DISSERTATION

ECO-PHYSIOLOGICAL STUDIES ON HORNED POPPY, (*GLAUCIUM SPP*.)

Submitted by

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ABSTRACT

ECO-PHYSIOLOGICAL STUDIES ON HORNED POPPY, (*GLAUCIUM SPP*.)

With increasing population demands on the world's water supply, there is a greater need for drastic water conservation methods especially in arid and semiarid regions. Salinity is considered the major factor limiting 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 related to genetic differences especially in genes relating to stress tolerance mechanisms and their interaction with environments. Horned Poppies (*Glaucium spp*) are members of the Poppy family, *Papaveraceae* native to the Mediterranean and Middle East. Some species have a wider distribution than others. *Glaucium* species have many similar characteristics and can be difficult to distinguish from each other. In arid and semiarid regions, climate and soil can make it difficult for many ornamental plants to grow. Therefore, nurseries are always interested in new plants that will survive well in these climates while satisfying customer's desire for new, beautiful plants. *Glaucium spp.* are currently being evaluated due to their drought and salt tolerance. However, no research has been done to evaluate and improve *Glaucium spp* seed germination under saline conditions or to test the interspecific difference in salinity or drought tolerance. The objectives of this study, therefore, was (1) to determine whether applications of ethephon, fusicoccin, kinetin, and thiourea could promote *Glaucium spp* seed germination under different salinity levels; (2) to determine the most effective concentrations of each growth regulator in enhancing horned poppy seed germination under saline conditions; (3) to evaluate the degree of salinity tolerance among the common Horned Poppy species that were available from Denver Botanic Gardens, *G. flavum, G. corniculatum, G. grandiflorum* and *G.*

*acutidentatum*s ; (4) determine which evaluation criteria are most closely associated with superior salinity tolerance; (5) to confirm selection criteria for evaluation of salinity tolerance in Horned Poppy species and (6) to evaluate the degree of drought tolerance among the common Horned Poppy species, *G. flavum, G. corniculatum, G. grandiflorum* and *G. acutidentatum*s.

An application of ethephon, fusicoccin, kinetin and thiourea were evaluated for their relative ability to promote *Glaucium spp* seed germination under different salinity levels and to determine the most effective concentrations of each growth regulator in enhancing *Glaucium spp* germination under saline conditions. On average, horned poppy germination percentage ranged from 30% in *Glaucium corniculatunm* to 74.8% in G. *flavum* under nonsaline condition, which were reduced to 15 and 50.2% at 15 and 30 dS m^{-1} salinity levels, respectively. Significant differences among tested chemicals were found at EC = 0.0 dS m^{-1} and EC = 15 dS m^{-1} levels but there was no significant difference at EC = 30 dS m^{-1}. A variation in germination percentage and rate among the tested species was indicated. *Glaucium flavum* achieved the highest germination percentage and rate under all salinity levels followed by *G. corniculatum* and *G. acutidentatum*. *G. grandiflorum* had the lowest germination percentage and rate. The effect of growth regulators varied from species to species and from one salinity level to the other. The current investigation demonstrated that 30 mM thiourea, 0.01 mM fusicoccin, 10.0 mM ethephon, and 1.5 mM kinetin increased seed germination percentage and rate of *Glaucium ssp* under saline conditions. Ethephon was the most effective growth regulator in ameliorating salinity effect on *Glaucium ssp* seed germination followed by thiourea, fusicoccin, and kinetin. All tested growth regulators had similar positive effects at the highest salinity level (EC = 30 dS m^{-1}).

The salinity tolerance of the four Horned Poppy species available from Denver Botanic Gardens, *G. flavum, G. corniculatum, G. grandiflorum and G. acutidentatum,* were compared to examine the effects of salinity (EC = 5, 15 and 25 dS m^{-1}) on plant characteristic that add up to its attractiveness, and to determine which tolerance mechanism was associated with superior salinity tolerance among the 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 green house. With higher salinity, leaf color declined over time to unacceptable ratings (below 6). In *G.flavum*, leaf color was not affected adversely under all salinity levels while all other species declined to the unacceptable rating of 5.3 (*G. acutidentatum*), 4 (*G. grandiflorun*), and 3.2 (*G. corniculatum*) at the salinity level of 25 dS m^{-1}. 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* acheived an average leaf area of 23 cm^2 while *G. acutidentatum* achieved leaf are of 21.3 cm^2 and *G.grandiflorum* and *G. corniculatum* achieved the same leaf area of 20.8 cm^2*in* the control treatment. *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. *G. corniculatum* had the lowest height of 32.7 cm the control treatment. 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. grandflorum* 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. acutidenatum*, 156, 274 and 428% in *G. grandflorum*, 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. acutidenatum*, *G. grandflorum* 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* demonstrated higher salinity tolerance at all salinity levels as compared to the other species. The proline accumulation might add to the salinity tolerance through osmoregulation or by acting as a carbon and nitrogen sink for stress recovery.

All 4 horned poppies were also evaluated for drought tolerance, (*G. flavum, G. corniculatum, G. grandiflorum and G. acutidentatums),* by examination of the effects of drought on plant aesthetics as well as the mechanisms associated with drought tolerance such as proline content, total non-structural carbohydrate content (TNC), shoot reducing sugar content (RSC) and Evapotranspiration rate (ET) as indicated by water use efficiency among the tested species. Lysimeter columns were used in this study which was replicated twice in the green house. *Glaucium spp.* were initiated from seeds. Seedlings were planted in potting mix, (Pro-Mix , Mycorrhizae and Biofunglcide). Fifty 3 leaf seedlings of each species were transplanted, each into PVS tubes (15 cm diameter and 50 cm long) containing commercial potting mix. The potting mix was mixed with sand in a 2:1 ratio to increase pore space. Water regimes applied included control (100% of the total evapotranspiration), as well as 75%, 50% and 25% of total evapotranspiration,(ET). With lower water regimes, leaf color declined over time to unacceptable ratings (below 6) in both *G. grandiflorun* and *G. corniculatum*. In *G. flavum* and *G. acutidentatum*, leaf color was not affected adversely under all water regimes. The effect of water

stress on leaf color among all species was highly significant. The decline in leaf color was high for all species at 50% and 25% ET. Leaf area decreased linearly in all species with increasing drought with a sharp drop at irrigation 25% of ET. *G.flavum* achieved the highest leaf area at all water regimes followed by *G. acutidentatum*, *G.grandiflorum* and *G. corniculatum*. *G.flavum* achieved the average leaf area of 24.3 cm^2 while *G. acutidentatum* achieved a leaf area of 22.2 cm^2. *G.flavum,* the average height was 45.8 cm while *G. acutidentatum* achieved an average height of 40.5 cm and *G.grandiflorum* at 30.0 cm. *G. corniculatum* had the lowest height of 27.8 cm at 100% ET. Increased water stress resulted in fewer flower buds, fewer flowers, and smaller flower area in all tested species. Also, the increasing drought decreased aesthetics of all *Glaucium spp*. to different degrees. *G. flavum* demonstrated greater transpiration efficiency (TE) since it was able to maintain its ET at lower irrigation rates while maintaining higher attractiveness when compared with *G. acutidentatum*, *G. grandiflorum* and *G. corniculatum* respectively. In *G. flavum*, as water regimes increased from control to 75, 50 and 25 % of the total evapotranspiration, average TNC decreased by 15.1, 30.3 and 48.0% and the average TNC decrease in *G. acutidentatum* shoots was 21.6, 40.1, and 53.7%. RSC response to different drought treatments followed a different trend than TNC. As water regimes increased from control to 75, 50 and 25% ET, average RSC increased by 40.7, 101.8 and 166.5 % in *G. flavum* and by 17.4, 40.0 and 103.4% in *G. acutidentatum.* The increase was 122.2, 39.6, and 90.6% in *G. grandiflorum* and 4.4, 26.5, and 62.5% in *G. corniculatum*, respectively. As water regimes decreased from control to 75, 50 and 25%, average proline content in shoots increased by 186, 325, and 472% in *G. flavum*, 163, 303 and 517% in *G. acutidentatum*, 160, 280 and 418% in *G. grandiflorum*, and 80, 190, and 340% in *G. corniculatum*, respectively. On the basis of the number of times in the best statistical category for leaf characteristics, plant height, flowering

characteristics, overall plant quality (attractiveness), water use efficiency, TNC, RSC, and Proline, *G. flavum* was found to have higher drought tolerance compared to *G. acutidentatum*, *G. grandiflorum* and *G. corniculatum*. In summary, as drought increased, *Glaucium spp*. exhibited reduction in leaf characteristics, plant Height, flowering characteristics, overall plant quality (attractiveness), TNC, and ET rate, and increased shoot total reducing sugars and Proline content. *G. flavum* showed higher drought tolerance at all water regimes compared to other tested species.

In summary, ethephon was the most effective growth regulator in ameliorating salinity effect on *Glaucium ssp* seed germination followed by thiourea, fusicoccin, and kinetin. 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 tested species. Proline accumulation could add to the salinity tolerance through osmoregulation or by acting as a carbon and nitrogen sink for stress recovery. Similarly, as drought increased, *Glaucium* spp. exhibited reduction in leaf characteristics, plant height, flowering characteristics, overall plant quality (attractiveness), TNC, and ET rate, and increased shoot total reducing sugars and proline content. *G. flavum* again showed greater tolerance at all water regimes (100%, 75%, 50% and 25% ET) when compared to the other tested species. Also, Proline accumulation was correlated with drought tolerance that could be done through osmoregulation or by acting as carbon and nitrogen sink for stress recovery.

