement. The multivariate statistical analyses applied in this study, i.e. clustering, correlation and path analyses were very effective in classifying *S. bigelovii* genotypes and revealed interrelationships among growth traits that could be selected towards *S. bigelovii* improvement in terms of seed and biomass yield.

**Keywords:** Domestication; halophytes; path analysis; dwarf glasswort; seawater agriculture
**Abbreviations:** SW_Seawater; ICBA_International Center for Biosaline Agriculture; MI_Masdar Institute of Science and Technology

## Introduction

Seawater agriculture is the cultivation of salt-tolerant crops irrigated with water pumped from the ocean, and has been explored in sandy soils of coastal deserts (Glenn et al., 2013). Apart from developing good management practices to support seawater farming in a sustainable manner, salt-tolerant plants with high productivity and commercial potential should be identified that could justify the cost of pumping seawater for irrigation. Scientists can either develop salt tolerance in traditional crops through conventional and molecular approaches (Roy et al., 2014; Ashraf and Akram, 2009; Yamaguchi and Blumwald, 2005; Flowers, 2004) or domesticate and improve wild halophytic species that would compare to traditional cultivations (Brown et al., 2014; Glenn et al., 1998). New crop varieties that are salt tolerant have not yet been bred that are successful on a commercial scale (Glenn et al., 2013; Flowers, 2004). In contrast, some halophytic species seem to be viable commercial alternatives to conventional crops (Panta et al., 2014). Several halophytes have demonstrated noteworthy resilience in a wide range of high salinity levels due to inherent salt tolerance (Brown et al., 2014; Rozema and Flowers, 2008; Flowers, 2004). *Salicornia bigelovii* has emerged as an important candidate over other halophytic species since it has demonstrated high yield, extreme salt tolerance (Ventura and Sagi, 2013), and has multiple potential uses as a fresh or pickled vegetable, animal forage, and a biofuel crop (Panta et al., 2014; Glenn et al., 2013).

*S. bigelovii* is an annual, euhalophytic species that naturally grows in southern California, Belize and Mexico (Glenn et al., 2013; Troyo-Dieguez et al., 1994), regions that are characterized by hot and arid climatic conditions comparable to the prevailing climate in the Arabian Peninsula. Several studies have demonstrated the crop potential of *S. bigelovii* in such regions and its exceptional adaptation to coastal desert ecosystems (Glenn et al., 2013; Zerai et al., 2010; Abdal, 2009; Grattan et al., 2008). As a prerequisite for successful cultivation, suitable *S. bigelovii* germplasm that has high productivity, is amenable to agricultural production, and is economically viable, needs to be identified and developed (Glenn et al., 1999). Field studies have already been carried out in the Arabian Peninsula in order to assess the suitability of coastal zones for *S. bigelovii* cultivation (Al-Yamani et al., 2013). Since there are no references for the natural occurrence of *S. bigelovii* in the region and no bred material is available for planting, wild collections of the halophytic species are introduced and undergo the domestication process. Germplasm characterization is needed in order to identify the functional traits of the best performing genotypes in terms of seed and biomass yield with promising breeding value (Mitchell et al., 2015; de Galarreta and Alvarez, 2001; Gepts, 2004). Through selection of plants for desirable traits, agronomic parameters are identified that ultimately improve crop productivity (Brown et al., 2014; Ruan et al., 2010; Zerai et al., 2010).

The quantification and evaluation of the genotypic differences is difficult when multiple growth characteristics are involved (Bresta et al., 2011). Using conventional approaches, genotypes are scored on single characters and the ranking is often inaccurate and cumbersome (Zeng et al., 2002; Zeng, 2005; Lee at al., 2005). Cluster analysis constitutes a multivariate statistical method that can be effectively used to characterize, classify and rank numerous plant cultivars (Jolliffe et al., 1989). Such technique has been used successfully to screen wheat, rice and potato genotypes under drought or salinity stress conditions which were further grouped into homogeneous groups based on their growth performance (Yasir et al., 2013; El-Hendawy et al., 2011, 2005; Zeng et al., 2002; Zeng, 2005; Khrais et al., 1998). It is also interesting to explore the relationships among the growth traits in order to define those that are highly correlated with high yield so that they can be further incorporated in a breeding program. Correlation and path coefficient methods offer this advantage. Correlation coefficients simply show mutual relationships between characteristics, whereas path analysis partitions correlations into direct and indirect effects, estimating the relative magnitude of each trait affecting specific factors such as seed and biomass yield (Garcia del Moral et al., 1991). Jaradat and Shahid (2012) used correlation analysis to examine the associations among twelve traits of nine *S. bigelovii* populations but no other information exists on the use of the aforementioned multivariate statistical techniques in the same halophytic species.

The current study constitutes a part of a series of experiments which try to unveil the crop potential of *S. bigelovii* in the Arabian Peninsula. The objectives of the study were to: a) Assess the growth performance of eleven wild collected *S. bigelovii* populations, which are in domestication process, under field conditions and irrigated with seawater (SW) based on multiple agronomic parameters using multivariate statistical approaches and b) Identify agronomic traits that are associated with high seed and biomass yield for further selection.

## Results

### *Variation among S. bigelovii populations for all growth parameters*

All growth parameters were differentiated by *S. bigelovii* genotype (Tables 1, 2 and 3). However, the F-test did not show statistically significant differences among *S. bigelovii* populations for root length (Table 1), seed weight per plant (Table 2), and duration of maturity (Table 3). Among spike characteristics, spike weight was characterized by the highest CV value (CV=47%), whereas spike width by the lowest (16%) (Table 1). For whole-plant and below-ground traits, wider range of variation was observed for number of spikes and dry root weight (CV=58% and 59% respectively) compared to number of branches and root length (CV=22% and 12% respectively). All biomass and seed yield measurements were characterized by high variation and CV values ranged from 30-73% (Table 2). The group of characteristics related to growth stages demonstrated the lowest CV values compared to the rest groups of traits and varied from 3% to 32% (Table 3). In particular, the duration of growth cycle and days to seed production was almost invariable among *S. bigelovii* populations (CV=3%). On the contrary, duration of seed production and duration of flowering were characterized by larger variation in CV values among the studied halophytic genotypes (CV=32% and 27% respectively).

MI, BC, CC, SP and VA populations received the highest values for all spike characteristics (Table 1). Spike length ranged from 88 mm to 116 mm on average, whereas it did not exceed 67 mm for the rest of genotypes. Spike weight and width values were also higher for all the aforementioned populations.

Height measurements greater than 44 cm were observed for MI, BC, CC, FL1 and SP plants. No clear trend could be observed for number of branches, root length and dry root weight among all *S. bigelovii* populations. Nevertheless, fewer spikes (60 on average) were observed for MI, BC, CC and SP plants, compared to the rest of genotypes for which the number of spikes reached almost 200.The distance between the first spike and the plant base is an important characteristic for plant collection when *S. bigelovii* plants are about to be harvested. FL1 spikes started growing from almost 15cm above the ground, whereas MI, BC, CC and SP spikes were approximately 11 cm from the plant base.

Fresh and dry shoot weight measurements revealed that other *S. bigelovii* genotypes such as FL1, FL2 and PR produced high biomass values comparable or even higher than MI, BC, CC and SP (Table 2). Although seed weight per plant did not show any statistical difference among halophytic populations, seed weight/m$^2$ highlighted the preponderance of MI, BC, CC and GA, which ranged from 27 to 38 g/m$^2$ on average. The number of seeds per spike and seeds weight per spike received higher values only for MI, BC, CC and SP populations.

