

Comparative responses of *Glaucium* spp. to salinity stress

Abstract

Salinity is considered as a major factor that reduces plant growth in arid and semiarid regions where soil salinity is naturally high, and precipitation is insufficient to achieve proper leaching. Plant species and cultivars within a species vary in their drought and salinity tolerance. These variations are associated with genes relating to stress tolerance mechanisms and their interaction with the environment. Horned Poppies (*Glaucium* spp.) are members of the Poppy family, Papaveraceae and are native to the Mediterranean and Middle East. The objectives of this study were to 1) evaluate the comparative salinity tolerance of Horned Poppy species, *G. flavum*, *G. corniculatum*, *G. grandiflorum* and *G. acutidentatum*, available from Denver Botanic Gardens; 2) examine the effects of salinity on plant characteristic associated with aesthetics of the species; and 3) determine tolerance mechanisms associated with superior salinity tolerance among tested species after evaluating the effects of salinity on total nonstructural carbohydrate content (TNC), shoot reducing sugar content (RSC), proline content and K^+/Na^+ in shoots of *Glaucium* spp. Lysimeter columns were used in this study which was replicated twice in the CSU Plant science greenhouse. With increase higher salinity levels from tap water (control) to EC levels of 5, 15, and 25 dSm⁻¹, leaf color declined over time to unacceptable ratings (below 6). In *G. flavum*, leaf color was least affected under all salinity levels while all other species declined to the unacceptable rating of 5.3 (*G. acutidentatum*), 4 (*G. grandiflorum*), and 3.2 (*G. corniculatum*) at the salinity level of 25 dSm⁻¹. Leaf area decreased linearly in all species with increasing salinity with a sharp drop at the salinity level of 25 dSm⁻¹. *G. flavum* achieved the highest leaf area at all salinity levels followed by *G. acutidentatum*, *G. grandiflorum* and *G. corniculatum*. The average leaf area of *G. flavum* when grown with tap water was 23 cm² while the *G. acutidentatum* average leaf area was 21.3 cm². *G. grandiflorum* and *G. corniculatum* average leaf area was 20.8 cm² with tap water. *G. flavum* achieved an average height of 56 cm while *G. acutidentatum* achieved an average height of 48 cm and *G. grandiflorum* had an average height of 35.1 cm in the control. *G. corniculatum* had the lowest height of 32.7 cm with the control treatment. In *G. flavum*, as salinity levels increased from control to 5, 15 and 25 dS m⁻¹, average TNC

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decreased by 15.7, 28.6 and 43.6% while the average TNC decrease in *G. acutidentatum* shoots was 17.6, 36.8 and 48%. The decrease in *G. grandiflorum* was 20, 40 and 48.6% while the decrease in *G. corniculatum* was 28.9, 49, and 53, respectively. As salinity levels increased from control to 5, 15 and 25 dS m⁻¹, average proline content in shoots increased by 218, 367, and 537% in *G. flavum*, 64.5, 296 and 510% in *G. acutidentatum*, 156, 273 and 428% in *G. grandiflorum*, and 79, 188, and 337% in *G. corniculatum*, respectively. Results indicated that K^+/Na^+ ratio was ≥ 1 at all salinity levels in *G. flavum*, as compared to *G. acutidentatum*, *G. grandiflorum* and *G. corniculatum*. In conclusion, as salinity increased, *Glaucium* spp. exhibited reduction in leaf characteristics, plant height, flowering characteristics, overall plant quality (attractiveness), TNC, and K^+/Na^+ ratio, and increased shoot total reducing sugars and proline content. *G. flavum* showed higher salinity tolerance at all salinity levels as compared to the other species. Proline accumulation could add to the salinity tolerance through osmoregulation or by acting as a carbon and nitrogen sink for stress recovery.

Keywords: Horned Poppies, salinity tolerance, proline, *Glaucium flavum*, *G. corniculatum*, *G. grandiflorum* and *G. acutidentatum***Abbreviations:** EC, electrical conductivity; TNC, total nonstructural carbohydrate content; RSC, shoot reducing sugar content

Introduction

Salinity is considered the major factor that reduces plant growth in arid and semiarid regions where soil salinity is naturally high, and precipitation is insufficient to achieve proper leaching. Saline environments affect plant growth in different to include a reduction in water uptake, gradual accumulation of ions to toxic levels, and a reduction of nutrient accessibility.¹ Increased use of brackish water and wastewater (effluent, recycled, or reclaimed water) has enhanced interest in the development of more salt tolerant landscape plants.^{2,3} The detrimental effects of salinity on plant growth include osmotic stress, ion toxicity, nutritional disturbances,⁵⁻⁶ damage to photosynthetic systems by excessive energy,⁷ and structural disorganization.⁸⁻¹⁰ Plants respond to salinity stress through a number of physiological changes including lowered leaf osmotic potential and/or a loss of turgor potential which can cause growth suppression.¹¹ Salt tolerant plants often mediate stress by osmotic adjustment, therefore minimizing changes in turgor potential which affect plant growth responses linked to carbon dioxide assimilation and cell elongation.¹²

Plant species and cultivars within a species vary in their drought and salinity tolerance.¹³⁻¹⁵ These variations are due to variations in genes relating to stress tolerance mechanisms and their interaction with the environments.¹⁶⁻¹⁹

Horned Poppies (*Glaucium* spp) are members of the Poppy family, Papaveraceae and are native to the Mediterranean and Middle East regions. Some species have a wider distribution than others. Horned poppies require full sun and well-drained soils for optimum growth. They should be spaced between 30 and 60 cm apart when grown by direct seeding in the fall and thinning them in the spring to the desired spacing. For earlier bloom, seeds are sown indoors 8 to 10 weeks prior to planting and then transplanted into the garden after danger of frost has passed. Germination takes 8 to 15 days at 15 to 18°C. Seedlings should be transplanted to individual pots when three leaves have formed but before the taproot has developed. Transplanting should be done without disturbing the root system. The crinkly, gray-green leaves also appear on the stems and below each flower. All horned poppies have blue-green foliage that is deeply pinnatifid to pinnatisect and typically grow 30-50 cm long. The leaves have varying degrees of texture from glaucous to villous. All leaves are lyrate to sub-lyrate shaped and have a rosette growth habit. They have solitary blooms on flower stalks that grow above the foliage. All species have four petals in their corolla and their pistil is surrounded

by stamens. They all develop long horned-shaped seed siliquiforms with the stigma remaining to cap off the top of the fruit. Species of interest in this study are *G. flavum*, *G. grandiflorum*, *G. acutidentatum* and *G. corniculatum*.