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I'm greatly indebted to the Libyan government, my sponsor, for giving me this great opportunity to catch up with advanced technology.

Special gratitude and love to my Father and my Mother who gave me everything to get this stage of my life. Also, I would like to express my deep warm love and gratitude to my wife for her understanding, patience and support ,and to my son Omar and my beautiful daughters, Maream and Mayar for giving me the joy of life.

DEDICATION

To my father who is the main supporter of me, to my mother who I am still need her ,and to my wife who is my main supporter here in Fort Collins, Colorado.

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CHAPTER 1

GLAUCIUM SPP. SEED GERMINATION AT DIFFERENT SALINITY LEVELS AS INFLUENCED BY GROWTH REGULATORS

SUMMARY

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. Horned Poppies (*Glacium spp*) are members of the Poppy family, *Papaveraceae* and native to the Mediterranean and Middle East regions. The easiest way to grow Horned Poppies is seeding where they are to bloom in the ground in fall and thinning them to the desired spacing the following spring**.** There are no reported seed germination trials on *Glaucium* species under saline conditions or how growth regulators may improve *Glaucium* seed germination percentage and speed. The objectives of this study were (1) to determine if applications of ethephon, fusicoccin, kinetin and thiourea could promote *Glaucium spp* seed germination under different salinity levels; (2) to determine the most effective concentrations of each growth regulator in enhancing *Glaucium spp* germination under saline conditions. Without pretreatment, *Glaucium spp* seeds have a low germination due to seed dormancy. Experiments were conducted to test the effect of the application of different concentrations of thiourea, fusicoccin, ethephon, and kinetin on horned poppy seed germination under three salinity levels. On average, horned poppy germination percentage ranged from 30% in *Glaucium corniculatunm* to 74.8% in *G. flavum* under nonsaline condition. Germination was reduced to 15 and 50.2% at 15 and 30 dS m^{-1} salinity levels, respectively. Analysis of variance indicated a significant difference among tested chemicals at EC = 0.0 dS m^{-1} and EC = 15 dS m^{-1} and EC = 30 dS m–1 (Table 1) in their effect

on enhancing Glaucium ssp seed germination percentage and rate of germination. In conclusion, a variation in germination percentage and rate among the tested species has been indicated. *Glaucium flavum* achieved the highest germination percentage and rate under all salinity levels followed by *G. acutidentatum*. and *G. grandiflorum* while *G. corniculatum* had the lowest germination percentage and rate. The effect of growth regulators varied from species to species and from one salinity level to the other. The current investigation demonstrated that 30 mM thiourea, 0.01 mM fusicoccin, 10.0 mM ethephon, and 1.5mM kinetin increased seed germination percentage and rate of *Glaucium ssp* under saline conditions. Ethephon was the most effective growth regulator in ameliorating salinity effect on *Glaucium ssp* seed germination followed by thiourea, fusicoccin, and kinetin. All tested growth regulators had similar positive effects at the highest salinity level (EC = 30 dS m^{-1}). Further research is needed to develop appropriate protocols for practical and effective treatment procedures for landscape use in saline areas.

INTRODUCTION

Salinity is considered as 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.

Horned Poppies (*Glacium spp*) are members of the Poppy family, *Papaveraceae*, native to the Mediterranean and Middle East regions. Some species have a wider distribution than others. *Glacium* species have many similar characteristics and can be difficult to distinguish from each other. Horned poppies grow best in full sun and in well-drained soil. The easiest way to grow Horned Poppies is seeding in the fall where they are to bloom and thinning them to the

desired spacing with spring germination**.** *G. flavum* Crantz is the most widely spread species in the genus. It's found along the coasts of Britain and the Atlantic Islands to the coasts of the Mediterranean Basin and the Black Sea (Grey-Wilson, 2000). It grows predominantly on sandy beaches and as a result it is commonly known as the Sea Horned Poppy. This implies that *G. flavum* is relatively salt tolerant due to its proxinity to the sea. *G. grandiflorum* Boiss & É. Huet is native in the southern part of the Caucasus Mountains inTurkey but is also found in Syria, Iran and the Sinai (Grey-Wilson, 2000). 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 & Bornm is endemic to Turkey where it is found on dry hillslopes and rocky places (Grey-Wilson, 2000). It is the most glabrous species with smooth sepals and ovaries. *G. corniculatum* (L.) J.H. Rudolph is native to the Mediterranean basin, Atlantic islands, Caucasus Mountains, Bulgaria, Romania, northern Iraq and northwestern Iran (Grey-Wilson, 2000; Davis, 1965). *G. corniculatum* also has some unique characteristics which are soft leaves, villous texture and sepals that are scabrous to hirsute. Although there is some conflicting information about *G. corniculatum*'s corolla, its petals have been observed to be yellow, orange or red (Davis, 1965) with a black basal spot (Grey-Wilson, 2000).

Previous research on *Glaucium flavum* seed germination has clearly indicated a need to stratify the seeds which have seed coat dormancy (hard seed). Previous research has also verified that *G. flavum* germinates better in cooler temperatures and that light can interact with germination at warmer temperatures (breaking a surface-germination avoidance mechanism) but light impedes germination at cooler temperatures. A combination of stratification type of scarification has

been tested too (Scott, 1963; Formanowicz and Koziowski, 1976; Mermerska, 1984; Thanos, et al. 1989; Walmsley and Davy, 1997; Elsner, 2007) with some success. Walmsley and Davy (1997) compared the seed germination of *G. flavum* under different levels of saline salt to mimic the sea water normally found on coasts. Germination in *G. flavum* dropped dramatically at 20% salinity. The authors indicated that *G. flavum* could be influenced by a salinity-enforced dormancy which would prevent it from germinating in winter when sea water inundates the beaches and force the seeds to wait until spring when rainwater would leach the salts from the sand and seeds. Unripened seed were also tested. They found that unripe seeds germinated readily and verified that *G. flavum* seeds have a hard seed coat dormancy as reported by Scott (1963). As seed aged, they showed an increased sensitivity to supraoptimal temperatures. Therefore, they concluded that reduced germination in older seeds is not necessarily due to lower viability but because of growing sensitivity to inappropriate conditions such that the optimal environmental conditions has a more narrow range; this is likely a symptom of decreased vigor.

The effects of germination regulating chemicals in enhancing seed germination under salinity conditions and alleviating salinity stress has been reported many plant species. Gul and Khan (2003) demonstrated a substantial enhancement in seed germination of the perennial halophyte Utah pickleweed (*Salicornia utahensis* Tidestrom) with the inclusion of ethephon,(an ethylene releasing compound) at 10 mM and kinetin 0.05 mM. Ethylene may stimulate seed germination (Whitehead and Nelson, 1992; Sutcliff and Whitehead, 1995), especially when seeds are exposed to salt and temperature stresses (Khan and Andreoli, 1993; Li et al., 1995). El-Keblawy et al. (2005) reported a positive effect of 0.05 mM kinetin in enhancing the germination at high salinity levels in mesquite [*Prosopis julifl ora* (Sw.) DC.]. While Khan and Ungar (2001a) reported that thiourea (10 mM) partially alleviated the inhibitory effects of salinity on

the germination of summer seeds of coastal dune grass [*Halopyrum mucronatum* (L.) Stapf], while kinetin (0.05 mM) alleviated the inhibitory effects of salinity on the germination of winter seeds. Fusicoccin is known to alleviate the effect of salinity on seed germination of halophytes (Ismail, 1990; Gul and Weber, 1998; Gul et al., 2000; Khan et al., 2002; Gul and Khan, 2003). Shahba et al. (2008 and 2009) have found a positive effect for thiourea, fusicoccin, kinetin and ethephon on saltgrass seed germination under saline conditions. It is still to be determined if other *Glacium* species will germinate under similar conditions and there have been no trials to improve *Glacium* species seed germination under saline conditions.

There is no reported seed germination trial on *Glaucium* species, under saline conditions and with use of growth regulators to improve seed germination percentage and rate. The objectives of this study were (1) to determine if applications of ethephon, fusicoccin, kinetin and thiourea could promote *Glaucium spp* seed germination under different salinity levels; (2) to determine the most effective concentrations of each growth regulator in enhancing *Glaucium spp* germination under saline conditions.

MATERIALS AND METHODS

Seed Acquisition. *Glaucium* seeds were acquired from Denver Botanic Garden's collection. *Glaucium* species studed were as follows; *G. flavum*, *G. acutidentatum*, *G. grandiflorum*, and *G. corniculutatum*. All four species are grown in the Rock Alpine Garden with an area of 43,560 ft^2 or approximately 4046.9 m^2. Seeds were stored at room temperature prior to initiation of these studies.

Viability Testing. A seed is considered viable if the embryo is alive and will germinate.The viability of the seeds was tested with a 1.0% concentration of 2, 3, 5-triphenyltetrazolium

chloride (TZ). The testing method used was in accordance with the standards set by the Association of Official Seed Analysts (AOSA) for seeds of the *Papaveraceae*. Two replications of 100 seeds from each seed lot were set on moist blotter paper to soften overnight. Off-center, longitudinal cuts were made into the seeds and they were then placed in petri dishes containing 1.0% TZ. The dishes were set in a dark store room at 19°C overnight (approximately hors). Viability was determined based on diagrams and descriptions of *Papaver sp*. seed staining in the AOSA handbook. Viability tests were performed on seeds prior to the combined stratification/scarification treatment.