All growth stages showed variability among *S. bigelovii* populations (Table 3). MI, BC, CC and SP genotypes reached flowering stage earlier (135 days on average) compared to the rest of the populations (176 days on average). On the contrary, flowering lasted more for the same halophytic genotypes (52 days on average), whereas the remaining genotypes completed anthesis within 43 days. Days to seed production did not differentiate to a large extent among *S. bigelovii* populations, which averaged 264 days approximately. The duration of seed production was extended in MI, BC, CC and SP populations (77 days on average) compared to the rest of the genotypes (47 days on average). Duration of maturity did not show any trend among *S. bigelovii* populations and ranged from 26 (LP) to 40 (FL2) days on average. Finally, the growth cycle of all halophytic genotypes averaged between 281 and 316 days. MI, BC, CC and SP plants completed their biological cycle in less than 298 days.

### *Ranking of S. bigelovii populations*

*S. bigelovii* genotypes were initially ranked based on each group of agronomic traits (Table 4). MI, SP, BC and CC were classified as the top populations that received the highest values for spike characteristics.VA was classified in the second rank for the same group of traits. Cluster ranking for whole-plant and below-ground traits revealed that PR was first in ranking followed by FL1 and FL2 in the second place.

**Table 1.** Descriptive statistics (minimum, maximum and coefficient of variation) for spike characteristics, morphological traits on whole-plant and below-ground parameters of eleven *S. bigelovii* populations irrigated with seawater. Values having the same letter are not significantly different ($LSD_{0.05}$). Multiple range test was conducted per trait for all *S. bigelovii* populations (per column).

| | Spike characteristics | | | | | | Whole-plant and below-ground characteristics | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Spike length (mm) | | Spike width (mm) | | Spike weight (g) | | Plant height (cm) | | FIRSTS PKD (cm) | | Number of branches | | Number of spikes | | Root length (cm) | | Dry root weight (g) | |
| *S. bigelovii* populations | Min-Max | | Min-Max | | Min-Max | | Min-Max | | Min-Max | | Min-Max | | Min-Max | | Min-Max | | Min-Max | |
| LA | 56.1-63.2 | a | 3.2-3.6 | bc | 0.186-0.227 | a | 33.1-39.1 | c | 10.1-13.7 | cd | 19.2-21.6 | bc | 67.5-77.0 | bc | 12.6-14.5 | b | 3.1-4.5 | bc |
| MI | 78.1-90.5 | bc | 3.9-4.8 | de | 0.348-0.420 | b | 43.7-46.6 | de | 9.4-11.5 | c | 21.1-22.9 | c | 54.5-71.5 | bc | 9.3-14.2 | ab | 1.5-2.7 | ab |
| FL2 | 42.9-57.7 | a | 3.2-3.9 | bcd | 0.148-0.254 | a | 28.1-43.8 | c | 7.7-10.8 | bc | 15.5-24.9 | bc | 126.5-181.0 | e | 11.0-13.3 | ab | 2.4-6.2 | bc |
| SP | 90.5-133.6 | d | 3.9-4.2 | cde | 0.458-0.606 | d | 40.7-48.9 | de | 9.9-11.6 | c | 18.0-24.5 | bc | 41.0-53.5 | ab | 8.7-14.6 | ab | 1.2-3.3 | ab |
| FL1 | 49.7-54.6 | a | 3.5-4.6 | bcde | 0.210-0.253 | a | 42.8-49.5 | de | 12.6-18.1 | e | 18.1-24.3 | c | 104.0-124.0 | de | 11.3-12.8 | ab | 3.2-6.8 | c |
| GA | 51.3-59.2 | a | 2.6-4.2 | b | 0.141-0.194 | a | 20.2-29.8 | b | 6.7-13.7 | bc | 11.4-23.0 | bc | 78.5-113.0 | cd | 11.6-15.2 | b | 1.4-4.5 | abc |
| BC | 81.6-90.0 | bc | 4.3-4.5 | e | 0.445-0.480 | bc | 38.4-48.5 | de | 9.9-13.6 | cd | 16.9-21.8 | bc | 45.5-99.0 | bc | 12.6-15.5 | b | 1.8-6.9 | bc |
| LP | 42.2-88.0 | ab | 2.2-2.8 | a | 0.087-0.234 | a | 19.3-31.7 | b | 6.1-7.6 | b | 12.5-18.5 | b | 27.0-103.0 | bc | 8.7-12.5 | ab | 1.2-3.9 | ab |
| CC | 78.9-102.7 | c | 4.0-4.5 | e | 0.436-0.514 | cd | 45.6-50.4 | e | 11.3-13.8 | cde | 15.7-23.6 | bc | 49.7-83.0 | bc | 10.2-15.2 | b | 2.5-5.2 | bc |
| PR | 53.8-67.2 | a | 3.1-3.4 | b | 0.162-0.190 | a | 38.6-43.7 | cd | 13.4-15.1 | de | 19.4-25.2 | bc | 185.4-222.0 | f | 9.0-9.8 | a | 7.9-9.0 | d |
| VA | 78.4-88.1 | bc | 4.2-4.7 | e | 0.414-0.563 | cd | 12.6-17.1 | a | 1.9-2.5 | a | 6.2-9.1 | a | 10.2-29.3 | a | 7.9-11.9 | a | 0.5-0.7 | a |
| $LSD_{(0.05)}$ | 9.1 | | 7.4 | | 32.1 | | 17.9 | | 11.0 | | 4.5 | | 18.5 | | 1.8 | | 6.0 | |
| F test | *** | | *** | | *** | | *** | | *** | | *** | | *** | | ns | | *** | |
| CV (%) | 28 | | 16 | | 47 | | 29 | | 34 | | 22 | | 58 | | 12 | | 59 | |

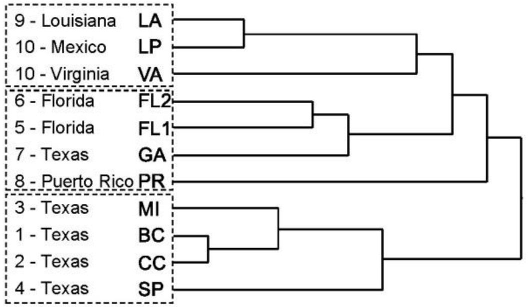

**Fig 1.** Hierarchical cluster analysis of *Salicornia bigelovii* genotypes based on all groups of plant attributes (spike characteristics, whole-plant and below-ground traits, seed and biomass yield and growth stages). The origin is placed next to every *Salicornia bigelovii* genotype. The numbers refer to genotype ranking in Table 4.