G. flavum Crantz is the most widely spread species in the genus. It's found in the coasts of Britain and the Atlantic Islands to the coasts of the Mediterranean Basin and the Black Sea.²⁰ It grows predominantly on sandy beaches and as a result it is commonly known as the Sea Horned Poppy. This likely indicates that *G. flavum* is salt tolerant. According to Davis,²¹ *G. flavum* is distinguished from other species by several characteristics. The sepals have crisp, pilose hairs on the surface and the petals can be solid yellow, red or reddish mauve. *G. flavum* is most often recognized for the yellow petals and is commonly referred to as the Yellow Horned Poppy. The ovary is densely papillose to tuberculate, basically a bumpy surface. The siliquae will retain the papillose to tuberculate texture. In Turkey, *G. flavum* normally flowers from May through the summer and even though it is most often found at sea level, it does grow into river valleys as well.²¹

G. grandiflorum Boiss & É. Huet is native to Turkey in the southern part of the Caucasus Mountains but it is also found in Syria, Iran and the Sinai.²⁰ Turkey is situated between the Mediterranean Sea and the Black Sea, where the precipitation ranges from 580 to 1300 mm/year. However, in the mountain ranges of the country there are variable climate conditions with harsh winters and drier conditions with a low precipitation of 400 mm/year. *G. grandiflorum* has features that distinguish it from other *Glaucium* species. It has only one main flower stem while other species have multiple flower stalks growing from the base of the rosette.²¹ The sepals have short, stiff hairs making the surface hirsute. The petals are dark orange to crimson red with a black spot at the base of the petal. The pedicle of the flower exceeds the subtending leaf, which differs from the other *Glaucium* species. There are two varieties of *G. grandiflorum*: var. *grandiflorum* and var. *torquatum*. *G. grandiflorum* var. *torquatum* has red petals with a black blotch and can be found in calcareous hillsides. *G. grandiflorum* var. *grandiflorum* is found in fields, banks and rocky slopes.

G. acutidentatum Hausskn & Borm is endemic to Turkey where it is found on dry hillslopes and rocky places.²⁰ *G. acutidentatum* is the most glabrous species with smooth sepals and ovaries. Although the ovary is smooth, the resulting siliquae is subtorulose. The petals are solid orange-buff in color. *G. acutidentatum* is found at elevations of 950-1400 m on dry hills.²¹

G. corniculatum (L.) J.H. Rudolph is native to the Mediterranean basin, Atlantic islands, Caucasus Mountains, Bulgaria, Romania, northern Iraq and northwestern Iran.^{20,21} *G. corniculatum* also has some unique characteristics. Its leaves have a soft, villous texture and its sepals are scabrous to hirsute. There is some conflicting information about *G. corniculatum*'s corolla. The petals are yellow, orange or red²¹ with a black basal spot.²⁰

The balance between carbohydrate production and consumption will impact the ability of a plant species to cope with salinity stress.²²⁻²⁴ The decline in salinity tolerance in some species can be associated with reduced carbohydrate availability and reduced effectiveness of Na⁺ exclusion and K⁺ active uptake and transport.²⁵⁻²⁸

Proline accumulates in larger amounts than other amino acids in salt stressed plants.²⁴ Proline accumulation is the first response of plants exposed to salt stress and water-deficit stress and is thought to reduce injury to cells.²⁹ Maggio et al.³⁰ suggested that proline may act as a

signaling/regulatory molecule able to activate multiple responses that participate in the adaptation process to elevated salinity levels. Rapid accumulation of proline in tissues of many plant species in response to salt, drought or temperature stress has been attributed to enzyme stabilization and/or osmoregulation.^{11,31} Ahmad et al.³² measured Proline content fluctuations under high salinity levels in salt tolerant and sensitive ecotypes of creeping bentgrass (*Agrostis stolonifera* L.) and concluded that the salt tolerant ecotype accumulated more proline in response to high salinity levels. Lee et al.²⁴ concluded that proline was the primary organic osmolyte for osmotic adjustment and proline accumulation was higher in salt tolerant seashore paspalum genotypes. However, other reports have indicated a negative effect of proline on salinity tolerance. Marcum (2002) has reported that proline accumulates in grasses under salinity stress at insufficient levels to achieve osmotic adjustment. Torello and Rice³³ concluded that Proline accumulation has no significant osmoregulatory role in salt tolerance of five turfgrass species ['Fults' alkaligrass (*Puccinellia distans* L. Parl.), 'Dawson' red fescue (*Festuca rubra* L. vat *trichophylla* Gaud.), 'Jamestown' red fescue (*Festuca rubra* L. vat *commutata* Gaud.), 'Adelphi' and 'Ram I' Kentucky bluegrass (*Poa pratensis* L.)] following their exposure to 170 mM NaCl salinity stress. Because of these contrasting reports on the role of proline in salt tolerance, its use as a selection criterion for salt tolerance has been questioned.³⁴ Thus, proper testing is required before making any conclusion regarding proline role in salinity tolerance in specific species.

The objectives of this study were to

1. Evaluate the comparative salinity tolerance of the common Horned Poppy species, *G. flavum*, *G. corniculatum*, *G. grandiflorum* and *G. acutidentatum*;
2. Examine the effects of salinity on plant characteristic that are associate with the aesthetics of Horned Poppy; and
3. Determine which tolerance mechanism is associated with superior salinity tolerance among the tested species.

Materials and methods

Lysimeter columns were used in this study which was replicated twice. All columns were placed in CSU, Plant Science greenhouse, Fort Collins, Co. *Glaucium* spp. plants were grown from seeds planted in potting mix, (Pro-Mix, Mycorrhizae and Biofungicide). Fifty seedlings, at the 3-leaf stage, of each species were transplanted one per PVS tubes (15 cm diameter and 50 cm long) containing commercial potting mix, (Pro-Mix, Mycorrhizae and Biofungicide). The plants were maintained under greenhouse conditions in the PVC tubes until full establishment and recovery from transplanting. The experimental design was a randomized complete Block (RCB). Each block represented one of the studied species and contained 16 tubes. Seedlings used in the experiments were chosen based on their similarity in size and number of leaves. Salinity treatments were control (Tap water), EC=5, EC=15 and EC=25 dSm⁻¹. Saline solutions were prepared using instant ocean salt mixture added to the irrigation water. Treatments were replicated four times. Salinity treatments were imposed after seedlings were fully established after transplanting. Soil leachate was collected biweekly to measure its EC, and adjustments were made as needed. Water use by the plants was measured weekly. Two tubes of each species were used as Lysimeter columns to monitor the weekly change in the evapotranspiration and the treatment amount adjusted accordingly. These tubes were watered with excess water, left to drain for 2 h, and the weight of each tube was recorded. Each tube was then re-weighed 24 h later. Over the course

of the experiments data were collected weekly on plant height, leaf color, leaf area, number of flower buds, size and number of flowers, quality and general aesthetics of the plants. Samples were collected for TNC, RSC, proline and tissue Na⁺ and K⁺ content analysis for each treatment. Visual quality was rated biweekly based on color, flower buds, flower number and size, height and uniformity using a scale of 0 (not attractive) to 10 (optimum attractiveness).