Germination test. Without pretreatment, *Glaucium spp* seeds have a low germination due to seed dormancy. To break seed dormancy, seeds were subjected to machine scarification.With the use of the MAT-OSU pneumatic seed scarifier, Mater Intel., Corvallis, OR). A series of pilot tests were performed to determine the appropriate sandpaper grit size, appropriate operation pressure of the scarifier, and scarification time for optimum results. In this experiment the seed scarifier was set at 112 MPa pressure with 60 Grit sandpaper used, and a scarification time of 4 min. In the stratification treatment, scarified seeds were placed on moist paper towels and stored at 4°C in darkness for 3 weeks. Three treatment factors were imposed: salinity, type of germination-regulating chemical, and concentrations of germination-regulating chemical. The experiment was set up in the growth chamber and repeated once. A split-split plot design with three replications was used. Salinity levels were considered as the whole plot factor, germination-regulating chemicals were the subplot factor, and the concentrations of these chemicals were the sub-subplot factor. Salinity levels were control (distilled water), and electrical conductivity (EC) of 5, 15 and 30 dS m^{-1} (salinity levels were determined based on prelimimary studies using various NaCl concentration to prepare EC solutions. Chemicals used to stimulate seed

germination were thiourea, fusicoccin, ethephon and kinetin. Four different concentrations of each were used: thiourea (0.00, 10, 20, and 30 mM), fusicoccin (0.0, 3.0, 5.0, and 10.0 μM), ethephon (0.0, 3.5 and10mM) and kinetin (0.0, 0.5, 1.0, 1.5 mM). All solution were prepared using NaCl solutions at 0, 15, and 30 dS m^{-1}, respectively.

Seeds were sown on sterile germination blotter papers lined in 9-cm diameter petri dishes. In each dish, 50 seeds were placed on each germination blot. Germination blots were moistened with 20 mL of each treatment solution. Petri dishes were sealed with parafilm and were placed in a germinator at the Colorado State Seed Laboratory. at 15°C at +/- 0.6°C. The germinator's light source was 6 cool, white fluorescent bulbs, which emits approximately 10.25 μmol s^{-1} m^{-2} of light. The germinator was set to give 16 hours of light and 8 hours of dark. Germination was recorded every other day after 2 days until 20. Seeds were considered germinated if the emerged radical was visible. Germination percentage was defined as the total percent germination in 20 d. The rate of germination was calculated by dividing the percentage of seeds germinated at each count by the number in days from the start of the germination test. The total of values obtained is the germination rate (Maguire, 1962). The experiment was repeated twice.

Data analysis. The data of the two experiments were subjected to ANOVA to test the experiment effect and the interaction between treatments and experiments. Experiments were not significant different. Therefore, data were pooled over experiments to test the effects of salinity levels and different concentrations of each chemical treatment on germination speed and percentage at individual salinity levels (SAS Institute, 2006). Means separation were performed at $P = 0.05$ by Fisher's LSD test when significant differences were found. The most effective concentration of each chemical treatment was chosen and subjected to ANOVA for chemical treatment comparison.

RESULTS AND DISCUSSION

Analysis of variance indicated significant species, salinity, chemical type, and concentration effects on the four *Glaucium ssp*. seed germination percent and rate. The interactions between species and growth regulators, chemical type and concentration, and among species, growth regulators and concentrations were also significant (Table 1-1). The increase in salinity significantly reduced the germination percentage and rate of the four *Glaucium ssp*. (Fig. 1-1). *G. flavum* achieved the highest germination percentage under non saline conditions (74.8%), followed by *G. acutidentatum* (50%), *G. grandiflorum* (39.7%), and *G. corniculatum* (30%). All *Glaucium species* were affected negatively by the increase in the salinity level. Effects of NaCl on seed germination could be osmotic and/or ionic either through reduction of water availability, interferance with some aspect of metabolism, or altering the balance of

Table 1. 1. Analysis of variance with mean squares and treatment significance of Glaucim Spp. seed germination rate (% d/1) and percentage (%) as affected by different growth regulators concentrations,and their interaction.

Source	Salinity Levels (dS/m)					
	0		15		30	
	rate	%	rate	%	rate	%
Species (S)	425.0**	2350.0**	110.0*	1512.0*	70.0**	249.0**
Growth regulators (T)	620.0**	3340.0**	120.0*	1210.0*	80.0**	369.0**
Concentrations (C)	800.6**	4215.0**	620.0**	3456.0**	540.0**	1590.0**
SxT	659.0**	1912.0**	299.0**	926.0**	199.0**	396.0**
TxC	755.2**	2212.0**	315.0**	1126.0**	250.0**	462.0**
SxTxC	856.2**	3115.0**	429.0**	2026.0**	340.0**	567.0**

*Significant at P<0.05
**Significant at P<0.001
†Not significant at P<.0.0 5

endogenous growth regulators in the seeds (Khan and Ungar, 1998a, 1998b; Khan and Ungar, 2001c; Ungar, 1991). Shahba et al. (2008 and 2009) found the same effect on saltgrass seed germination under saline conditions.

Thiourea significantly improved germination percentage (Fig. 1-2) and germination rate (Fig. 1-3) of the four *Glaucium ssp*. at all salinity levels. Analysis of variance and a means separation test indicated that 30.0 mM of thiourea was the most effective concentration of those tested in improving both germination rate and percentage at all salinity levels (Table 1-2). The level of 30.0 mM of thiourea increased germination percentage at EC = 0.0 dS m–1 from 74.8 to 92.2%, at EC = 15 dS m–1 from 68.7 to 73.8%, and at EC = 30 dS m–1 from 50.2 to 64.7% in *G. flavum*. Thiourea concentration of 30.0 mM had a similar effect on germination rate (Table 1-3). In this species, the level of 30.0 mM of thiourea increased germination rate at EC = 0.0 dS m–1 from 20.8 to 29.0%, at EC = 15 dS m–1 from 17.2 to 19.3, and at EC = 30 dS m–1 from 13.0 to 17.2. In *G. acutidentatum,* the concentration at 30.0 mM improved seed germination percentage and rate the greatest of those tested. At an EC = 0.0 dS m^{-1}, germination percentage increased from 50.0 to 82.0%, while seeds at EC = 15 dS m^{-1} increased from 45.2 to 60.5%, and at EC = 30 dS m^{-1} increased from 35.0 to 50.7%. Thiourea had a similar effect on germination rate. The level of 30 mM achieved the greatest germination rate followed by 20 mM and 10 mM (Table 1-3). The level of 30.0 mM of thiourea increased germination rate of *G. acutidentatum* at EC = 0.0 dS m^{-1} from 9.3 to 19.0, at EC = 15 dS m^{-1} from 7.8 to 13.0, and at EC = 30 dS m^{-1} from 5.5 to 8.5. The level of 30 mM increased germination percentage at EC = 0.0 dS m^{-1} from 39.7 to 72.2%, at EC = 15 dS m^{-1} from 29.5 to 52.7%, and at EC = 30 dS m^{-1} from 19.7 to 32.5% in *G. grandiflorum*. In the same species, the level of 30.0 mM of thiourea increased germination rate at EC = 0.0 dS m^{-1} from 10.9 to 17.4, at EC = 15 dS m^{-1} from 8.5 to 13.6, and at EC = 30 dSm^{-1}