**Table 2.** Descriptive statistics (minimum, maximum and coefficient of variation) for biomass and seed yield parameters of eleven *S. bigelovii*populations irrigated with seawater. Values having the same letter are not significantly different ($LSD_{0.05}$). Multiple range test was conducted per trait for all *S. bigelovii* populations (per column).

| | Biomass parameters | | | | Seed yield parameters | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fresh shoot weight per $m^2$($kg/m^2$) | | Dry shoot weight per plant (g) | | Seed weight per plant (g) | | Seed weight per $m^2$ ($g/m^2$) | | Number of seeds per g | | Number of seeds per spike | | Seed weight per spike(g) | |
| *S. bigelovii* populations | Min-Max | | Min-Max | | Min-Max | | Min-Max | | Min-Max | | Min-Max | | Min-Max | |
| LA | 2.3-2.7 | bcd | 35.8-51.0 | bc | 1.0-1.4 | ab | 9.2-16.3 | ab | 2991-4569 | bc | 43.0-53.6 | bc | 0.0094-0.0179 | c |
| MI | 1.5-3.3 | bcd | 43.5-58.3 | bc | 1.8-2.7 | c | 34.0-40.4 | cd | 2909-3636 | bc | 109.9-123.6 | e | 0.0302-0.0400 | ef |
| FL2 | 2.5-3.8 | d | 34.0-108.1 | cd | 1.7-2.8 | bc | 14.7-21.8 | ab | 1785-2274 | a | 17.8-34.7 | ab | 0.0015-0.0194 | abc |
| SP | 2.5-3.0 | cd | 34.8-61.6 | bc | 1.5-3.1 | bc | 9.7-32.7 | ab | 2890-4141 | bc | 116.2-149.5 | e | 0.0280-0.0456 | ef |
| FL1 | 2.9-4.4 | d | 55.8-83.1 | cd | 1.1-2.5 | bc | 6.2-42.7 | abc | 2298-4386 | bc | 21.0-30.3 | ab | 0.0051-0.0091 | abc |
| GA | 1.7-1.8 | bc | 15.4-35.1 | ab | 1.3-2.6 | bc | 13.3-40.2 | bcd | 3044-4265 | bc | 29.8-95.6 | cd | 0.0074-0.0313 | cd |
| BC | 2.2-4.6 | d | 34.0-79.7 | bcd | 1.5-2.3 | bc | 19.0-48.7 | d | 2653-3584 | ab | 114.7-144.0 | e | 0.0351-0.0544 | f |
| LP | 0.7-2.3 | ab | 13.3-52.4 | ab | 0.6-2.0 | ab | 3.3-17.7 | ab | 3406-4910 | c | 2.5-43.2 | a | 0.0003-0.0135 | ab |
| CC | 2.2-3.3 | cd | 34.3-61.4 | bc | 1.6-2.8 | bc | 28.7-54.3 | d | 2632-4008 | bc | 118.4-139.4 | e | 0.0383-0.0484 | f |
| PR | 1.2-3.9 | cd | 70.2-99.6 | d | 1.1-1.4 | abc | 10.6-16.2 | ab | 7219-8044 | d | 1.7-2.8 | a | 0.0002-0.0004 | a |
| VA | 0.0-0.5 | a | 5.7-13.4 | a | 0.6-0.8 | a | 2.1-3.1 | a | 2998-4005 | bc | 75.1-79.4 | d | 0.0231-0.0284 | de |
| $LSD_{(0.05)}$ | 4.6 | | 4.2 | | 2.2 | | 3.8 | | 14.5 | | 33.8 | | 16.1 | |
| F test | *** | | *** | | ns | | *** | | *** | | *** | | *** | |
| CV (%) | 40 | | 43 | | 30 | | 59 | | 37 | | 70 | | 73 | |

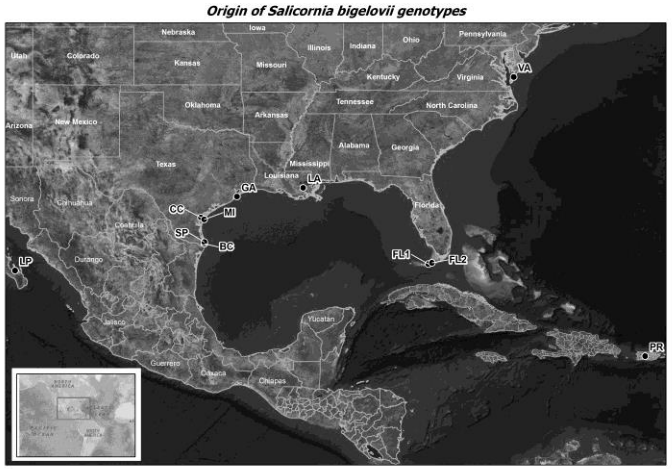

**Fig 2.** Map showing the sampling sites of *Salicornia bigelovii* populations. Quinby, Virginia (VA); Grand Island, Louisiana (LA); Galveston Island, Texas (GA); Corpus Christi Bridge, Texas (CC); Mustang Island, Texas (MI); South Padre Island, Texas (SP); Boca Chica, Texas (BC); La Paz, Mexico (LP); Geiger Key, Florida (FL1); Little Torch Key, Florida (FL2); Puerto Rico (PR).

**Table 3.** Descriptive statistics (minimum, maximum and coefficient of variation) for developmental stages of eleven *S. bigelovii* populations irrigated with seawater. Values having the same letter are not significantly different ($LSD_{0.05}$). Multiple range test was conducted per trait for all *S. bigelovii* populations (per column).

| | Growth stages | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Days to flowering (days) | | Duration of flowering (days) | | Days to seed production (days) | | Duration of seed production (days) | | Duration of maturity (days) | | Growth cycle (days) | |
| *S. bigelovii* populations | Min-Max | | Min-Max | | Min-Max | | Min-Max | | Min-Max | | Min-Max | |
| LA | 162.0-176.0 | de | 35.0-52.0 | abcd | 260.2-273.4 | c | 44.2-62.4 | ab | 26.8-35.6 | ab | 294.6-301.6 | bc |
| MI | 122.0-126.0 | a | 46.0-84.0 | de | 247.2-262.4 | ab | 56.4-84.0 | bc | 29.6-35.2 | ab | 276.8-297.6 | ab |
| FL2 | 179.0-190.0 | ef | 25.0-36.0 | a | 262.2-266.2 | bc | 40.0-62.2 | ab | 27.4-52.6 | b | 293.4-318.8 | cd |
| SP | 143.0-157.0 | bc | 39.0-61.0 | bcde | 264.8-269.2 | c | 55.2-82.8 | bc | 24.2-32.0 | ab | 289.0-300.4 | bc |
| FL1 | 175.0-190.0 | ef | 27.0-31.0 | a | 263.8-269.6 | c | 52.6-58.6 | abc | 21.0-38.0 | ab | 290.6-301.8 | bc |
| GA | 126.0-165.0 | bc | 49.0-52.0 | bcde | 260.4-270.6 | c | 46.4-94.6 | bc | 28.2-35.6 | ab | 288.6-306.2 | bc |
| BC | 126.0-161.0 | ab | 36.0-50.0 | abc | 251.8-270.6 | abc | 59.6-97.6 | c | 25.2-45.4 | ab | 295.8-301.6 | bc |
| LP | 175.0-200.0 | fg | 29.0-39.0 | ab | 247.4-272.0 | abc | 11.4-68.0 | a | 21.6-32.6 | a | 272.0-304.6 | ab |
| CC | 126.0-126.0 | a | 45.0-80.0 | cde | 260.2-263.2 | abc | 57.2-89.2 | bc | 28.6-42.0 | b | 291.8-303.0 | bc |
| PR | 201.0-201.0 | g | 46.0-46.0 | abcd | 275.2-284.0 | d | 28.2-37.0 | a | 30.8-45.0 | ab | 306.0-323.0 | d |
| VA | 156.0-159.0 | cd | 61.0-72.0 | e | 250.2-256.6 | a | 19.2-39.6 | a | 21.8-32.4 | a | 278.4-282.6 | a |
| $LSD_{(0.05)}$ | 18.3 | | 4.1 | | 3.6 | | 3.7 | | 1.3 | | 3.6 | |
| F test | *** | | *** | | *** | | *** | | ns | | *** | |
| CV (%) | 16 | | 27 | | 3 | | 32 | | 14 | | 3 | |