Total nonstructural carbohydrate content, RSC, tissue Na⁺ and K⁺ and proline content were determined at the termination of the experiment. Shoot tissue at the termination of the experiment was harvested and washed with cold distilled water to remove plant debris for carbohydrate analysis. Approximately 5 g samples from the treatments were freeze-dried (Genesis 25 LL Lyophilizer, Virtis, Gardiner, NY). After freeze-drying, samples were ground with a Wiley mill, sieved through a screen with 425 µm openings, and kept in airtight vials at -20 °C. TNC was measured using the method described by Chatterton et al.³⁵ In brief, 25 mg freeze-dried samples were transferred to 5 ml of 0.1% clarase solution and incubated at 38°C for 24 h. Then, 0.5 ml of hydrochloric acid (50%, v/v) was added to the incubation solution. After the solution was incubated at room temperature for 18 h, the pH value of the solution was adjusted to between 5 and 7 with 10 and 1 N NaOH. This resulting solution was used to determine TNC content using a spectrophotometer at 515 nm wavelength (model DU640; Beckman).

To measure the free reducing sugar, 25 mg of the freeze dried, ground, and sieved sample was extracted with 10 ml of 0.1 M phosphate buffer (pH=5.4) for 24 h at room temperature. An extracted aliquot (0.2 mL) was used to determine the reducing sugar content by using the same method as was used to measure TNC.

To measure ion content, about 5 g of shoots were harvested, washed with deionized water, and dried at 70°C for 24 h. Dried shoots were ground in a Wiley mill and passed through a screen with 425µm openings. Approximately 1g of dried and screened sample was weighed and ashed for 7 h at 500°C. Ash was dissolved in 10 ml of 1N HCl and diluted with deionized water. Solution aliquots were analyzed for Na⁺ and K⁺ by inductively-coupled plasma atomic emission spectrophotometry (ICP-AES) (Model 975 plasma Atomcomp, Thermo Jarrell Ash Corp., Franklin, Mass.).

Actual proline tissue accumulation levels were determined according to the method of Bates et al.³⁶ as modified by Torello and Rice³³ with approximately 0.5g fresh weight of tissue. Samples were ground with liquid nitrogen in a mortar. Each sample was homogenized in 10 ml of 3% aqueous sulfosalicylic acid followed by agitation for 1h prior to filtration through #2 Whatman filter paper. After filtration 2 ml of extract from each sample was reacted with 2 ml of ninhydrin reagent (1.25 mg of ninhydrin in 30 mL of glacial acetic acid and 20 mL of 6 M H₃PO₄) and 2 ml of glacial acetic acid followed by 1 h of heating at 100°C in an enclosed water bath. Samples were then quickly cooled by immersion in an ice bath and total proline was determined spectrophotometrically at 520 nm. Actual proline tissue accumulation levels were determined by subtracting mean control data from salinity treatment data for all cultivars during the entire experimental period.

Data analysis

The data of the two experiments were subjected to ANOVA to test the experiment effect and the interaction between treatments and experiments. The experimental run was not significant. Therefore, data were pooled over experiments to test the effects of salinity,

species and their interactions using ANOVA.³⁷ Leaf characteristics (color and area), number of buds, and flower characteristics (number and size) were analyzed on individual measurement dates to examine salinity, and species effects over time. Means were separated by least significant difference at the 0.05 level of probability. Regression analysis was performed to determine the relationship between the measured parameters at the end of the study (dependent variables) and the salinity levels (independent variable).

Results and discussion

Leaf characteristics

Leaf color. Comparisons of leaf color among species and salinity levels clearly showed significant differences (Table 1). With higher salinity, leaf color declined over time to unacceptable ratings (below 6). In *G. flavum*, leaf color was not as adversely affected under all salinity levels as all other species which declined to the unacceptable rating of 5.3 (*G. acutidentatum*), 4 (*G. grandiflorum*), and 3.2 (*G. corniculatum*) at the salinity level of 25 dSm⁻¹ (Figure 1). Leaf data from other species showed similar responses to the increasing salinity.

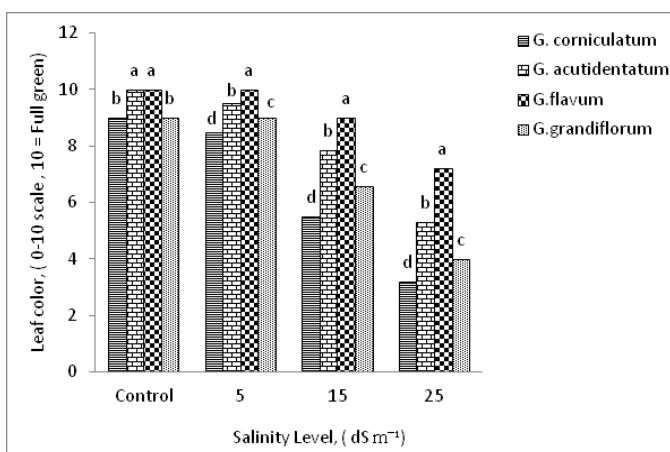


Figure 1 Effect of different salinity levels on leaf color of four *Glaucium* spp. Columns labeled with different letters are significantly different at P=0.05 within each salinity level.

Bayat et al.³⁸ found a 61% decrease in chlorophyll index as a result of 300 mM NaCl in Persian petunia.³⁸ The upper leaves of butterhead lettuce had a dark-green color, while lower leaves showed chlorosis under high salinity.³⁹ It seemed that salinity targeted chlorophyll formation processes which resulted in pale or yellow leaves.⁴⁰ High level of salinization decreases the content of pigment fractions (chlorophyll a and b) through the suppression of the enzymes that are associated with the synthesis of photosynthetic pigments.^{11,41,42} Salt stress opens porphyrin rings and through fusion, harmful solutes are transferred to the vacuole. The presence of these solutes results in the loss of the green color of leaves⁴³ and ultimately reduces the chlorophyll concentration in the leaf.⁴⁴⁻⁴⁶ The interference of salt ions on the newly synthesized protein molecules has another negative effect on the chlorophyll biosynthesis.⁴² Salinity affects turfgrasses and other landscape plant by reducing their value.⁴⁷⁻⁵²

Leaf area. Analysis of variance indicated significant differences among species and among salinity levels and their interactions (Table 1). Linear regression indicated a significant negative association between leaf area and salinity levels (Table 2). Leaf area decreased linearly in all species with increasing salinity with a sharp drop at the

salinity level of 25 dS m⁻¹. *G. flavum* achieved the highest leaf area at all salinity levels followed by *G. acutidentatum*, *G. grandiflorum* and *G. corniculatum*. *G. flavum* achieved an average leaf area of 23 cm² while *G. acutidentatum* achieved an average leaf area of 21.3 cm² and with both *G. grandiflorum* and *G. corniculatum* with a leaf area of 20.8 cm² in the control treatment (Figure 2). Leaf area decreased from 23 to 20, 17.6 and 10.8 cm² in *G. flavum*, from 21.3 to 18.8, 14.3, and 8.2 cm² in *G. acutidentatum*, from 20.8 to 18.2, 11.8, and 6 cm² in *G. grandiflorum*, and from 20.8 to 18.1, 10.9, and 4.5 cm² in *G. corniculatum* when the salinity increased from the control to 5, 15 and 25 dSm⁻¹, respectively (Figure 2). Leaf area followed the similar trend of leaf color since healthy leaves generally have a greater leaf area. Previous reports indicated similar salinity effect on leaf area⁵³ in other species. Continuous exposure to elevated root-zone salinity progressively decreased leaf size over time.^{54,55}