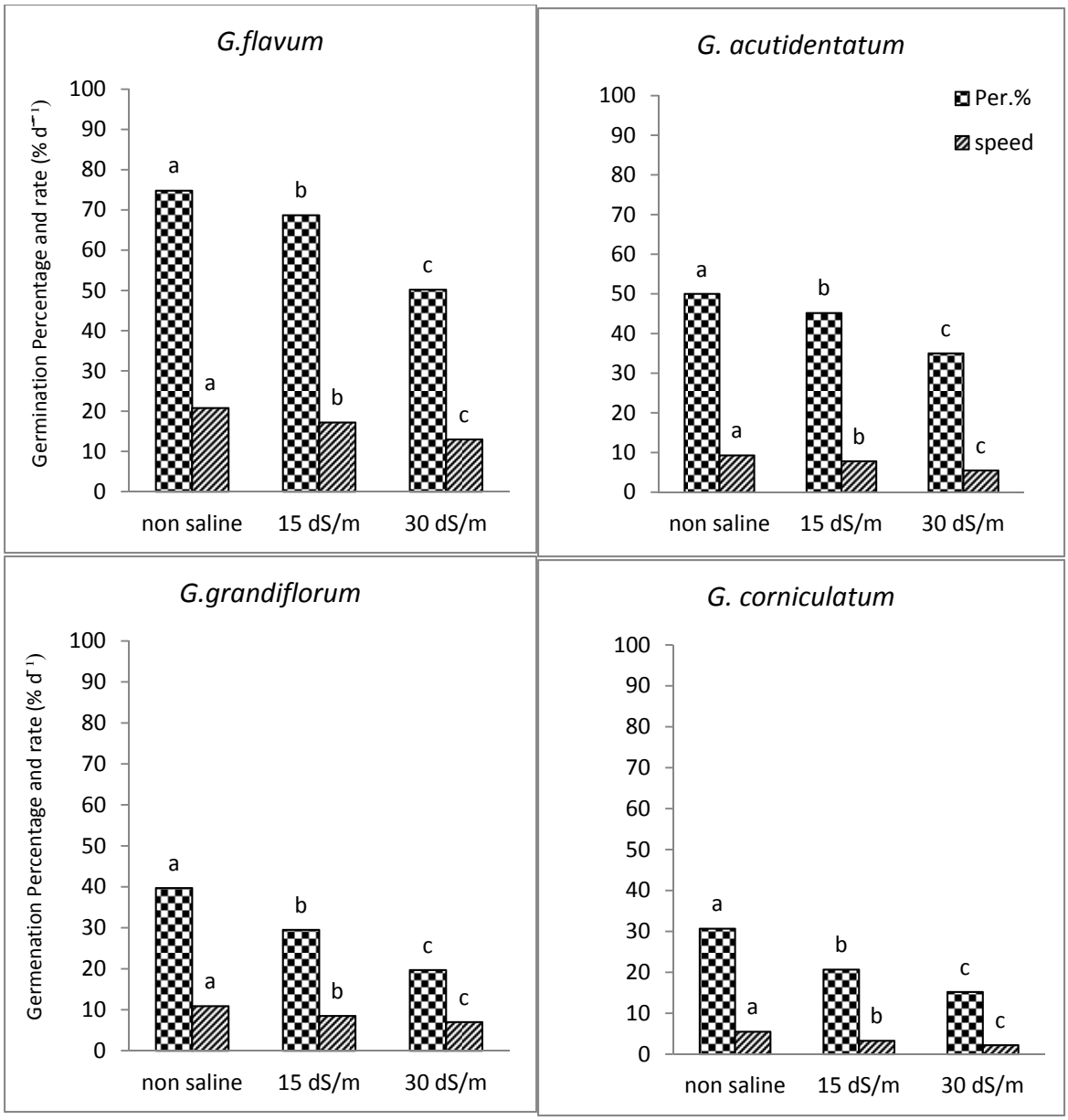

Figure 1. 1: Effect of different salinity levels on *Glaucium. spp*. seed germination percentage and rate . Columns labeled with different letters in either percentage or the rate are significantly different at P=0.05 with each salinity level.

from 7.0 to 9.5. *G. corniculatum* had the lowest germination percentage and rate even under the optimum thiourea concentration.The level of 30.0 mM of thiourea increased germination percentage at EC = 0.0 dS m^{-1} from 30.7 to 58.0%, at EC = 15 dS m^{-1} from 20.7 to 38.0%, and at EC = 30 dS m^{-1} from 15.2 to 30.0. Thiourea concentration of 30.0 mM had a similar trend of effect on germination rate (Table 1-3). The level of 30.0 mM of thiourea increased germination speed at EC = 0.0 dS m^{-1} from 5.5 to 14.5%, at EC = 15 dS m^{-1} from 3.3 to 7.5, and at EC = 30 dS m^{-1} from 2.2 to 5.0. The role of thiourea in alleviating salinity effects on seed germination has been well established in many halophytes (Khan & Gul, 2006; Ungar, 1991 and 1995; Shahba et al., 2008). These results are consistent with previous investigations that demonstrated the effectiveness of thiourea in ameliorating salinity-induced inhibition of germination. Thiourea has alleviated the salinity induced dormancy in summer seeds of *Halopyrum mucronatum* (Khan and Ungar, 2001a), *Sporobolus arabicus* (Khan and Ungar, 2001b), *Salicornia rubra* (Khan et al., 2002), *Atriplex prostrata* (Khan et al., 2003), *Zigophyllum simplex* (Khan and Ungar, 1997), *Aeluropus lagopoides* (Gulzar and Khan, 2002), *Triticum aestivum* L. (Siddiqui et al., 2006) and *Disticlis spicata* Grene (Shahba et al., 2008). Thiourea has been used partially alleviated the germination inhibition under saline conditions in mesquite (El-Keblawy et al., 2005), seaside arrow grass (Khan and Ungar, 2001c), and iodine bush (Gul et al., 2000). The fact that thiourea stimulates seed germination and reduces the negative effects of salinity on germination indicates its importance as a compatible osmoregulator (Gul et al., 2000). Eashi et al. (1979) found that nitrogenous compounds such as thiourea could promote germination by acidification and softening of cell walls, or by activating the pentose phosphate pathway. Salinity causes a reduction in growth promotors (cytokinins and gibberellins) and increases in ABA in seeds (Kabar and Baltepe, 1990).

Table 1. 2. Effect of different concentrations of ethophon, fusicoccin, thiourea and kinetin on *Glaucium spp* seed germination percentage under different salinity levels

G. Regulator	Level	*G. acutidentatum*			*G. corniculatum*			*G.flavum*			*G.grandiflorum*		
		Salinity Level (dS/m)			Salinity Level (dS/m)			Salinity Level (dS/m)			Salinity Level (dS/m)		
		0	15	30	0	15	30	0	15	30	0	15	30
Thiourea	10 mM	60.7c	56.7b	45b	36.7c	20.7c	16.5c	78.8c	69.8b	53.2c	44.5c	35.3c	26.5c
	20 mM	61.2b	56.7b	45b	54.5b	38.5b	21.2b	84.3b	69.8ab	59.2b	60.5b	42.3b	29.5b
	30 mM	82a	60.5a	50.7a	58.0a	38.0a	30.0a	92.2a	73.8a	64.7a	72.2a	52.7a	32.5a
	Control	50d	45.2c	35c	30.7d	20.7c	15.2d	74.8d	68.7c	50.2d	39.7d	29.5d	19.7d
Fusicoccin	0.003mM	55.0c	40d	20.8d	26.8d	22.5b	15.5b	79.2c	68.7c	50.2c	40.7b	20.8d	17d
	0.005mM	57.7b	47.7b	25.8c	30.3c	20.5d	15d	84.3b	69.8b	50.7b	40.7b	30.0b	21.2b
	0.01 mM	80.3a	62.8a	45.0a	56.0a	36.0a	21.0a	91.3a	72.7a	51.3a	71.7a	50.7a	31.2a
	Control	50d	45.2c	35b	30.7b	20.7c	15.2c	74.8d	68.7c	50.2c	39.7c	29.5c	19.7c
Ethophon	3 mM	64.0c	50.5c	40.2b	46.8c	39.2b	20b	80c	69.2c	51.7c	51.8c	29.7c	20.7c
	5 mM	65.7b	51b	35.3c	50.7b	36.7c	17c	83b	74.5b	55.7b	60.7b	40.7b	35.0b
	10 mM	87.7a	66.8a	53.7a	70.0a	48.0a	25.0a	97.3a	77.5a	62.3a	76.3a	61.7a	42.5a
	Control	50d	45.2d	35d	30.7d	20.7d	15.2d	74.8d	68.7d	50.2d	39.7d	29.5d	19.7d
Kinetin	0.5 mM	55.2c	36.2d	31.7d	40.3c	24.7c	15d	76.3c	65.7d	49.2d	59.7c	43.8c	25.8bc
	1.0 mM	61.0b	46.2b	35.5b	41.3b	32b	17b	78.8b	69b	50.5b	61.7b	45.8b	27.8b
	1.5 mM	84.0a	64a	48.0a	60.7a	45.8a	22.5a	94.0a	72.2a	55.7a	73.7a	55.8a	35.0a
	Control	50d	45.2c	35c	30.7d	20.7d	15.2c	74.0d	68.7c	50.2c	39.7d	29.5d	19.7d

Values followed by the same letters within a column for each growth regulator are not significantly different (P=0.05).

Table 1. 3. Effect of different concentration of ethophon, fusicoccin, thiourea and kinetin on *Glaucium spp* seed germination rate under different salinity levels.

G. Regulator	Level	*G. acutidentatum*			*G. corniculatum*			*G.flavum*			*G.grandiflorum*		
		Salinity Level (dS/m)			Salinity Level (dS/m)			Salinity Level (dS/m)			Salinity Level (dS/m)		
		0	15	30	0	15	30	0	15	30	0	15	30
Thiourea	10 mM	13.2c	11b	7.4c	9.2c	6.5c	3.5c	22.8c	18c	14c	11.8c	10.1c	8.3c
	20 mM	13b	11b	7.4b	11.9b	7.2b	3.8b	26.2b	18.5b	14.6b	15.1b	11.4b	8.8b
	30 mM	19a	13a	8.5a	14.5a	7.5a	5a	29a	19.3a	17.2a	17.4a	13.6a	9.5a
	Con.	9.3d	7.8c	5.5d	5.5d	3.3d	2.2d	20.8d	17.2d	13d	10.9d	8.5d	7d
Fusicoccin	0.003mM	10.4c	5.5d	3.2c	4.5d	3.8b	2.5b	21.5c	17.6c	14c	11b	7.1d	6.1d
	0.005mM	11.2b	6.4b	3d	5c	2.4d	2d	25.2b	18.5b	14.1b	11b	9b	7.4b
	0.01mM	17.7a	11.8a	7.7a	12.8a	6.8a	5a	27.7a	19.1a	14.5a	17.4a	13.1a	9.2a
	Con.	9.3d	7.8c	5.5b	5.5b	3.3c	2.2c	20.8d	17.2d	13d	10.9c	8.5c	7c
Ethophon	3 mM	15.8c	8.5c	5.4d	8.3c	6.4c	2.7b	21.3c	18.3c	14c	13.4c	8.8c	7.5c
	5 mM	16.3b	8.8b	6.2b	9b	7.5b	2.7b	24b	19.4b	15.2b	15b	11b	9b
	10 mM	23a	14.6a	12a	18.2a	8.8a	4.5a	31a	20.3a	17.7a	19.2a	15.3a	11.5a
	Con.	9.3d	7.8d	5.5c	5.5d	3.3d	2.2c	20.8d	17.2d	13d	10.9d	8.5d	7d
Kinetin	0.5 mM	10.7c	6d	4.9d	7.8c	2.7d	1.6c	20.8c	15d	11.8d	14.8c	11c	8.1c
	1.0 mM	13.5b	8b	5.4c	8b	3.8b	2.2b	22b	17.7b	12.4c	15.1b	11.9b	8.8b
	1.5 mM	21a	15.7a	10a	15.3a	8.1a	4a	29.3a	18.8a	15.3a	17.6a	14.1a	10a
	Con.	9.3d	7.8c	5.5b	5.5d	3.3c	2.2b	20.8c	17.2c	13b	10.9d	8.5d	7d

Values followed by the same letters within a column for each growth regulator are not significantly different (P=0.05).