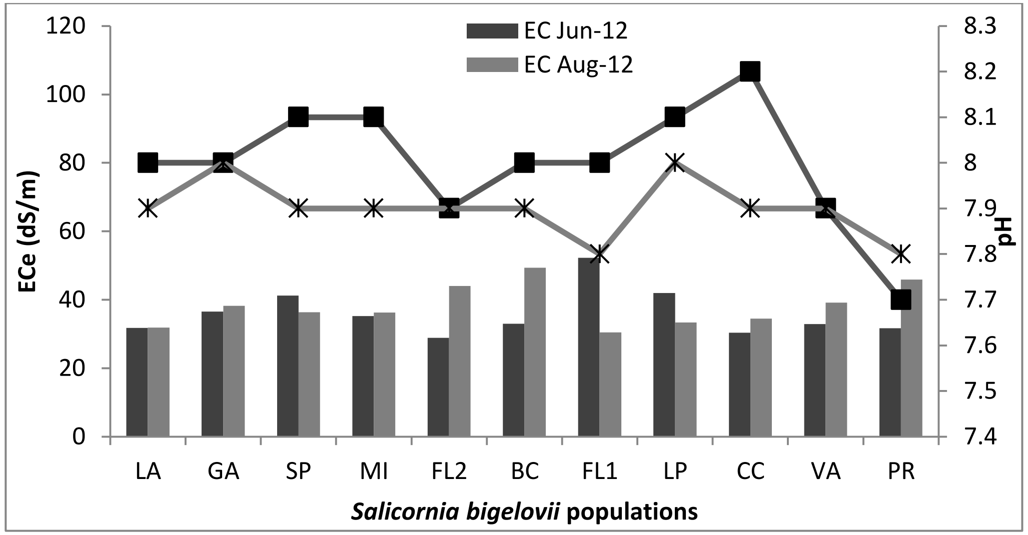

**Fig 3.** Salinity ($EC_e$) and pH measured for soil samples collected from the root zone (0-25 cm soil depth) from different plotsin Juneand August 2013.

**Table 4.** Ranking of *S. bigelovii* genotypes for their salt tolerance in terms of five groups of plant attributes based on cluster analysis (Ward method).

| *S. bigelovii* populations | Cluster group ranking based on[f]: | | | | | Sum[g] | Genotype ranking[h] |
|---|---|---|---|---|---|---|---|
| | Spike characteristics[a] | Whole-plant and below-ground traits[b] | Biomass Yield[c] | Seed Yield[d] | Growth stages[e] | | |
| LA | 3 | 4 | 3 | 5 | 4 | 19 | 9 |
| MI | 1 | 5 | 3 | 1 | 1 | 11 | 3 |
| FL2 | 4 | 2 | 1 | 2 | 5 | 14 | 6 |
| SP | 1 | 5 | 3 | 1 | 2 | 12 | 4 |
| FL1 | 4 | 2 | 1 | 2 | 4 | 13 | 5 |
| GA | 3 | 3 | 4 | 3 | 2 | 15 | 7 |
| BC | 1 | 4 | 2 | 1 | 1 | 9 | 1 |
| LP | 6 | 3 | 4 | 5 | 4 | 22 | 10 |
| CC | 1 | 4 | 3 | 1 | 1 | 10 | 2 |
| PR | 5 | 1 | 2 | 4 | 5 | 17 | 8 |
| VA | 2 | 6 | 5 | 6 | 3 | 22 | 10 |

a. Spike characteristics: Spike length, spike width, spike weight,

b. Whole-plant and below-ground traits: Plant height, number of branches, number of spikes, root length, dry root weight

c. Biomass yield measurements: Fresh biomass / $m^2$, dry shoot weight / plant

d. Seed yield measurements: seed weight/$m^2$, seed weight/plant, number of seeds per g, number of seeds per spike, seed weight per spike

e. Growth stages: days to flowering, duration of flowering, days to seed production, duration of seed production, duration of maturity, growth cycle

f. The means of the growth characteristics (see Material and Methods) were used in order to get the cluster groups. The rankings were obtained in the order from the highest to the lowest cluster means (data not shown).

g. The ranking numbers based on the cluster group rankings were added for each group of characteristics per genotype in order to calculate the total sum.

h. Genotypes ranking was based on the sums of the previous rankings. The genotype with the smallest sum was characterized as the most tolerant.

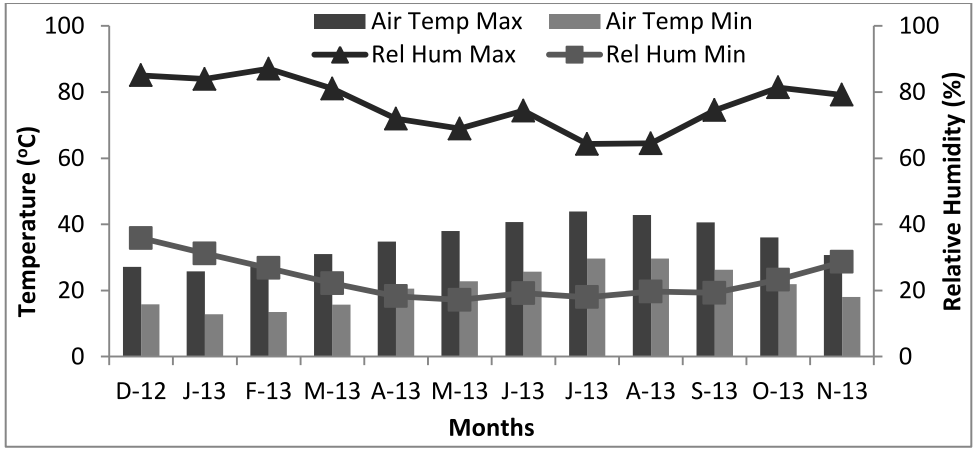

**Fig 4.** Minimum and maximum temperatures and relative humidity recorded for the whole growing season from sowing to harvest.