The decline in leaf growth was the earliest response of exposure to salinity stress.^{56,57} Plants cope with salinity stress by decreasing their leaf area to conserve energy.⁴² Leaf area decreased gradually with increasing salinity in *Withania somnifera* under salt stress,⁴² and in *Salvadora persica*.^{42,58} Salinities above 25 mM resulted in a decrease in leaf area in mangrove species⁵⁹ as well. In aquatic plants, high salinity or a prolonged exposure to salt causes greater leaf mortality than leaf gain per plant and eventual death.⁶⁰⁻⁶² This may be a direct effect of salt on rate of cell division, to a slower rate of cell expansion, or a decrease in the duration of cell expansion. If cell division was affected, even if cell growth potential was not affected, final leaf size would be limited due to reduced cell number.⁵⁵ The rapid response to the increase in salinity is mainly osmotic and resulted in inhibition of leave formation. The long-term response is a result of ionic toxicity that accelerates senescence of mature leaves.⁶³

Table 1 Analysis of variances with mean square and treatment significance of leaf color, leaf area, plant height, number of buds, number of flowers, flower area, plant quality (attractiveness), total non-structure carbohydrate content (TNC), shoot reducing sugar content (RSC), Proline content and shoot K⁺/Na⁺ ratio in *Glaucium* spp

Parameters	Source		
	Species (S)	Salinity (EC)	S X EC
Leaf color (0-10 scale)	9.2**	75.5**	69.5*
Leaf area (cm ²)	2.3**	3.44**	2.2*
Plant height (cm)	4.42**	3.88**	3.25*
Number of buds	39.7**	88.0**	29.4*
Number of flowers	4.4**	7.9**	3.9*
Flower area (cm ²)	2.9**	1.7**	3.7*
Plant quality (0-10 scale)	9.5**	8.6**	6.1*
K ⁺ /Na ⁺	139.0**	54.0**	299.0*
TNC (mgg ⁻¹ dry wt)	1690**	1771**	2895*
RSC (mgg ⁻¹ dry wt)	47.0**	892.0**	521.0*
Proline content (μgg ⁻¹ fresh wt.)	1870**	2997**	1897*

*Significant at P≤0.05.

**Significant at P≤0.01.

Table 2 Linear regression of different parameters of *Glaucium* spp. measured at the end of the experiment vs. salinity levels, Control (c), 5, 15, 25 dSm⁻¹

Species	Parameter					
	Plant quality (0-10 scale)		Leaf area (cm ²)		Flower area (cm ²)	
	Regression	R ²	Regression	R ²	Regression	R ²
<i>G. acutidentatum</i>	Y=6.20-0.22 X	0.84**	Y=122.5-1.22 X	0.88**	Y=320.5-7.1 X	0.80**
<i>G. corniculatum</i>	Y=5.06-0.33 X	0.68*	Y=131.2-1.16 X	0.89**	Y=303.3-2.8 X	0.75*
<i>G. flavum</i>	Y=8.6-0.16 X	0.92**	Y=122.8-1.32 X	0.83**	Y=313.3-0.8 X	0.94**
<i>G. grandiflorum</i>	Y=2.92-0.18 X	0.85**	Y=133.2-1.55 X	0.75*	Y=299.0-8.8 X	0.77*

*Significant at P≤0.05.

**Significant at P≤0.01.

Plant height

Generally, there was a significant decrease in plant height as salinity increased. As salinity increased there were significant differences among the species in plant height (Table 1). *G. flavum* achieved an average height of 56 cm while *G. acutidentatum* averaged 48 cm and *G. grandiflorum* had an average height of 35.1 cm. *G. corniculatum*

was the shortest with an average height of 32.7 cm in the control treatment (Figure 3). Plant height decreased from 55.5 to 51.6, 35.4 and 29.9 cm in *G. flavum*, from 47.7 to 38.4, 26.7, and 16.7 cm in *G. acutidentatum*, from 35.1 to 28.5, 6.3, and 5.8 cm in *G. grandiflorum*, and from 32.7 to 27.3, 5.3, and 4.5 cm in *G. corniculatum* as the salinity level increased from the control to 5, 15 and 25 dS m⁻¹, respectively (Figure 3). By comparing the decrease in plant height

under the salinity level of 25.0 dS m⁻¹ to the corresponding control treatment, the decrease in plant height in *G. flavum* was 7, 36 and 46% while was 19.7, 44 and 65% in *G. acutidentatum*. The decrease was 19, 82 and 83% in *G. grandiflorum* and 16.5, 83.8, and 86% in *G. corniculatum* at 5, 15 and 25 dSm⁻¹, respectively (Figure 3). Several reports have demonstrated the negative effects of salinity on plant height^{1,4,57,64-66} in various plant species. Studies on Brassica have shown severe reduction in plant height due to salinity.¹ In Brassica, increasing salinity levels from 0 to 12 dSm⁻¹ resulted in reduction in plant height from 68.68 cm to 50.66 cm. Also, there were significant differences among Brassica genotypes in their response to salinity.¹ Similarly, significant variations of impact of increasing salinity were reported for rapeseed cultivars including interaction of salinity-cultivars for plant height.^{1,66} The reduction in growth parameters such as height could be attributed to several effects such as the osmotic stress and /or ionic toxicity⁴ which is more harmful to plants during the succulent seedling stage in addition to the stressful effects of ion uptake.^{67,57} Salinity stress favors the growth of roots rather than shoots which results in a decrease in plant height. Marcum⁶⁸ reported root mass increased under saline conditions of several grasses at mowing heights ranging from 35 to 75 mm. Also, root growth stimulation under saline conditions has been reported in several salinity tolerant grasses as well.^{49,69} Fu et al.⁴⁷ found an increase in total root mass ranging from 30% to 66% and 39% to 89% at the 25.4 mowing height as compared to 12.7 and 6.4 mm mowing heights, respectively when salinity was between the control level and 10 dS m⁻¹. Shahba²⁷ and Shahba et al.²⁸ reported an increase in root mass of Bermuda grass cultivars and seashore paspalum cultivars when salinity level increased from control to 20.0 dS m⁻¹. Rozema and Visser⁷⁰ indicated that increased rooting and the associated increase in root absorbing area is an adaptive mechanism to the osmotic and nutrient deficiency stresses occurs under saline conditions with the result of a reduction in shoot mass and plant height. Unfortunately, we have not measured the change in root mass in this study to assure this effect in *Glaucium* spp.