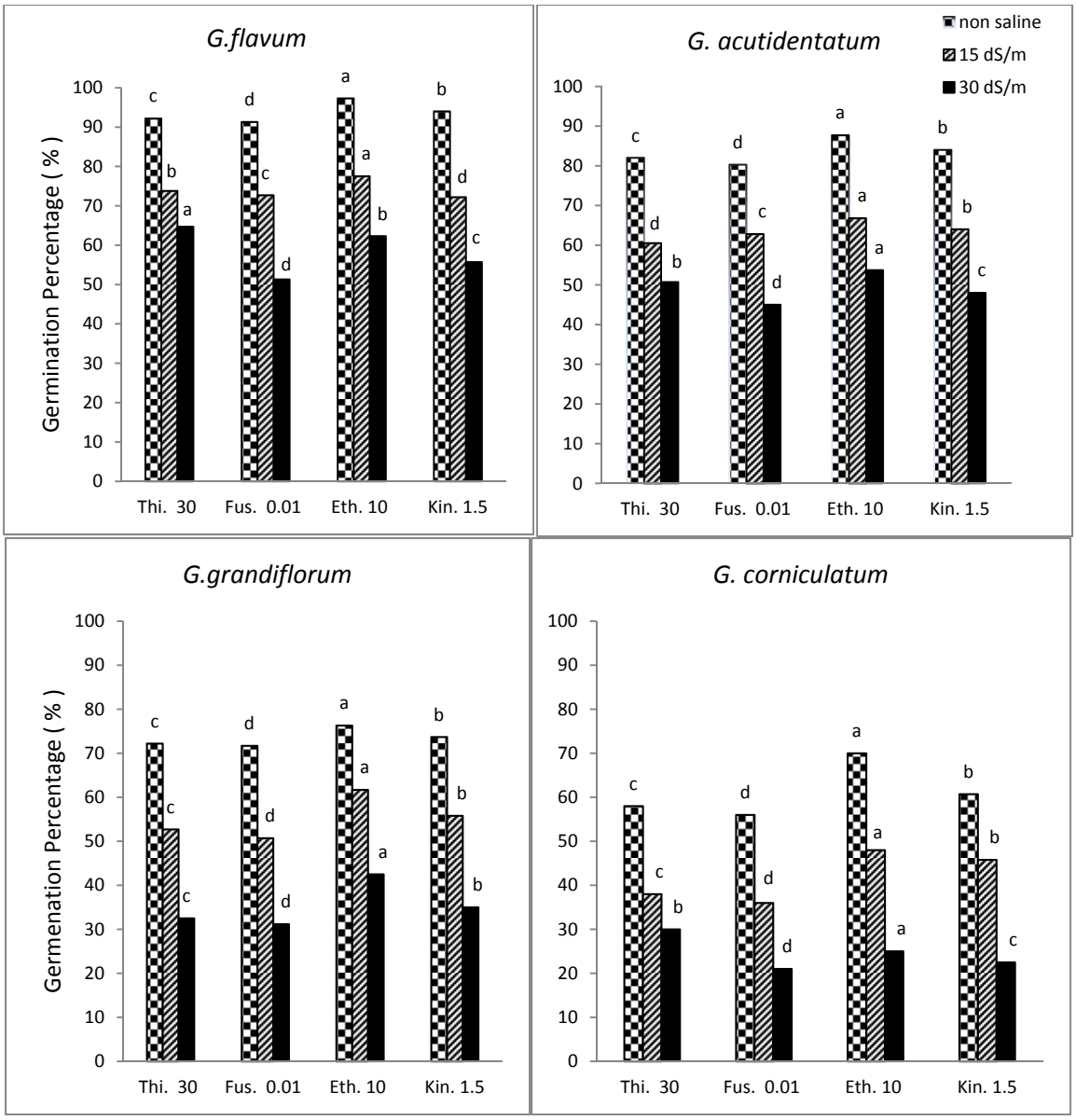

Figure 1. 2: Effect of different plant growth regulaters (Thiourea 30 mM, Fusicoccin 0.01 mM , Ethophon 10 mM , Kinetin 1.5 mM) on *Glaucium. spp*. seed germination percentage under different levels of salinity . Columns labeled with different letters are significantly different at P=0.05 within each salinity level.

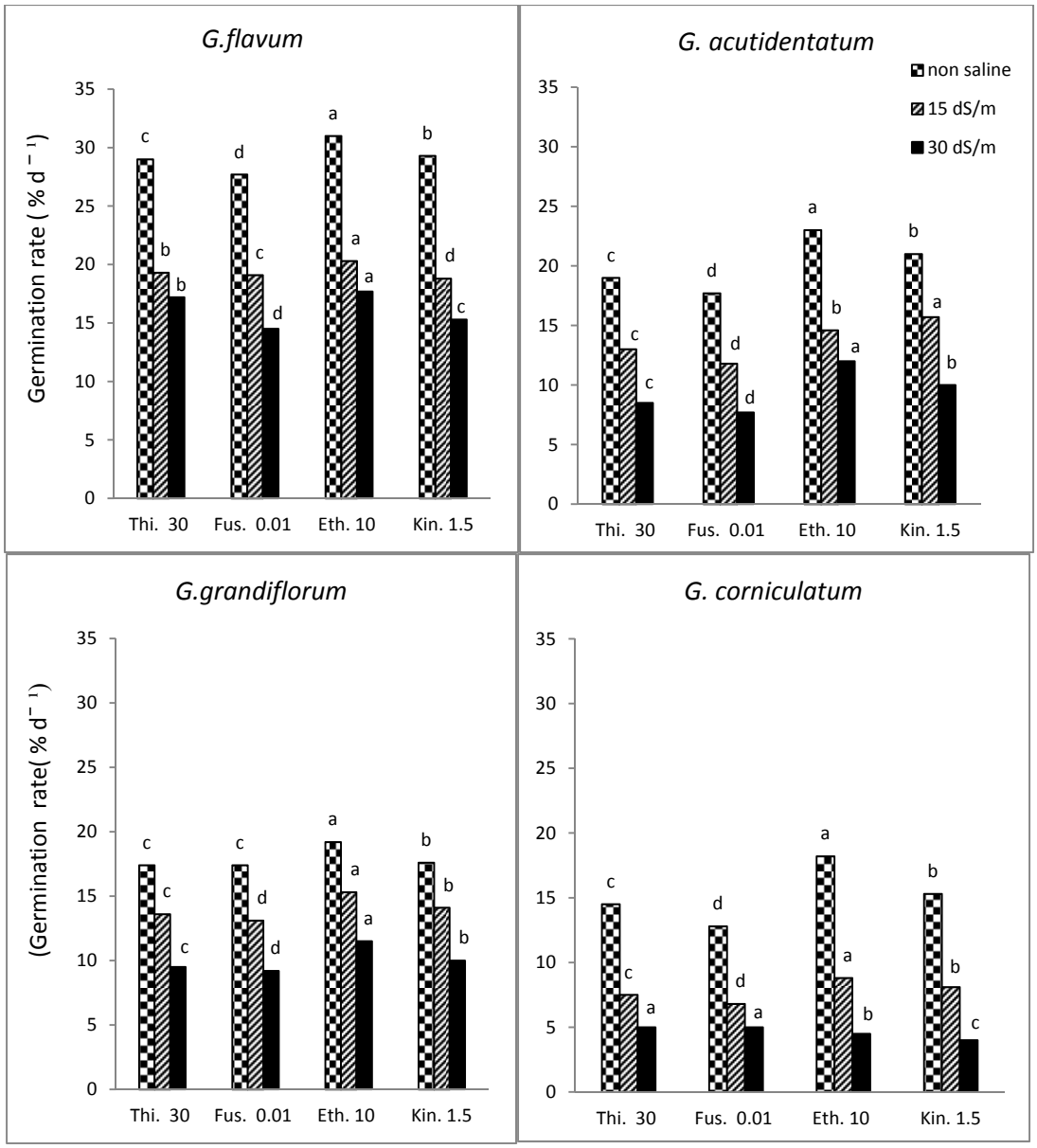

Figure 1. 3: Effect of different plant growth regulaters (Thiourea 30 mM, Fusicoccin 0.01 mM , Ethophon 10 mM , Kinetin 1.5 mM), on germination rate of *Glaucium. spp*. under different levels of salinity . Columns labeled with different letters are significantly different at P=0.05 within each salinity level.

Thiourea may counteract this effect, controlling the adverse changes through a balance of hormonal promotors and inhibitors.