**Table 5.** Significant (p<0.01 and p<0.05) correlation coefficients among all growth parameters for eleven *S. bigelovii* populations irrigated with seawater (SW).

| | | Spike characteristics | | | Whole-plant and below-ground characteristics | | | | | | Biomass parameters | | Seed yield parameters | | | | | Growth stages | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SPL | SPW | SPWT | PH | FIRSTSPKD | BR | SPK | RL | DRWT | FRSHWTSQ | DSHWT | SWTPL | SWTSQ | SSG | SSSP | SWTSP | DTOFL | DUFL | DTOSP | DUSP | DUM | GC |
| Spike characteristics | SPL | 1 | | | | | | | | | | | | | | | | | | | | | |
| | SPW | 0.551$^{**}$ | 1 | | | | | | | | | | | | | | | | | | | | |
| | SPWT | 0.874$^{**}$ | 0.748$^{**}$ | 1 | | | | | | | | | | | | | | | | | | | |
| Whole-plant and below-ground characteristics | PH | | 0.377$^{*}$ | | 1 | | | | | | | | | | | | | | | | | | |
| | FIRSTSPKD | | | | 0.771$^{**}$ | 1 | | | | | | | | | | | | | | | | | |
| | BR | | | | 0.687$^{**}$ | 0.623$^{**}$ | 1 | | | | | | | | | | | | | | | | |
| | SPK | -0.481$^{**}$ | | -0.550$^{**}$ | | 0.515$^{**}$ | 0.383$^{*}$ | 1 | | | | | | | | | | | | | | | |
| | RL | | | | | | 0.412$^{*}$ | | 1 | | | | | | | | | | | | | | |
| | DRWT | | | -0.351$^{*}$ | 0.455$^{**}$ | 0.716$^{**}$ | 0.354$^{*}$ | 0.832$^{**}$ | | 1 | | | | | | | | | | | | | |
| Biomass parameters | FRSHWTSQ | | | | 0.801$^{**}$ | 0.778$^{**}$ | 0.527$^{**}$ | 0.503$^{**}$ | 0.350$^{*}$ | 0.598$^{**}$ | 1 | | | | | | | | | | | | |
| | DSHWT | | | | 0.700$^{**}$ | 0.671$^{**}$ | 0.540$^{**}$ | 0.768$^{**}$ | | 0.824$^{**}$ | 0.767$^{**}$ | 1 | | | | | | | | | | | |
| Seed yield parameters | SWTPL | | 0.434$^{*}$ | | 0.644$^{**}$ | 0.390$^{*}$ | 0.452$^{**}$ | | 0.557$^{**}$ | | 0.600$^{**}$ | 0.557$^{**}$ | 1 | | | | | | | | | | |
| | SWTSQ | | 0.460$^{**}$ | | 0.595$^{**}$ | 0.391$^{*}$ | 0.396$^{*}$ | | 0.472$^{**}$ | | 0.517$^{**}$ | | 0.683$^{**}$ | 1 | | | | | | | | | |
| | SSG | | -0.400$^{*}$ | | | | 0.045 | 0.417$^{*}$ | -0.408$^{*}$ | 0.471$^{**}$ | | | | | 1 | | | | | | | | |
| | SSSP | 0.756$^{**}$ | 0.665$^{**}$ | 0.842$^{**}$ | 0.357$^{*}$ | | | -0.573$^{**}$ | | -0.406$^{*}$ | | | 0.384$^{*}$ | 0.532$^{**}$ | -0.432$^{*}$ | 1 | | | | | | | |
| | SWTSP | 0.680$^{**}$ | 0.729$^{**}$ | 0.838$^{**}$ | 0.373$^{*}$ | | | -0.503$^{**}$ | | | | | 0.398$^{*}$ | 0.560$^{**}$ | -0.494$^{**}$ | 0.968$^{**}$ | 1 | | | | | | |
| Growth stages | DTOFL | -0.509$^{**}$ | -0.670$^{**}$ | -0.672$^{**}$ | | | | 0.493$^{**}$ | -0.387$^{*}$ | 0.363$^{*}$ | | | -0.430$^{*}$ | -0.643$^{**}$ | 0.495$^{**}$ | -0.905$^{**}$ | -0.906$^{**}$ | 1 | | | | | |
| | DUFL | 0.502$^{**}$ | | 0.492$^{**}$ | | | | -0.438$^{*}$ | | -0.363$^{*}$ | -0.397$^{*}$ | -0.431$^{*}$ | | | | 0.469$^{**}$ | 0.386$^{*}$ | -0.444$^{**}$ | 1 | | | | |
| | DTOSP | | | | | 0.415$^{*}$ | | 0.638$^{**}$ | | 0.506$^{**}$ | 0.363$^{*}$ | 0.472$^{**}$ | | | 0.443$^{**}$ | | -0.358$^{*}$ | 0.442$^{**}$ | | 1 | | | |
| | DUSP | | 0.530$^{**}$ | 0.364$^{*}$ | 0.601$^{**}$ | | | | 0.500$^{**}$ | | 0.477$^{**}$ | | 0.675$^{**}$ | 0.720$^{**}$ | -0.515$^{**}$ | 0.651$^{**}$ | 0.692$^{**}$ | -0.734$^{**}$ | | | 1 | | |
| | DUM | | | | 0.407$^{*}$ | 0.395$^{*}$ | | 0.458$^{**}$ | | 0.524$^{**}$ | 0.610$^{**}$ | 0.528$^{**}$ | 0.450$^{**}$ | 0.550$^{**}$ | | | | | | | 0.384$^{*}$ | 1 | |
| | GC | | | | 0.440$^{*}$ | 0.554$^{**}$ | | 0.755$^{**}$ | | 0.702$^{**}$ | 0.654$^{**}$ | 0.680$^{**}$ | 0.370$^{*}$ | | | | | | | 0.766$^{**}$ | | 0.696$^{**}$ | 1 |

**Correlation is significant at the 0.01 level, * Correlation is significant at the 0.05 level.

**Table 6.** Path coefficient analysis showing direct and indirect effects of several traits on seed weight/m$^2$ for all *S. bigelovii* populations.

| | | Indirect effects through: | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Traits | Direct effect | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Total Indirect effect | Total Effect / Correlation |
| Number of branches (1) | 0.311 | | -0.025 | 0.012 | 0.011 | -0.001 | 0.008 | 0.044 | 0.037 | 0.085 | 0.396 |
| Number of spikes (2) | -0.066 | 0.119 | | 0.057 | -0.200 | 0.122 | -0.191 | -0.008 | 0.200 | 0.099 | 0.033 |
| Spike length (3) | -0.119 | -0.031 | 0.032 | | 0.264 | -0.167 | 0.198 | 0.037 | -0.045 | 0.288 | 0.168 |
| Number of seeds per spike (4) | 0.349 | 0.010 | 0.038 | -0.090 | | -0.239 | 0.351 | 0.095 | 0.019 | 0.183 | 0.532 |
| Seed weight per spike (5) | -0.247 | 0.001 | 0.033 | -0.081 | 0.338 | | 0.352 | 0.102 | 0.061 | 0.807 | 0.560 |
| Days to flowering (6) | -0.388 | -0.006 | -0.033 | 0.061 | -0.316 | 0.224 | | -0.107 | -0.078 | -0.254 | -0.643 |
| Duration of seed production (7) | 0.145 | 0.095 | 0.004 | -0.031 | 0.227 | -0.174 | 0.285 | | 0.168 | 0.574 | 0.720 |
| Duration of seed maturity (8) | 0.437 | 0.026 | -0.030 | 0.012 | 0.015 | -0.035 | 0.069 | 0.056 | | 0.114 | 0.550 |

**Table 7.** Path coefficient analysis showing direct and indirect effects of several traits on fresh shoot biomass /m$^2$ for all *S. bigelovii* populations.

| Traits | Direct effect | Indirect effects through: | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | Total Indirect effect | Total Effect / Correlation |
| Plant height (1) | 0.749 | | -0.049 | 0.026 | -0.032 | 0.134 | 0.079 | 0.802 |
| Number of branches (2) | -0.071 | 0.515 | | 0.020 | -0.002 | 0.092 | 0.625 | 0.527 |
| Root dry weight (3) | 0.057 | 0.340 | -0.025 | | 0.037 | 0.214 | 0.567 | 0.597 |
| Days to Flowering (4) | 0.103 | -0.236 | 0.001 | 0.021 | | 0.062 | -0.151 | -0.063 |
| Growth cycle (5) | 0.305 | 0.330 | -0.021 | 0.040 | 0.021 | | 0.370 | 0.655 |