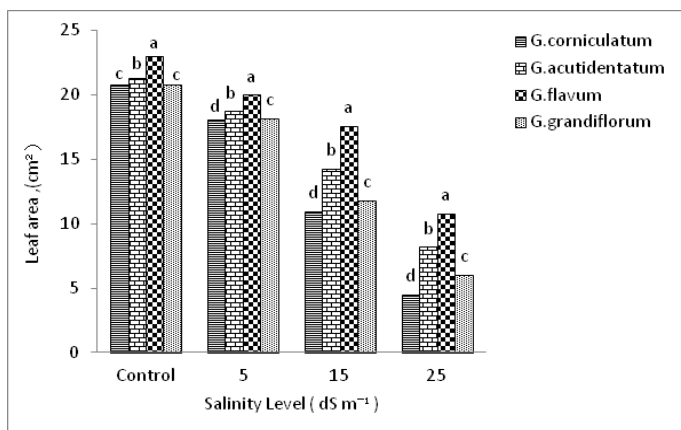


Figure 2 Effect of different Salinity levels on leaf area of four *Glaucium* spp. Columns labeled with different letters are significantly different at P=0.05 within each salinity level.

Flowering characteristics

Number of flower buds. Numbers of flower buds varied significantly among *Glaucium* spp., salinity levels and their interaction (Table 1). Increased salinity levels resulted in fewer flower buds (Figure 4). In *G. flavum*, as salinity levels increased from control to 5, 15 and 25.0 dS m⁻¹ average buds number decreased by 11.5, 36 and 65 %

respectively. The decrease was greater in *G. acutidentatum* where the average number of flower buds decreased by 36, 60 and 79 % when salinity increased from control to 5, 15 and 25dSm⁻¹, respectively. *G. grandiflorum* and *G. corniculatum* did not produce any flower buds at the salinity levels of 15 and 25 dS m⁻¹. This study showed that salinity significantly affected the production of flower buds. At the control treatment, all species produced flower buds with the highest number produced by *G. flavum* (30.5), followed by *G. acutidentatum* (29), *G. grandiflorum* (11) and the lowest number of flower buds was produced by *G. corniculatum* (10) (Figure 4).

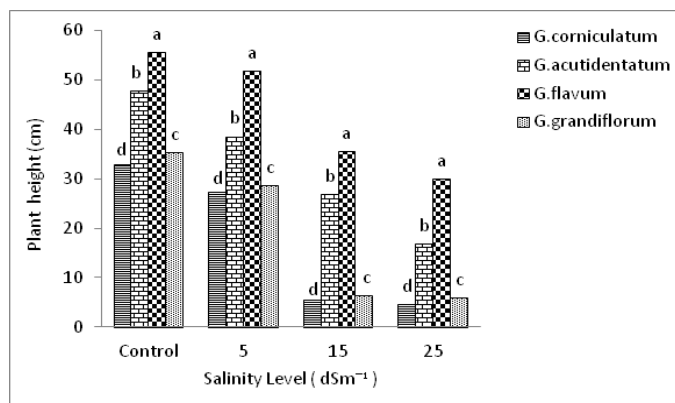


Figure 3 Effect of different Salinity levels on the height of four *Glaucium* spp. Columns labeled with different letters are significantly different at P=0.05 within each salinity level.

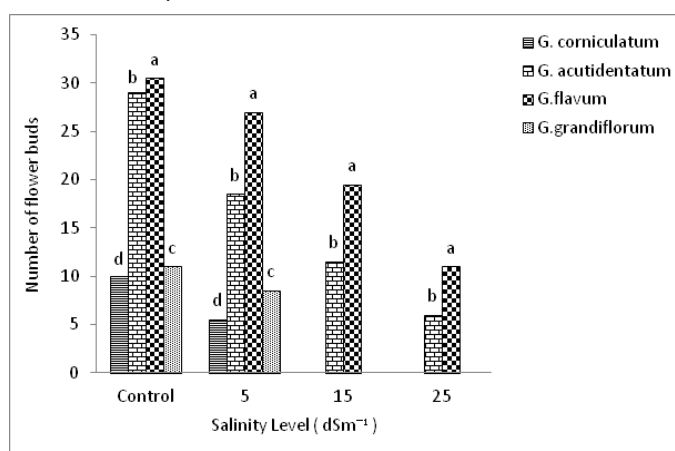


Figure 4 Effect of different Salinity levels on number of flower buds of four *Glaucium* spp. Columns labeled with different letters are significantly different at P=0.05 within each salinity level.

Number of flowers. The number of actual flowers is another indicator of plant vigor. Number of flowers varied significantly (P<0.05) among species, salinity levels and their interaction (Table 1). The number of flowers declined with higher salinity. The decline in flower number under higher salinity was more severe and more rapid in less salinity tolerant species (*G. grandiflorum* and *G. corniculatum*) and was moderate in *G. flavum* and *G. acutidentatum* (Figure 5). *G. flavum* produced the greatest number of flower under all salinity levels when compared to the other species (Figure 5). *G. corniculatum* had the lowest number of flowers at all salinity levels. *G. flavum* (28.5) had the greatest number of flowers in the control treatment followed by *G. acutidentatum* (24), *G. grandiflorum* (9) and *G. corniculatum*

(8.2) in average. Only *G. flavum* and *G. acutidentatum* developed at the salinity levels of 15 and 25 dSm⁻¹ (Figure 5). Flower area. Comparisons of flower area among species and among salinity levels and their interaction clearly showed significant differences (Table 1). Flower area decreased linearly with increasing salinity level. Regressions were strongly linear, with slope more negative with less tolerant species (Table 2). At the control treatment, flower area was the greatest in *G. flavum* (23 cm²) followed by *G. acutidentatum* (17 cm²), and *G. grandiflorum* (10.5 cm²). *G. corniculatum* had the smallest total flower area at the control treatment (8.9 cm²). The decline in flower area under higher salinity was more severe and more rapid in species (*G. grandiflorum* and *G. corniculatum*) that were less tolerant to salinity and was moderate in *G. flavum* and *G. acutidentatum* (Figure 6). *G. flavum* showed greater flower area under all salinity levels compared to other species (Figure 6).

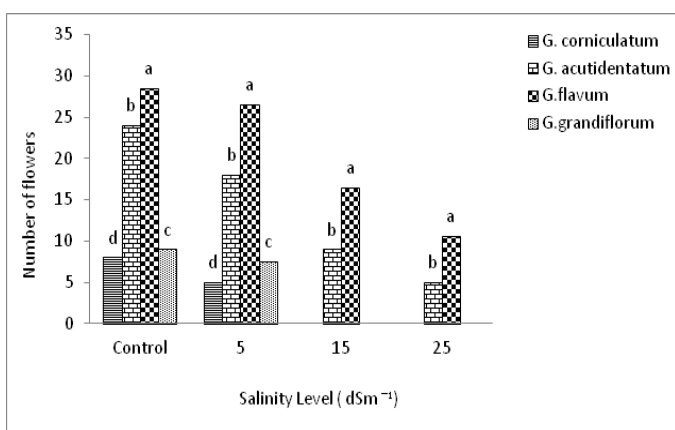


Figure 5 Effect of different Salinity levels on the number of flowers of four *Glaucium* spp. Columns labeled within different letters are significantly different at P=0.05 within each salinity level

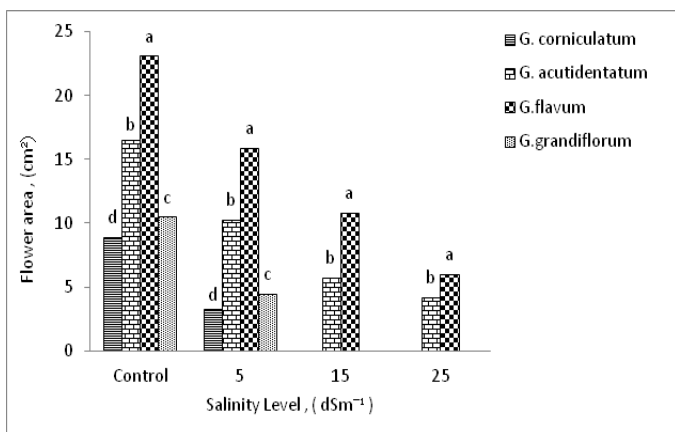


Figure 6 Effect of different Salinity levels on flower area of four *Glaucium* spp. Columns labeled with different letters are significantly different at P=0.05 within each salinity level.