Germination percentage (Fig.1-2) and rate (Fig. 1-3) of *Glaucium ssp*. were significantly improved by the presence of fusicoccin in the germination solution on the blotter paper at all salinity levels. Analysis of variance and means separation test indicated that the level of 0.01 mM of fusicoccin was the optimum level of those tested in ameliorating the effect of salinity on *Glaucium ssp*. seed germination at all salinity levels. It increased germination percentage from 74.8 to 91.3, from 68.7 to 72.7 and from 50.2 to 51.3 in the control treatment, at EC = 15 dS m^{-1} and at EC = 30 dS m^{-1} respectively in *G. flavum* (Table 1-2). Fusicoccin concentration of 0.01mM similarly affected on germination rate (Table 1-3). At a level of 0.01 mM fusicoccin increased germination rate at EC = 0.0 dS m^{-1} from 20.8 to 27.7%, at EC = 15 dS m^{-1} from 17.2 to 19.1, and at EC = 30 dS m^{-1} from 13.0 to 14.5. In *G. acutidentatum,* the level of 0.01 mM was also optimum in improving seed percentage and rate (Tables 1-2 and 1-3). At the control treatment, germination percentage increased from 50.0 to 80.3%, while at EC = 15 dSm^{-1} it increased from 45.2 to 62.8%, and at EC = 30 dS m^{-1} from 35.0 to 45.0% (Fig. 1-2). Fusicoccin demonstrated a similar effect on germination rate. The level of 0.01 mM achieved the greatest germination rate followed by 0.005 mM and 0.003 mM. The level of 0.01 mM of fusicosccin increased germination rate of *G. acutidentatum* at EC = 0.0 dS m^{-1} from 9.3 to 17.7, at EC = 15 dS m^{-1} from 7.8 to 11.8, and at EC = 30 dS m^{-1} from 5.5 to 7.7 (Fig. 1-3). In *G. grandiflorum*, the level of 0.01 mM of fusicoccin again achieved the greatest increase in germination percentage (Table 1-2) and rate (Table 1-3) followed by the level of 0.005 mM and the level of 0.003 mM. The level of 0.01 mM increased germination percentage at EC = 0.0 dS m^{-1} from 39.7 to 71.7%, at EC = 15 dS m^{-1} from 29.5 to 50.7%, and at EC = 30 dS m^{-1} from 19.7 to

31.2% (Fig. 1-2). Once again, the level of 0.01 mM of fusicoccin on increasing germination rate at EC = 0.0 dS m^{-1} from 10.9 to 17.4, at EC = 15 dS m^{-1} from 8.5 to 13.1, and at EC = 30 dSm^{-1} from 7.0 to 9.2 (Fig. 1-3). *G. corniculatum* had the lowest germination percentage and rate under all fusicoccin treatments (Table 1-2). The level of 0.01 mM of fusicoccin increased germination percentage at EC = 0.0 dS m^{-1} from 30.7 to 56.0%, at EC = 15 dS m^{-1} from 20.7 to 36.0%, and at EC = 30 dS m^{-1} from 15.2 to 21.0 in *G. corniculatum* (Fig. 1-2). Fusicoccin concentration of 0.01 mM similarly effected germination rate (Table 1-3). An increased germination rate at EC = 0.0 dS m^{-1} from 5.5 to 12.8%, at EC = 15 dS m^{-1} from 3.3 to 6.8, and at EC = 30 dS m^{-1} from 2.2 to 5.0 (Fig. 1-3).

Fusicoccin contains three fused carbon rings and another ring which contains an oxygen atom and five carbons. Alleviation of salinity effect on seed germination by fusicoccin has been reported in many halophytes such as *Zygophyllum qatarensis* Hadidi (Ismail, 1990), Utah pickleweed (Gul and Khan, 2003), triangle orache (*Atriplex prostrata* Boucher ex DC.) (Khan et al., 2003) and *Disticlis spicata* Grene (Shahba et al., 2008). Conversely, El-Keblawy et al. (2005) reported the failure of fusicoccin to stimulate germination in mesquite. Fusicoccin may stimulate ATPase during the early phases of germination to facilitate proton extrusion and K+ uptake (Marre, 1979). Cocucci et al. (1990) studied the response of radish (*Raphanus sativus* L.) seeds to osmotic medium and fusicoccin during the early germination stages and indicated that fusicoccin counteracted the inhibitory effect of salinity in the medium by enhancing H+ extrusion and synthesis of malic acid. Lutsenko et al. (2005) suggested that fusicoccin affects the ionic balance, especially the K+/Na+ ratio, aiding in ionic homeostasis in seed and embryo. Salinity stress enhances ABA production, which has an inhibitory effect on seed germination. Fusicoccin has been reported to remove the inhibitory effect of ABA on seed germination by

accelerating development and by replacing the requirements for light and endogenous hormones in breaking dormancy (Lado et al., 1975).

Ethephon (2-chloroethyl phosphonic acid), significantly improved germination percentage (Fig. 1-2) and germination rate (Fig. 1-3) at all salinity levels. Ethephon at 10.0 mM was the optimum concentration of those tested in relieving the inhibitory effects of salinity on *Glaucium ssp*. germination percentage (Table 1-2) and rate (Table 1-3). The level of 10.0 mM of ethephon increased germination percentage at EC = 0.0 dS m^{-1} from 74.8 to 97.3%, at EC = 15 dS m^{-1} from 68.7 to 77.5%, and at EC = 30 dS m^{-1} from 50.2 to 62.3% in *G. flavum* (Fig. 1-2). Ethephon concentration of 10.0 mM had a similar effect on germination rate (Fig. 1-3). In *G. flavum*, the level of 10.0 mM of ethephon increased germination rate at EC = 0.0 dS m^{-1} from 20.8 to 31.0%, at EC = 15 dS m^{-1} from 17.2 to 20.3, and at EC = 30 dS m^{-1} from 13.0 to 17.7. In *G. acutidentatum,* the level of 10.0 mM was also the best in improving seed percentage and rate. At EC = 0.0 dS m^{-1}, germination percentage increased from 50.0 to 87.7%, at EC = 15 dS m^{-1} from 45.2 to 66.8%, and at EC = 30 dS m^{-1} from 35.0 to 53.7%. The level of 10 mM achieved the highest germination rate followed by 5.0 mM and 3.0 mM (Table 1-3). The level of 10.0 mM of ethephon also increased germination rate of *G. acutidentatum* at EC = 0.0 dS m^{-1} from 9.3 to 23.0, at EC = 15 dS m^{-1} from 7.8 to 14.6, and at EC = 30 dS m^{-1} from 5.5 to 12.0. In *G. grandiflorum,* the level of 10.0 mM of ethephon once again achieved the greatest increase in germination percentage (Table 1-2) and rate (Table 1-3) followed by the level of 5.0 mM and the level of 3.0 mM. The level of 10 mM increased germination percentage at EC = 0.0 dS m^{-1} from 39.7 to 71.7%, at EC = 15 dS m^{-1} from 29.5 to 50.7%, and at EC = 30 dS m^{-1} from 19.7 to 31.2. In *G. grandiflorum* , the level of 10.0 mM of ethephon increased germination rate at EC = 0.0 dS m^{-1} from 10.9 to 17.4, at EC = 15 dS m^{-1} from 8.5 to 13.1, and at EC = 30 dSm^{-1} from 7.0 to 9.2.

G. corniculatum had the lowest germination percentage and rate even under the optimum ethephon concentration. The level of 10.0 mM of ethephon increased germination percentage at EC = 0.0 dS m^{-1} from 30.7 to 70%, at EC = 15 dS m^{-1} from 20.7 to 48.0%, and at EC = 30 dS m^{-1} from 15.2 to 30.0 (Fig. 1-2). Ethephon concentration of 10.0 mM had a similar trend of effect on germination rate (Fig. 1-3). In *G. corniculatum,* the level of 10.0 mM of ethephon increased germination rate at EC = 0.0 dS m^{-1} from 5.5 to 18.2, at EC = 15 dS m^{-1} from 3.3 to 8.8, and at EC = 30 dS m^{-1} from 2.2 to 5.0.

In research on the role of ethephon on seed germination, it reduced dormancy in the seeds of several species the rough a reversal of the inhibitory effect of abscisic acid (ABA) and osmotic stress was (Schonbeck and Egley, 1981; Gulzar and Khan, 2002; Gul and Khan, 2003, Shahba et al., 2008). Ethephon significantly ameliorated the effect of salinity, ranging from 36 to 54 dS m–1 in iodine bush [*Allenrolfea occidentalis* (S. Wats.) Kuntze] (Gul and Weber, 1998), Utah pickleweed (Gul and Khan, 2003), and dropseed (*Sporobolus ioclados* Nees ex Trin) (Gulzar and Khan, 2002).However, the effectiveness of ethephon in ameliorating salinity-induced dormancy is variable among plant species. It partially alleviated salinity induced dormancy in *Arthrocnemum indicum* (Wild.) Moq. (Khan et al., 1998) and saltwort (*Salicornia rubra* A. Nels.) (Khan et al., 2002). In contrast, it had no effect on seed germination of seaside arrow grass (*Triglochin maritima* L.) under various salinity level (Khan and Ungar, 2001c).