**Table 8.** Grouping of traits, their names and measurements.

| Grouping of traits | Trait names and description of their measurement |
|---|---|
| Spike characteristics | Spike length (SPL, mm)<br>Spike width (SPW, mm)<br>Spike weight (SPWT, g) |
| Whole-plant and below-ground characteristics | Plant height (PH, cm)<br>Distance between first spike and plant base (FIRSTSPKD, cm)<br>Number of branches (BR)<br>Number of spikes (SPK)<br>Root length (RL, cm)<br>Dry root weight (DRWT, g) |
| Biomass parameters | Fresh shoot weight/m$^2$ (FRSHWT, kg/m$^2$)<br>Dry shoot weight per plant (DSHWT, g) |
| Seed yield parameters | Seed weight per plant (SWTPL, g)<br>Seed weight/m$^2$ (SWTSQ, g/m$^2$)<br>Number of seeds per g (SSG)<br>Number of seeds per spike (SSSP)<br>Seed weight per spike (SWTSP, g) |
| Growth stages | Days to flowering (DTOFL)<br>Duration of flowering (DUFL)<br>Days to seed production (DTOSP)<br>Duration of seed production (DUSP)<br>Duration of seed maturity (DUM)<br>Growth Cycle (GC) |

This finding indicated that PR had the highest values for the majority of characteristics, whereas MI, SP, BC and CC populations were grouped in lower rankings due to lower values. Based on classification for biomass yield, FL1 and FL2 were categorized in the first rank followed by BC and PR genotypes. In addition, MI, SP and CC populations received even lower values for both fresh biomass/m$^2$ and dry shoot weight per plant and were placed in inferior classes. Cluster analysis conducted for seed yield parameters revealed that MI, BC, SP and CC genotypes were the most productive among *S. bigelovii* genotypes. Finally, cluster ranking based on growth stages showed that MI, BC and CC shared common characteristics, whereas SP and GA differentiated to a lesser extent compared to FL1 and FL2 genotypes. Overall ranking showed that BC genotype was ranked as the most productive among all halophytic populations. Then CC, MI, SP, FL1 and FL2 followed in descending order.

Taking into account these findings, four categories of halophytic populations were distinguished with the following characteristics: Group 1 consists of MI, SP, BC and CC genotypes. The plants have long, wide and heavy spikes, good plant height, an average number of spikes, average biomass, high seed yield, a short stage to anthesis, a long duration of the seed setting phase and a shorter growth cycle. These genotypes could be used as parents in a breeding program to produce high seed yielding varieties in a shorter growing period for biofuel production. Group 2 includes only the BC genotype because it produced more biomass in a shorter growth cycle compared to MI, SP and CC populations. This advantage could be interesting from breeders' point of view if dwarf glasswort is cultivated for green biomass to be used as fresh or pickled vegetable. FL1 and FL2 genotypes were categorized in Group 3. These plants are characterized by short and light spikes of medium width, a high number of spikes, high biomass, low seed yield, long duration of flowering and prolonged growth cycle. These two genotypes could be incorporated in a breeding program to improve *S. bigelovii* plants biomass for animal forage. Finally, Group 4 is comprised of LA, VA, GA, PR and LP genotypes where overall growth performance was not promising. However, some of these populations partially demonstrated high values for some traits such as VA which spikes attributes had high values and PR which was characterized by high biomass yield and the longest growth cycle among all *S. bigelovii* genotypes.

Hierarchical clustering taking into account all plant attributes (Fig. 1) coincided with the final ranking based on the sum of the partial rankings (Table 4). MI, BC, CC and SP, the most productive genotypes, were grouped in one cluster. All four of them originated from southern part of Texas. FL1 and FL2 populations collected from Florida, assembled in a separate cluster. All the rest of *S. bigelovii* genotypes were clustered based on their poor growth performance.

### *Associations and interrelationships among growth parameters*

Almost 50% of the 276 possible pair-wise correlations were significant (p≤0.01 and p<0.05), out of which 14% and 86% were positive and negative respectively (Table 5). All measurements taken on spikes were highly positively associated with each other (>0.500). In addition, all or some of them were negatively correlated with number of spikes, dry root weight, number of seeds per g and days to flowering. In contrast, positive correlations were observed with plant height, all seed yield parameters except from number of seeds per g, duration of flowering and duration of seed production. No spike characteristic was related to biomass parameters. Whole-plant and below-ground characteristics were also positively correlated. High and positive correlations were also noted with both biomass yield measurements. Positive or negative associations were observed for whole-plant and below-ground characteristics with seed yield parameters and growth stages. Biomass parameters were positively associated with few seed yield characteristics (seed weight/plant and seed weight/m$^2$) and most of the growth stages apart from duration of flowering. Seed weight per plant and seed weight/m$^2$ were significantly and positively correlated with all seed yield parameters, excluding number of seeds per g, since no correlation was observed. Number of seeds per spike and seed weight per spike were only negatively correlated with number of seeds per g. Positive or negative or negligible correlations were observed with growth stages. Finally, not many significant correlations (positive or negative) were observed among developmental stages.

Path-coefficient analysis was used to separate the total correlation into direct and indirect effects of eight and four characteristics contributing to seed (Table 6) and biomass (Table 7) yield respectively for all screened *S. bigelovii* genotypes. This method can reveal interrelationships between yield and growth parameters that are probably masked through simple correlation analysis.

Based on Subramanian and Subramanian (1994) criteria, number of branches, number of seeds per spike, duration of seed maturity and days to flowering had a high direct effect on seed yield (direct effect>0.3) (Table 6). In particular, the first three parameters had positive (0.311, 0.349 and 0.437 respectively), whereas the last one negative (-0.388) direct effect on seed production. Seed weight per spike exhibited moderate (-0.247), spike length and duration of seed production low (-0.119 and 0.145 respectively) and number of spikes showed negligible (-0.066) direct effects.

Both correlation coefficients and direct effects of number of branches (0.396 and 0.311 respectively), number of seeds per spike (0.532 and 0.349 respectively), duration of seed maturity (0.550 and 0.437 respectively) and days to flowering (-0.643 and -0.388 respectively) had either high positive or negative values, implying that correlation explained the true relationship between the growth parameters and seed yield. Hence, the results indicated that seed yield in these eleven *S. bigelovii* populations depended upon high number of branches and seeds per spike and prolonged seed maturity. Shorter period for *S. bigelovii* plants to reach flowering stage also contributed to higher seed yield.

Seed weight per spike and duration of seed production were characterized by high and positive correlation coefficients (0.560 and 0.720 respectively) but by negative and low direct effects respectively. In this case, the indirect effects should be considered for the cause of correlation. Number of seeds per spike and days to flowering were the causal factors that contributed mostly to the total sum of indirect effects (0.807) for seed weight per spike. A combination of various components such as number of seeds per spike, seed weight per spike, days to flowering and duration of seed maturity also counteracted the direct effect of the duration of seed production.

Both number of spikes and spike length demonstrated negligible and low correlation coefficients respectively. Their direct effects were also low and negative. Number of seeds per spike, seed weight per spike and days to flowering affected more the overall sum of indirect effects either positively or negatively.