The results are similar to several previous reports in other species. Soil salinity resulted in significant flower bud mortality in blueberry⁷¹ while Falcon et al.⁷² reported a continuous yield reduction of rose as salinity increase. Küçükahmetler⁷³ suggested that the effect of salinity on flower bud formation could be curvilinear, i.e. the plant can overcome and adjust to the salinity effect over time. Unfortunately, this assumption was not the case in this study and may be due to the short

duration of the study. The suppression of plant growth under saline conditions may be associated with decreased availability of water or to the toxicity of sodium chloride.⁷⁴ Furthermore, the hydrolysis of reserved foods to produce energy necessary for survival reduces the amount of resources available for flower formation. Salinity stress imposes additional energy requirements on plant cells and less carbon is available for growth and flower primordial initiation.^{6,65,74} Salinity effect on flower formation is likely an indirect result of its effect on photosynthesis (Pn) efficiency as well. Although Pn is less sensitive to salinity when compared to other growth parameters,⁴⁷ it does add additional support to the superior salinity tolerance of *G. flavum* as compared to the other tested species. Previous studies documented the adverse effects of salinity on Pn in several species.^{25,27,28,75,76} In creeping bentgrass, Liu and Cooper⁷⁶ reported a 20% decrease in Pn when salinity increased from 0.0 to 16 dS m⁻¹, while Qian and Fu²⁵ reported a 40% decrease for the same species when salinity increased from control (0.2 dS m⁻¹) to 15 dSm⁻¹. Increasing salinity and reduction in mowing heights of Bermudagrass²⁷ cultivars and seashore paspalum cultivars²⁸ additively decreased canopy photosynthesis. Photosynthetic capacity is reduced in the presence of high salinity due to stomata closure, damage to photosynthetic systems by excessive energy, structural disorganization or reduction in photochemical quenching.^{31,77}

Razmjoo et al.⁶⁵ has related the negative effects of salinity on flower number to its early effect on the growth and production of strong a shoot system. Pessaraki and Touchane⁷⁸ found that the reduction in biomass production due to salinity stress is more obvious than the reduction in shoot lengths in bermudagrass. The decrease in plant biomass production due to salinity may be attributed to low or medium water potential, specific ion toxicity, or ion imbalance.⁴ In addition; elevated salinity may adversely affect photosynthesis and as a result adversely affect plant biomass production through less accumulation of carbon products.⁵⁶

The reduction in the number of flowers usually is more drastic than other growth parameters under high salinity as it is a cumulative effect.⁶⁵ High salinity in irrigation water has been reported to reduce flowering intensity, fruit set, number of fruits, and fruit growth.⁷⁹⁻⁸¹ Flower yield of China aster was reduced from 49.8 g/plant to 26.3 g/plant when salinity was increased from control to only 4 dSm⁻¹.⁷³

Plant quality (attractiveness)

Plant quality (attractiveness) varied significantly among species and salinity levels. The interaction between species and salinity levels was significant too (Table 1). Plant quality decreased linearly with increasing salinity level in all species. Regressions were strongly linear, with slope more negative in the less tolerant species (Table 2). Increasing salinity decreased the attractiveness of all *Glaucium* spp. although to different degrees (Figure 7). Under the control treatment, there was no difference between *G. flavum*, and *G. acutidentatum* and both did equally well and achieved the maximum quality (10, 10), while there was a significant difference between *G. grandiflorum* (9.5) and *G. corniculatum* (9) (Figure 7). The treatment of 5 dSm⁻¹ did not have a significant effect on the quality of *G. flavum*, although it significantly reduced the quality of the other species (Figure 7). All species were adversely affected at the salinity level of 15 dS m⁻¹, where, *G. flavum* had a limited decline (9) followed by *G. acutidentatum* (8), *G. grandiflorum* (6.24) and *G. corniculatum* (5.5) (Figure 7). The decline in quality and attractiveness under the higher salinity level of 25 dS/m was more severe in all species; however, *G. flavum* had the

highest quality at this level which indicates its relative superior salinity tolerance (Figure 7). The response of plants to salinity is determined by their general growth characteristics and by their physiological mechanisms of salt tolerance. The range of salinity in which the plant is able to survive varies according to the species;⁸² In several species growth may be affected by either the absence of or excess of NaCl in the substrate.^{59,62,83–86} The ability to limit Na⁺ transport into the shoots, and to reduce the Na⁺ accumulation in the rapidly growing shoot tissues, is critically important for maintenance of high growth rates and protection of the metabolic process in elongating cells from the toxic effects of Na⁺ for example, the quality of lilies (plant height, flower bud length and flower diameter) decreased as salinity levels increased.⁷³

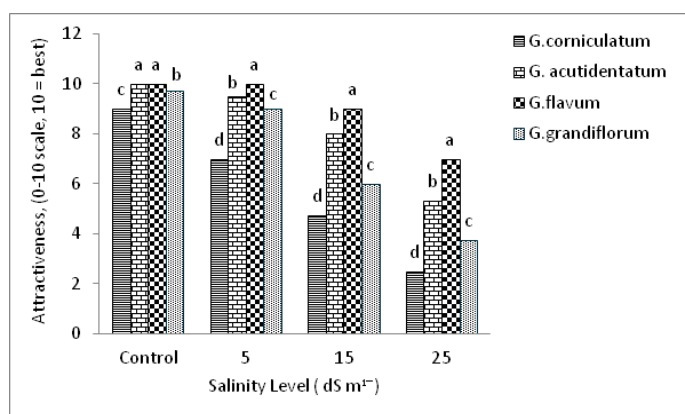


Figure 7 Effect of different salinity levels on the attractiveness of four *Glaucium* spp. Columns labeled with different letters are significantly different at $P=0.05$ within each salinity level.