Kinetin treatments enhanced *Glaucium ssp* seeds germination percentage (Table 1-2) and rate (Table 1-3) under all salinity levels. Analysis of variance and mean separation tests indicated that the level of 1.5 mM of kinetin was the optimum of those tested in improving seed germination percentage (Fig. 1-2) and rate (Fig. 1-3) under all salinity levels. Kinetin at 1.5 mM treatment of *G. flavum* increased germination percentage from 74.8 to 94.0, from 68.7 to 72.2

and from 50.2 to 55.7 in the control, at EC of 15 dS m^{-1}, and at EC = 30 dS m^{-1} respectively in *G. flavum* (Fig. 1-2). The Kinetin concentration of 1.5 mM had a similar effect on germination rate (Fig. 1-3), in *G. flavum*. It increased germination rate at EC = 0.0 dS m^{-1} from 20.8 to 29.3%, at EC = 15 dS m^{-1} from 17.2 to 18.8, and at EC = 30 dS m^{-1} from 13.0 to 15.3. In *G. acutidentatum,* the level of 1.5 mM was again the optimum in improving seed germination percentage and rate (Tables 1-2 and 1-3). The control treatment germination percentage increased from 50.0 to 84.0%, at EC = 15 dS m^{-1} from 45.2 to 64.0%, and at EC = 30 dS m^{-1} from 35.0 to 48.0% (Fig. 1-2). Kinetin had a similar effect on germination rate in this species with 1.5 mM with the greatest effect followed by 1.0 mM and 0.5 mM. The level of 1.5 mM of kinetin increased germination rate of *G. acutidentatum* at EC = 0.0 dS m^{-1} from 9.3 to 21.0, at EC = 15 dS m^{-1} from 7.8 to 15.7, and at EC = 30 dS m^{-1} from 5.5 to 10.0 (Fig. 1-3). In *G. grandiflorum*, the level of 1.5 mM of kinetin again achieved the greatest increase in germination percentage (Table1- 2) and rate (Table1- 3) followed by the level of 1.0 mM and the level of 0.5 mM. The level of 1.5 mM increased germination percentage at EC = 0.0 dS m^{-1} from 39.7 to 73.7%, at EC = 15 dS m^{-1} from 29.5 to 55.8%, and at EC = 30 dS m^{-1} from 19.7 to 35.0% (Fig. 1-2). It's also increased germination rate in *G. grandiflorum* at EC = 0.0 dS m^{-1} from 10.9 to 17.6, at EC = 15 dS m^{-1} from 8.5 to 14.1, and at EC = 30 dSm^{-1} from 7.0 to10.0 (Fig. 1-3). *G. corniculatum* had the lowest germination percentage and rate under all kinetin treatments (Table 1-2). The level of 1.5 mM of kinetin did however increase germination percentage at EC = 0.0 dS m^{-1} from 30.7 to 60.7%, at EC = 15 dS m^{-1} from 20.7 to 45.8 0%, and at EC = 30 dS m^{-1} from 15.2 to 22.5 (Fig. 1-2). The Kinetin concentration of 1.5 mM had a similar effect on germination rate (Table 1-3). Where it increased rate EC = 0.0 dS m^{-1} it from 5.5 to 15.3%, at EC = 15 dS m^{-1} from 3.3 to 8.1, and at EC = 30 dS m^{-1} from 2.2 to 4.0 (Fig.1-3).

Kinetin has been demonstrated to ameliorate the salinity-induced germination inhibition in Utah pickleweed (Gul and Khan, 2003), *Brassica campestris* L. (Ozturk et al., 1993), *Zygophyllum simplex* L. (Khan and Ungar, 1997), *Halopyrum mucronatum* (Khan and Ungar, 2001a), *Salicornia rubra* (Khan et al., 2002), and *Disticlis spicata* Grene (Shahba et al., 2008). It has also been shown to partially ameliorate salinity inhibitory effects on seed germination of mesquite (El-Keblawy et al., 2005), seaside arrow grass (Khan and Ungar, 2001c), and *Aeluropus lagopoides* (L.) Trin. ex Thw. (Gulzar and Khan, 2002). However, it had no effect on the salinity-induced dormancy in *Sporobolus ioclados* Nees ex Trin and *Urochondra setulosa* Trin (Gulzar and Khan, 2002), *Salicornia pacifica* Standl. (Khan and Weber, 1986), *Zygophyllum qatarense* (Ismail, 1990), *Sporobolus arabicus* Boiss (Khan and Ungar, 2001b), *Cressa cretica* L. and *Suaeda fruticosa* auct. non Forsk. *Salsola imbricate* Forssk. and *Haloxylon stocksii* Boiss. (Gulzar and Khan, 2002; 2003). Khan and Ungar (2001a) suggested that the addition of kinetin likely overcomes the deficiency in growth-promoting substances that are inhibited in salt-stressed seeds. The increase in seed germination under high salinity after exogenous application of kinetin was attributed to the ability to enhance water uptake during germination (Sastry and Shekhawat, 2001). Miller (1961) suggested that kinetin eff ect on the breaking of dormancy and promotion of seed germination may result from its combination of influences on cell division and enlargement. Also, kinetin enhances the biosynthesis of ethylene. As discussed previously, ethylene or ethylene-releasing compounds enhance germination when seeds are exposed to salt stress.

Analysis of variance indicated a significant difference among tested chemicals at EC = 0.0 dS m^{-1} , EC = 15 dS m^{-1} and at EC = 30 dS m–1, (Table1-1), in their effect on enhancing *Glaucium ssp* seed germination percentage and germination rate . In conclusion, the tested

species varied significantly in their variation in germination percentage and rate. *Glaucium flavum* achieved the greatest germination percentage and rate under all salinity levels followed by *G. acutidentatum* and *G. grandiflorum* while *G. corniculatum* had the lowest germination percentage and rate. The effect of growth regulators varied from species to species and from one salinity level to another. The current investigation showed that 30 mM thiourea, 0.01 mM fusicoccin, 10.0 mM ethephon, and 1.5mM kinetin increased seed germination percentage and rate of *Glaucium ssp* under saline conditions. Ethephon was the most effective growth regulator in ameliorating salinity effect on *Glaucium ssp* seed germination followed by kinetin, thiourea, and fusicoccin. All tested growth regulators had similar positive effects at the highest salinity level (EC = 30 dS m^{-1}). More research is needed to develop appropriate protocols for practical and effective treatment procedures for landscape use restoration of saline areas.

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CHAPTER 2

COMPARATIVE RESPONSES OF *GLAUCIUM SPP.* TO SALINITY STRESS

SUMMARY

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) *e*xamine 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 tab water (control) to EC levels of 5,15, and 25 dS m^{-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. grandiflorun*), and 3.2 (*G. corniculatum*) at the salinity level of 25 dS m^{-1}. 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*. The average leaf area of *G.flavum* when grown with tap water was 23 cm^2 while the *G. acutidentatum* averge leaf area was 21.3 $cm^{2.}$. *G.grandiflorum* and *G. corniculatum* averge leaf area was 20.8 cm^2 with tab 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 decreased by 15.7, 28.6 and 43.6% while the average TNC decrease in *G. acutidenatum* shoots was 17.6, 36.8 and 48%. The decrease in *G. grandflorum* 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 roline 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. grandflorum*, 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. acutidenatum*, *G. grandflorum* 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.

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 (Rameeh el al., 2012). Increased use of brackish water and wastewater (effluent, recycled, or reclaimed water) has enhanced interest in the development of more salt tolerant landscape plants (Carrow and Duncan, 1998; Marcum et al., 1998). The detrimental effects of salinity on plant growth include osmotic stress, ion toxicity, nutritional disturbances (Greenway and Munns, 1980; Lauchli, 1986; Cheeseman, 1988), damage to photosynthetic systems by excessive energy (Brugnoli and Bjorkman, 1992), and structural disorganization (Flowers et al., 1985; Delfine et al., 1998; Romero-Aranda et al., 1998). 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 (Levitt, 1980). 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 (Harivandi et al., 1992).

Plant species and cultivars within a species vary in their drought and salinity tolerance (Epstein et al., 1980; Pasternark, 1987; Saranga et al., 1992). These variations are due to variations in genes relating to stress tolerance mechanisms and their interaction with the environments (Shanon, 1985; Bohnert et al., 1995; Igartua, 1995; Duncan and Carrow, 1999).

Horned Poppies (*Glaucium* spp) are members of the Poppy family, Papaveracea 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 18oC. 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. Stems of Horned Poppy branch and form a rosette of leaves. The crinkly, gray-green leaves also appear on the stems and below each flower. The golden-yellow flowers may be up to 5 cm in diameter. There are also orange or red flowers. The roots of the horned poppy are considered poisonous.

All horned poppies have blue-green foliage that is deeply pinnatified 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 sublyrate 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 completely 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 (Grey-Wilson, 2000). 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 (1965), *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 (Davis, 1965).

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 (Grey-Wilson, 2000). 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 (Davis, 1965). 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 & Bornm is endemic to Turkey where it is found on dry hillslopes and rocky places (Grey-Wilson, 2000). *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 (Davis, 1965).

G.corniculatum (L.) J.H. Rudolph is native to the Mediterranean basin, Atlantic islands, Caucasus Mountains, Bulgaria, Romania, northern Iraq and northwestern Iran (Grey-Wilson, 2000; Davis, 1965). *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 red (Davis, 1965) with a black basal spot (Grey-Wilson, 2000).

The balance between carbohydrate production and consumption will impact the ability of a plant species to cope with salinity stress (Huang and Fry, 1999; Lee et al., 2008a, 2008b) . 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 (Qian and Fu, 2005; Lee et al., 2007; Shahba, 2010b, shahba, 2012).

Proline accumulates in larger amounts than other amino acids in salt stressed plants (Lee et al., 2008b). Proline accumulation is the first response of plants exposed to salt stress and water-deficit stress and is though to reduce injury to cells (Ashraf and Foolad 2007). Maggio et al. (2002) 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 (Flowers et al., 1977; Levit, 1980). Ahmad et al. (1981) 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. (2008b) 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 Rice (1986) 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 NaC1 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 (Ashraf and Harris, 2004). 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. acutidentatums;* 2) *e*xamine the effects of salinity on plant characteristic that are associate with the aesthetics of Horned Popy; and 3) determine which tolerance mechanism are 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 Biofunglcide). 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 Biofunglcide). 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 expermiments 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 dS m^{-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 thought a screen with 425 μm openings, and kept in airtight vials at –

20 ^{o}C. TNC was measured using the method described by Chatterton et al. (1987). 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 ^{o}C for 24 h. Dried shoots were ground in a Wiley mill and passed through a screen with 425 μm openings. Approximately 1 g of dried and screened sample was weighed and ashed for 7 h at 500 ^{o}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. (1973) as modified by Torello and Rice (1986) 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 ^{o}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 (SAS Institute, 2006). 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 2-1). 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. grandiflorun*), and 3.2 (*G. corniculatum*) at the salinity level of 25 dS m^{-1} (Fig. 2-1). Leaf data from other species showed similar responses to the increasing salinity.