Plant height and growth cycle showed high direct effects and correlations (0.749 and 0.305 respectively), exhibiting strong and positive relationship with fresh biomass production (Table 7). Number of branches and dry root weight were characterized by high correlations (0.527 and 0.597 respectively), whereas their direct effect was negligible. Days to flowering demonstrated very low direct and total effect. Among all growth parameters, plant height distinguished with the highest indirect effect. Growth cycle was another parameter that indirectly contributed to the effect of the rest parameters on biomass yield.

## Discussion

Several studies have been conducted on *S. bigelovii* germplasm evaluation (Zerai et al., 2010; Jaradat and Shahid, 2012; Shahid et al., 2013). However, they were tested under greenhouse conditions and a limited number of traits were investigated. The current study constitutes a field evaluation of *S. bigelovii* irrigated with high saline water focusing on a bigger range of agronomic characteristics including root characteristics. Full strength SW irrigation diversified *S. bigelovii* genotypes and differentiated their performance at various growth stages. High intraspecific variation was observed among *S. bigelovii* populations, for the majority of traits as measured by CV indices. These findings are in accordance with Jaradat and Shahid (2012) who also observed significant variability among selections made from natural *S. bigelovii* populations. Hence, the wild germplasm tested in the present study, reflects significant genetic variability that could be valuable for breeding purposes. This high genetic polymorphism could be attributed to the cross pollinating nature of the species (Zerai et al., 2010). The observed intraspecific variation in response to salinity is consistent with studies on other halophytic plants such as *Phragmites australis*, *Distichlis spicata*, *Schoenoplectus* spp. and *Spartina* spp. (Richards et al., 2005; Utomo et al., 2009; Howard, 2010). Differences in growth characteristics among populations may be attributed to various mechanisms that control salt tolerance under high saline conditions (Zeng, 2005), such as ion uptake and compartmentalization, solute accumulation and osmotic protection and antioxidant regulation (Gupta and Huang, 2014; Youssef, 2009). Likewise, the variation noted in growth among *S. bigelovii* populations in the present study, could be attributed to the differential response of plants to counteract the deleterious effects imposed by salinity through deploying various mechanisms on physiological basis. The fact that FL1 and FL2 plants produce more biomass compared to the rest *S. bigelovii* populations may be attributed to photosynthesis-related genes. It has been stated that halophytes such as *Spartina townsendii*, *Beta vulgaris* ssp. *maritima* and *Plantagocoronopus* have developed strategies to maintain low photosynthetic rates even at high salinity levels (Koyro, 2006). FL1 and FL2 populations were characterized by short spikes, high number of spikes compared to high productive populations such as BC, CC, SP and MI, which obtained longer spikes and much fewer spikes. Additionally, when FL1 and FL2 plants were irrigated with high saline water, they produced twice as much seed, than those irrigated with brackish groundwater (25 dS/m) treatment (unpublished data). These two genotypes could be introduced in a breeding program targeted to develop improved lines for biomass production. These results are in agreement with other authors who identified genotypes with excellent spike traits and high seed or/and biomass yield, suggesting that these could be selected for diversified plant production (Shahid et al., 2013; Jaradat and Shahid, 2012; Zerai et al., 2010).

Several traits such as number of seeds per spike, number of spikes and spike length received higher values compared to the ones obtained by Jaradat and Shahid (2012) and Shahid et al. (2013). In particular, the number of seeds per spike was doubled or tripled the values reported by the same authors. However, *S. bigelovii* genotypes screened by Jaradat and Shahid (2012) and Shahid et al. (2013) were also treated with high saline water (salinity≈45 dS/m) and were more productive in terms of seed and biomass yield compared to the halophytic populations tested in the present study. This could be attributed to the fact that they constituted selected germplasm and bred varieties.

Ranking of *S. bigelovii* genotypes, based on cluster analyses conducted for different groups of agronomic traits, facilitated their evaluation and increased the accuracy of the rankings. Cluster analysis is a multivariate technique that has the major advantage to perform simultaneous analysis of multiple parameters (Zeng et al., 2002). The results indicated that various *S. bigelovii* genotypes have been identified for different commercial uses. Only BC accession excelled for both seed and biomass production. Previous research studies also adopted this statistical approach for screening rice, wheat and potato genotypes for salt-tolerance taking into account various characteristics at different growth stages (El-Hendawy et al., 2005, 2011; Zeng, 2005; Khrais et al., 1998). *S. bigelovii* genotypes originating from Texas were the best adapted in the local environment compared to the rest germplasm. This could be attributed to the fact that the place of origin resembles the place of domestication in several characteristics (Zohary et al., 2012). The most important factor common in both areas is the hot and arid climate that defines plants ecophysiology.

Pearson correlation and path analyses are useful to detect strong intercorrelations among growth parameters that could be simultaneously improved through breeding processes. Correlation analysis revealed that all spike characteristics were strongly correlated, implying that they could be improved through a single selection process. High and positive correlation was also reported for the same characteristics by Jaradat and Shahid (2012). However, the number of spikes was highly and negatively correlated with spike and seed yield components, supporting the finding that highly productive genotypes were characterized by lower number of spikes. Root weight parameters exhibited positive and strong association with either biomass or seed yield parameters. Salinity reduces both shoot and root development, but the decrease is lower in roots compared to shoots (Fageria et al., 2012). Finally, most of the growth stages were highly correlated with both seed and biomass yield exerting their significance for the final production.

Path analysis has the advantage that unveils interrelationships among multiple parameters which may be masked in correlation analysis and as a result the relative importance of the potential causal factors are measured (Whittaker et al., 2009; Ehsani-Moghaddam and De Ell, 2009). Several yield components that have direct bearing on seed or biomass production have been selected and introduced in path analyses (Fellahi et al., 2013; Tripathi et al., 2011; Cyprien and Kumar, 2011; Ali et al., 2008; Garcia del Moral et al., 2005; Subramanian and Subramanian, 1994). The analysis revealed for the current *S. bigelovii* genotypes that number of seeds per spike, number of branches, days to flowering and duration of seed maturity were characterized by high direct effects which were also reflected in the correlations coefficients. Therefore, direct selection for higher number of seeds per spike and number of branches would be enough to increase the seed production. Number of seeds per spike actually demonstrated the maximum direct effect on seed yield compared to the rest of the yield components. Branches production is the first developmental stage in *S. bigelovii* that it may exercise a direct impact on all other agronomic parameters that are developed later. Shortened period to reach flowering stage and increased duration of seed maturity would also contribute to increased grain yield. The final number of seeds per spike may depend upon the number of primordia produced by the apical meristem, the duration of seed production and grain-filling period as observed in barley and wheat (Garcia del Moral et al., 1991; Moragues et al., 2006). Although longer vegetative stage influences positively the number of seeds in barley, the opposite is observed for dwarf glasswort. This may be attributed to the longer growth cycle of the last crop and to the fact that prolonged period to anthesis exposes plants to unfavourable conditions (increasing temperature in combination with high salinity). When duration of seed maturity is prolonged, seeds growth continues and more assimilates are translocated (Garcia del Moral et al., 2005). Although duration of seed production was highly and positively correlated with seed yield the true effect was low. This could be attributed to a reduction in spikelet fertility, since a delay in floret pollination may increase embryo abortion, and decrease the capacity of seed setting. Similar trend was observed for seed weight per spike which was positively associated with seed production, however, the direct effect was negative and moderate. Number of seeds per spike and days to flowering exercised compensatory indirect effect on both parameters. Garcia del Moral et al. (2003) also observed that similar associations among yield components did not clearly appear in the simple correlations, but their compensatory effects were evident through path analysis. Higher-yielding genotypes in terms of both seed and biomass yield were selected for further improvement in the breeding programs carried out in Tucson, Arizona, whereas early flowering and reduced biomass lines were favored in the Eritrea selections (Zerai et al., 2010). Hence, genotypes were selected based on the climatic conditions of the areas where *S. bigelovii* were cultivated. Finally, *S. bigelovii* genotypes could be improved for biomass production by selecting higher plants with prolonged growth cycle, since these two growth parameters were characterized by both high direct effects and correlation coefficients. Plant height was also observed to be one of the main characteristics that discriminated between *S. bigelovii* breeding populations (Jaradat and Shahid, 2012). Although our data did not reveal any relationship between days to flowering and biomass production, Jaradat and Shahid (2012) supported that delayed anthesis contributed to higher production of fresh shoots with high market value.