Salinity may directly or indirectly inhibit cell division and enlargement and finally the growth of the whole plant. Some above ground visible morphological symptoms of plants are marginal yellowing/browning of foliage, premature leaf fall, twig and branch die back, loss of vigor and stunted growth.⁴⁵ Several previous studies indicated similar results to the findings on Horny poppy. Fu et al.⁴⁷ indicated a decline in the turf quality of ‘Brilliant’ Kentucky bluegrass and ‘L-93’ creeping bentgrass irrigated with high salinity water when compared to those irrigated with fresh water. Salinity caused a decline in the quality of bermudagrass cultivars²⁷ and in seashore paspalum cultivars.²⁸ Razmjoo et al.⁶⁵ related the negative effects of salinity on plant quality to its early effect on the growth and production of a strong shoot system and as a result flowering ability. The reduction in biomass production due to salinity stress is more obvious than the reduction in shoot lengths in bermudagrass and more effective on the quality.⁷⁸ The decrease in plant biomass production due to salinity may be attributed to low or medium water potential, specific ion toxicity, or ion imbalance.⁴ In addition, elevated salinity may adversely affect photosynthesis and as a result adversely affect plant biomass production through less accumulation of carbon products.⁵⁶ The reduction in the number of flowers usually is more drastic than other growth parameters under high salinity as it is a cumulative effect.⁶⁵ Fewer and smaller or weak flowers adversely affect the attractiveness of any landscape plant.

Shoot total nonstructural carbohydrates and total reducing sugar content

Shoot TNC varied significantly among species, salinity levels and their interaction (Table 1). Increasing salinity decreased shoot TNC of

Glaucium spp. (Table 3). Regression analysis indicated a significant negative linear relationship between salinity levels and TNC contents (Table 3). In *G. flavum*, as salinity levels increased from control to 5, 15 and 25 dS m⁻¹, average TNC decreased by 15.7, 28.6 and 43.6% and the average TNC decrease in *G. acutidenatum* shoots was 17.6, 36.8 and 48%. The decrease in *G. grandiflorum* was 20, 40 and 48.6% while the decrease in *G. corniculatum* was 28.9, 49, and 53%, respectively. A decline in TNC was recorded, most likely due to continued defoliation that removed photosynthetic tissues due to whole plant stress. High salinity escalated the reduction in TNC which resulted from the reduction in the shoot system. Shoot RSC varied significantly among species, salinity levels and their interactions (Table 1). RSC response to different salinity treatments followed a different trend than TNC (Table 4). Reducing sugars in plants mainly consists of glucose and fructose.^{87,88} While nonstructural carbohydrates are energy reserves in plants, soluble reducing sugars are thought to play an important role in salinity, drought and freezing tolerance as osmoregulators and as protectants as they prevent cell desiccation.⁸⁹ Regression analysis indicated a significant positive association between salinity and RSC content in all species at all salinity levels (Table 4). As salinity levels increased from control to 5, 15 and 25 dS m⁻¹, average RSC increased by 37, 89 and 147 % in *G. flavum* and by 17.2, 43.8 and 106% in *G. acutidenatum*. The increase was 15.1, 26.3, and 70.9% in *G. grandiflorum* and 4, 23.3, and 61% in *G. corniculatum*, respectively. Similar results were observed by Qian and Fu⁴⁷ who found a decrease in TNC and an increase in RSC with increasing salinity level in shoots of L-93 creeping bentgrass. Shahba²⁷ also found an increase in RSC and a decrease in TNC with increases salinity which was maximized at lower mowing heights in bermudagrass species (Tifgreen, Tifdwarf and Tifway) and seashore paspalum cultivars.²⁸ Carbon reduction could be related to the salt resistance mechanisms that are energy dependent. The results suggested that carbohydrate availability was a limiting factor for shoot growth under high salinity stress that results in shoot reduction.

Soluble carbohydrates may interact with membrane phospholipids and proteins to stabilize their structures and prevent desiccation under salinity stress.⁸⁹ In agreement with the horned poppy findings, Lee et al.^{23,24} reported a positive association between salinity tolerance and reducing sugars accumulation in seashore paspalums. Also, Qian and Fu²⁵ indicated that salinity increased glucose and fructose content in bentgrass. TNC serves as the resource for the increased RSC under increased salinity conditions i.e. the relationship between TNC and RSC is a source sink relation.

Shoot proline content

Shoot proline content varied significantly among species, salinity levels and their interaction (Table 1). Increasing salinity increased shoot proline content of *Glaucium* species (Table 5). As salinity levels increased from control to 5, 15 and 25 dS m⁻¹, average Proline content in shoots increased by 218, 367, and 537% in *G. flavum*, 64.5, 296 and 510% in *G. acutidenatum*, 156, 273 and 428% in *G. grandiflorum*, and 79, 188, and 337% in *G. corniculatum*, respectively. Regression analysis indicated a significant positive association between salinity and Proline content in all species (Table 5). Although the role of proline accumulation in salinity tolerance is well documented in this study, it has been questioned by others.³⁴ These results suggested a positive role for proline in *Glaucium* species salinity tolerance. A positive effect of proline accumulation in salinity tolerance was also reported in seashore paspalum cultivars.²⁷ Accumulation of proline in plant tissues in response to salinity stress has been attributed to

enzyme stabilization and/or osmoregulation.^{8,11} It likely enhances membrane stability and mitigates the effect of NaCl on cell membrane disruption and protein structure, act as a sink for carbon and nitrogen for stress recovery and can buffer cellular redox potential under

salinity stress.²⁹ Maggio et al.³⁰ suggested that proline may act as a signaling/regulatory molecule able to activate multiple responses that participate in the adaptation process to elevated salinity levels.

Table 3 Total nonstructural carbohydrates (TNC) in shoots of *Glaucium* spp. measured at the end of the experiment vs. salinity levels, control (c), 5, 15, 25 dSm⁻¹

Species	TNC (mg g ⁻¹ dry wt)				Regression	R ²
	Salinity level (dS m ⁻¹)					
	C	5	15	25		
<i>G. acutidentatum</i>	125.6b†	103.8b	79.2b	65.2b	Y=131.1-1.1X	0.91**
<i>G. corniculatum</i>	103.8d	79.7d	53.8d	49.2d	Y=119.1-1.2X	0.92**
<i>G. flavum</i>	133.2a	112.2a	95.5a	74.9a	Y=116.6-0.9X	0.89**
<i>G. grandiflorum</i>	109.9c	88.2c	66.5c	56.5c	Y=125.5-1.2X	0.82**

† Values followed by the same letters within a column for each cultivar are not significantly different ($P=0.05$) based on a Fisher's LSD test.

*Significant at $P\leq 0.05$.

** Significant at $P\leq 0.01$.

Table 4 Total reducing sugar content (RSC) in shoots of *Glaucium* spp. measured at the end of the experiment vs. salinity levels, Control (c), 5, 15, 25 dSm⁻¹

Species	RSC (mg g ⁻¹ dry wt)				Regression	R ²
	Salinity level (dSm ⁻¹)					
	c	5	15	25		
<i>G. acutidentatum</i>	19.2	22.5b†	27.6b	39.6b	Y=21.7+0.12 X	0.82**
<i>G. corniculatum</i>	15.9	16.6d	19.6d	25.6d	Y=16.3+0.11 X	0.72*
<i>G. flavum</i>	18.9	25.9a	35.8a	46.8a	Y=11.5+0.30 X	0.92**
<i>G. grandiflorum</i>	17.9	20.6c	22.6cb	30.6c	Y=16.3+0.15 X	0.79*

† Values followed by the same letters within a column for each cultivar are not significantly different ($P=0.05$) based on a Fisher's LSD test.