## Materials and Methods

### *Plant material and field preparation*

Field study was conducted at the experimental station of ICBA in Dubai, United Arab Emirates. The soil at the experimental plots was characterized as sandy in texture (sand 98%, silt 1% and clay 1%), calcareous, porous, moderately alkaline, with organic matter of <0.5 and salinity of 1.2 dS/m.

Seeds of eleven *S. bigelovii* wild populations (LA, MI, FL2, SP, FL1, GA, BC, LP, CC, PR, VA) were provided by MI in Abu Dhabi, through a collaborative research project between ICBA and MI. The origin of *S. bigelovii* genotypes are presented in Fig 2. LP, PR, VA, LA, populations were collected from Mexico, Puerto Rico, Virginia and Louisiana respectively, MI, CC, BC, SP and GA genotypes were collected from Texas, and FL1 and FL2 populations from Florida.

Initially, organic compost was incorporated in soil at a rate of 40 t/ha. *S. bigelovii* seeds were sown on 1st of December 2012 at a density of 3 g/m$^2$. Seeds were covered lightly with soil and then with agryl sheets to impede seed losses. Seeds/seedlings were irrigated with full-strength seawater (SW) (salinity≈55 dS/m). The size of each experimental plot was 4m$^2$ and the planting distance was 25x25cm. Drippers were used to irrigate *S. bigelovii* plants with a 30% leaching fraction. A randomized complete block design was applied with three replications per population. Soil samples were collected from the root zone (0-25 cm soil depth) and were analyzed for pH and salinity at the beginning of June (vegetative stage) and August (maturity stage) (Fig. 3). Soil salinity varied between 28.9 and 52.3 dS/m for both sampling dates. Soil salinity values measured in August either increased by 3% to 34%, stabilized or decreased as low as to 68% compared to the ones obtained in June. Soil pH ranged from 7.7 to 8.2. The climate in the study area is very hot and humid especially during summer season. Minimum and maximum temperatures and relative humidity are presented in Fig 4. Maximum temperatures ranged from 31$^o$C (March) to 41$^o$C (June) and from 44$^o$C (July) to 43$^o$C (August) during flowering stage and grain filling period respectively.

### *Data collection*

*S. bigelovii* plants were harvested at optimum vegetative stage (end of May) from two rows equal to 1 m$^2$ and fresh biomass/m$^2$ was measured. When plants reached maturity stage which lasted from the end of August till the beginning of October, they were harvested and data were collected from ten tagged plants per experimental plot. Twenty two growth parameters were monitored in total and divided in five groups based on the functionality of the traits (adapted from Mitchell et al. 2015; Perez-Harguindeguy et al., 2013; Chen et al., 2012). All the agronomic parameters are listed in Table 8. These traits included 3 spike characteristics, 6 whole-plant and below-ground traits, 2 biomass parameters, 5 seed yield traits and 6 developmental stages. Whole-plant and below-ground traits, dry shoot weight per plant, seed weight per plant and number of seeds per g were calculated as the average of measurements taken from 10 tagged plants. Seed weight / m$^2$ was calculated from the whole plot excluding the two lines from which plants were harvested to measure fresh biomass/m$^2$ (Total area=3 m$^2$). Spike characteristics and seed yield related to spikes (number of seeds per spike and seed weight per spike) were measured based on the average of 6 randomly selected spikes at maturity. Days to flowering were counted when 5% of the plants started flowering in the plot. Duration of flowering was the period from the onset of flowering till 100% of the plants were in full bloom. Days to seed production started immediately after the end of flowering. Duration of seed production was defined as the period that started from the completion of anthesis stage till the beginning of seeds maturity. It is well known that *S. bigelovii*plants mature gradually. Duration of maturity started from the day when 10% of the plant was mature (10% of the plant was brown in colour) and ended, when the plant was completely mature (100% of the plant was brown in colour). Growth cycle started from the day of sowing till the day of harvest.

### *Data analysis*

Descriptive statistics (minimum, maximum values and coefficient of variation) were calculated for all the growth parameters studied. Analysis of variance (ANOVA) was used to examine whether there were significant statistical differences among *S. bigelovii*populations for all the studied parameters. LSD multiple range test was used to compare the means ($p \leq 0.01$ and $p<0.05$). Cluster analysis was applied to score and group *S. bigelovii* populations based on the aforementioned five groups of variables. As a result, *S. bigelovii* populations were classified based on their growth performance. In this way, genotypes that were clustered within groups tended to be similar to each other and dissimilar among groups. Group rankings were produced based on Ward's clustering method using the means of the growth parameters. Correlation analysis was performed using Pearson coefficient, in order to identify the associations among growth parameters. Path way analysis was conducted to supplement correlation analysis and investigate the direct and indirect impact of the interrelationships of several growth parameters on seed and biomass yield of *S. bigelovii* genotypes. It is different from simple correlation analysis, since it focuses on the causes and their relative importance, whereas the latter simply measures the mutual association ignoring the causation. For the direct and indirect effects a value of >1 is considered as very high, 0.3-0.99 high, 0.2-0.29 moderate, 0.1-0.19 low and <0.1 as negligible (Subramanian and Subramanian, 1994). Statistical analyses were carried out with the statistical packages JMP 8.0 (SAS Institute, Inc., Cary, NC, USA) and Statgraphics Centurion XVII (StatPointInc, Virginia, USA).

## Conclusion

*S. bigelovii* has a great potential to be cultivated in coastal areas as vegetable, forage and oilseed crop without competing productive agricultural lands and freshwater resources. The current study examined several groups of traits for eleven *S. bigelovii* genotypes which were irrigated with full-strength SW. Classification and ranking of the halophytic populations revealed that BC, CC, SP and MI were high yielding genotypes and have good potential for seed yield improvement through breeding. FL1, FL2 and BC populations could also be further investigated for enhancement of biomass production. The study also unveiled interesting interrelationships among growth parameters that could be used for further selection to increase seed production such as number of seeds per spike, number of branches, days to flowering and duration of seed maturity. Plant height and growth cycle could be also selected for biomass improvement. The multivariate statistical approaches presented in this study were very useful in ranking *S.*

*bigelovii* genotypes and elucidating the interrelationships among growth traits, thus providing breeders with valuable information to improve grain and biomass yield.

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