*Significant at $P\leq 0.05$.

** Significant at $P\leq 0.01$.

Table 5 Proline content in shoots of *Glaucium* spp. measured at the end of the experiment vs. salinity levels, Control (c), 5, 15, 25dSm⁻¹

Species	Proline content (µg g ⁻¹ fresh wt)				Regression	R ²
	Salinity level (dSm ⁻¹)					
	c	5	15	25		
<i>G. acutidentatum</i>	250.0	646.0b†	990.0b	1527.0b	Y=218.3+22.9 X	0.87**
<i>G. corniculatum</i>	233.7	418.0d	670.0d	1018.0d	Y=150.7+19.7X	0.77*
<i>G. flavum</i>	268.7	855.0a	1255.0a	1712.0a	Y=142.4+12.6 X	0.92**
<i>G. grandiflorum</i>	230.6	590.0c	860.0c	1215.0c	Y=189+20.7 X	0.81*

† Values followed by the same letters within a column for each cultivar are not significantly different ($P = 0.05$) based on a Fisher's LSD test.

*Significant at $P\leq 0.05$.

**Significant at $P\leq 0.01$.

Shoot and root K⁺/Na⁺ ratio

Shoot and root K⁺ and Na⁺ varied significantly among species, salinity levels and their interaction (Table 1). Increasing salinity decreased shoot K⁺/Na⁺ ratio (Table 6). As salinity increased, Na⁺ content increased and K⁺ content decreased. Regression analysis indicated a significant negative linear relationship between salinity levels and K⁺/Na⁺ ratios (Table 6). Wyn Jones et al.⁹⁰ suggested a threshold K⁺/Na⁺ ratio of 1 for normal growth of plants subjected

to salinity. Results indicated that K⁺/Na⁺ ratio was ≥ 1 at all salinity levels in *G. flavum*, as compared to *G. acutidentatum*, *G. grandiflorum* and *G. corniculatum* (Table 3). Similar results were reported by Qian and Fu,²⁵ who studied the response of creeping bentgrass, Shahba,²⁷ who studied the response of bermudagrass cultivars to salinity and mowing heights and Shahba et al.²⁸ who studied the response of seashore paspalum cultivars to salinity and mowing heights, there was an increase in Na⁺ and a decrease in K⁺ concentration in shoots

with increasing salinity level, this was more obvious at higher salinity levels where a reduction in shoot size was observed. Storey and Wyn Jones⁹⁰ suggested that the capacity to maintain high shoot K⁺/Na⁺ ratios is an important element of salt tolerance, especially in species which lack foliar salt-excretion mechanisms such as *Glaucium* species. Shannon⁹¹ studied salt tolerance of 32 lines of tall wheatgrass and found an association between salinity tolerance and the maintenance of K⁺/Na⁺ ratio ≥ 1 . This suggested that the increase in mowing height may help limit shoot Na⁺ and improve K⁺ concentrations in the shoot. Also, it has been demonstrated that salinity tolerance in

several grass species (Poaceae) is associated with the exclusion of Na⁺ from shoot and the capacity to maintain high shoot K⁺/Na⁺ ratio.^{27,28,33,47,92,93} Greive et al.⁹⁴ found an increase in Na⁺ concentration and a decrease in K⁺ ion concentration when salinity increased from 15 to 25 dS m⁻¹ and concluded that neither K⁺/Na⁺ ratios nor K⁺:Na⁺ selectivity coefficients appear to be satisfactory indicators of relative salt tolerance of the forages examined in their study. Similarly, Lee et al.²³ concluded that K⁺/Na⁺ ratio did not appear to be related to salinity tolerance of seashore pasplum ecotypes.

Table 6 Effect of different salinity levels on K⁺/Na⁺ ratio of *Glaucium* spp. linear regression of different K⁺/Na⁺ ratios of measured at the end of the experiment vs. salinity levels, Control (c), 5, 15, 25 dSm⁻¹

Species	K ⁺ /Na ⁺ ratio				Regression	R ²
	Salinity level (dS m ⁻¹)					
	C	5	15	25		
<i>G. acutidentatum</i>	2.8b	2.1b	1.1b	0.6b	Y=128.0-0.91 X	0.77*
<i>G. corniculatum</i>	2.0d	1.3d	0.5d	0.2c	Y=112.4-1.1 X	0.84**
<i>G. flavum</i>	3.2a	2.5a	1.5a	1.0a	Y=121.1-1.66 X	0.82**
<i>G. grandiflorum</i>	2.4c	1.7c	0.7c	0.3c	Y=121.7-1.65 X	0.82**

†Values followed by the same letters within a column for each cultivar are not significantly different (P=0.05) based on a Fisher's LSD test.

*Significant at P0.05.

** Significant at P0.01.

The proteins of the cell membrane play a significant role in the selective distribution of ions within the plant cells. These proteins include 1) Primary H⁺-ATPases that generate the H⁺ electrochemical gradient. This gradient controls ion transport through the plasma membrane with high selectivity for K⁺ over Na⁺, 2) K⁺/Na⁺ antiports in the plasma membrane for pumping excess Na⁺ out of the cell, 3) Na⁺/H⁺ antiports in the tonoplast for extruding Na⁺ into the vacuole.^{34,95} The selectivity of K⁺ over Na⁺ in root uptake is an energy dependent process and more likely regulated to a substantial degree by H⁺ gradients across the plasma membrane which are maintained by H⁺-ATP ase activity. Low mowing height depletes TNC reserves which induce ATP deficit, resulting in the lack of energy to control active ion selectivity in uptake and transport.^{25,27,28}

Salt stress responses are tissue- and salinity-specific. For long-term performance and persistence in field situations under salt stress, it is essential that grasses exhibit both root and shoot tissue salinity tolerance.² On the basis of the number of times in the best statistical category for leaf characteristics, plant height, flowering characteristics, overall plant quality (attractiveness), TNC, RSC, proline content and K⁺/Na⁺ ratio, *G. flavum* was found to have higher salt tolerance when compared to *G. acutidenatum*, *G. grandiflorum* and *G. corniculatum*. Also, increasing salinity had less adverse effects on overall responses of all species when they were able to maintain a larger shoot system.

In summary, as salinity increased, *Glaucium* spp. exhibited reduction in leaf characteristics, plant height, flowering characteristics, overall plant quality (attractiveness), TNC, and K⁺/Na⁺ ratio, and increased shoot total reducing sugars and proline content. *G. flavum* showed greater salinity tolerance at all salinity levels when compared to the other species tested. Proline accumulation could add to the salinity tolerance through osmoregulation or by acting as carbon and nitrogen sink for stress recovery.

Acknowledgments

None.

Conflicts of interest

Authors declare that there is no conflict of interest.

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