Table 2. 1. Analysis of variances with mean square and treatment significance of leaf color, leaf area, plant height, number of buds, number of flowers, flower are, 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 (mg g^{-1} dry wt)	1690**	1771**	2895*
RSC (mg g^{-1} dry wt)	47.0**	892.0**	521.0*
Proline content (µg g^{-1} fresh wt.)	1870**	2997**	1897*

* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.

Table 2. 2. Linear regression of different parameters of *Glaucium spp.* measured at the end of the experiment vs. salinity levels, Control (c), 5, 15, 25 dS m^{-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
G. acutidentatum	Y = 6.20 – 0.22 X	0.84**	Y = 122.5 – 1.22 X	0.88**	Y = 320.5 – 7.1 X	0.80**
G. corniculatum	Y = 5.06 – 0.33 X	0.68*	Y = 131.2 – 1.16 X	0.89**	Y = 303.3 – 2.8 X	0.75*
G. flavum	Y = 8.6 – 0.16 X	0.92**	Y = 122.8 – 1.32 X	0.83**	Y = 313.3 – 0.8 X	0.94**
G. *grandiflorum*	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 $P \leq 0.05$.
** Significant at $P \leq 0.01$.

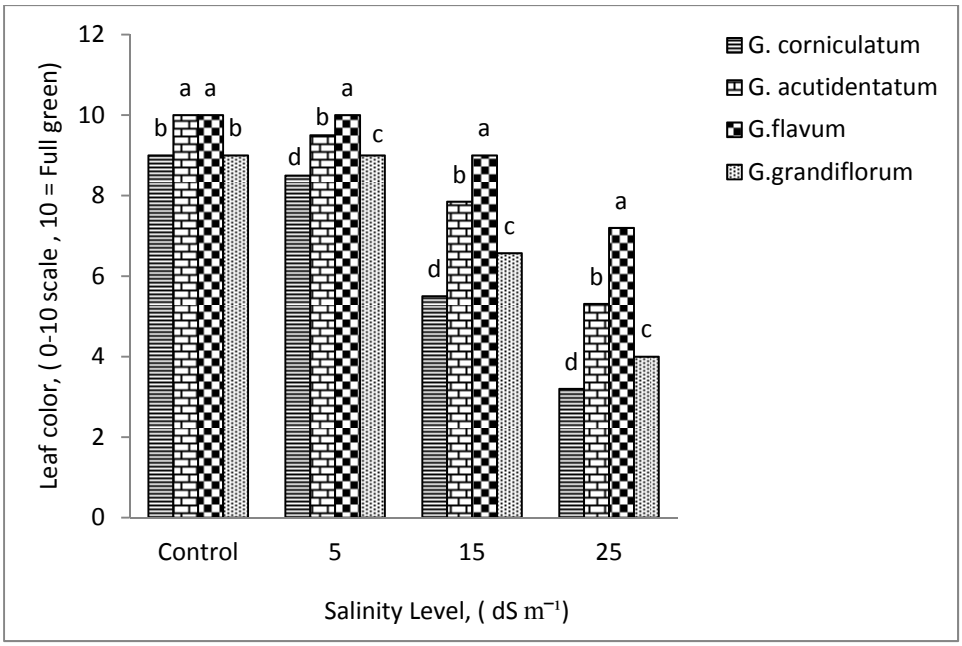

Figure 2. 1: Effect of different salinity levels on leaf color of four *Glaucium spp*. Columns labeled with different letters are significantly different at P=0.05 within each salinity level.

Bayat et al. (2012) found a 61% decrease in chlorophyll index as a result of 300 mM NaCl in Persian petunia (Bayat et al. , 2012). The upper leaves of butterhead lettuce had a dark-green color, while lower leaves showed chlorosis under high salinity (Choi and Lee, 1999). It seemed that salinity targeted chlorophyll formation processes which resulted in pale or yellow leaves (Kubis et al., 2004). 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 (Murkute et al., 2006; Levitt, 1980; Jaleel et al., 2008). Salt stress opens porphyrin rings and through fusion, harmful solutes are transfered to the vacuole. The presence of these solutes results in the loss of the green color of leaves (Parida and Das, 2005) and ultimately reduces the chlorophyll concentration in the leaf (El-Desouky and Atawia, 1998; Aggarwal, et al., 2012; Enteshari and Hajbagheri, 2011). The interference of salt ions on the newly synthesized protein molecules has another negative effect on the chlorophyll biosynthesis (Jaleel et al., 2008). Salinity affects turfgrasses and other landscape plant by reducing their value (Fu et al., 2005; Francois, 1988; Dudeck et al., 1983; Marcum and Murdoch, 1990, Shahba, 2010a, 2010b; Marcum, 2001).

Leaf area. Analysis of variance indicated significant differences among species and among salinity levels and their interactions (Table 2-1). Linear regression indicated a significant negative association between leaf area and salinity levels (Table 2-2). 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* acheived an average leaf area of 23 cm^2 while *G. acutidentatum* achieved an averge leaf area of 21.3 cm^2 and with both *G.grandiflorum* and *G. corniculatum* with a leaf area of 20.8 cm^2 in the control treatment (Fig.2-2). Leaf area decreased

from 23 to 20, 17.6 and 10.8 cm^2 in *G. flavum*, from 21.3 to 18.8, 14.3, and 8.2 cm^2 in *G. acutidentatum*, from 20.8 to18.2, 11.8, and 6 cm^2 in *G.grandiflorum*, and from 20.8 to 18.1, 10.9, and 4.5 cm^2 in *G. corniculatum* when the salinity increased from the control to 5, 15 and 25 dSm^{-1}, respectively (Fig. 2-2). 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 area (Abdul Jaleel et al., 2007) in other species. Continuous exposure to elevated root-zone salinity progressively decreased leaf size over time (Munns et al., 1988; Volkmar, et al., 1998). The decline in leaf growth was the earliest response of exposure to salinity stress (Munns and Termaat, 1986; Chartzoulakis and Klapaki, 2000). Plants cope with salinity stress by decreasing their leaf area to conserve energy (Jaleel et al., 2008). Leaf area decreased gradually with increasing salinity in *Withania somnifera* under salt stress (Jaleel et al., 2008), and in *Salvodora persica* (Dagar, et al. 2004; Jaleel et al., 2008). Salinities above 25 mM resulted in a decrease in leaf area in mangrove species (Clough, 1984) 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 (Srivastava and Jefferies, 1995; Warwick and Bailey, 1998; Su´arez and Medina, 2005). 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,(Volkmar,etal.,1998).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 (Munns and Tester, 2008).

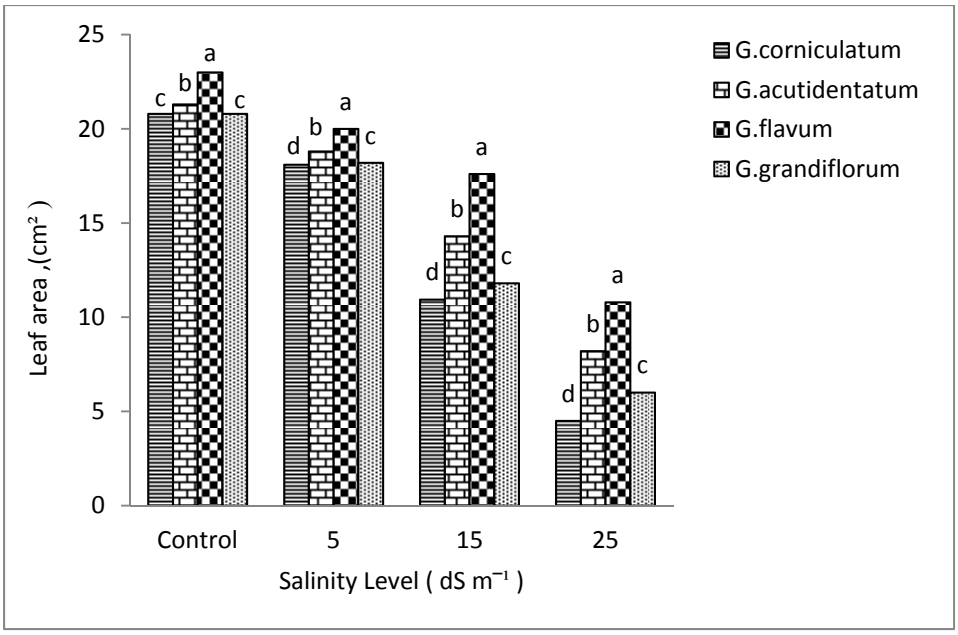

Figure 2. 2: Effect of different Salinity levels on leaf area of four *Glaucium spp*. Columns labeled with different letters are significantly different at P=0.05 within each salinity level.

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 2-1). *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 (Fig.2-3). 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 (Fig. 2-3). 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 dS m^{-1}, respectively (Fig. 2-3). Several reports have demonstrated the negative effects of salinity on plant height (Ungar, 1996; Razmjoo et al., 2008; Chartzoulakis and Klapaki, 2000; Rameeh et al., 2012; Bybordi, 2010; Greenway and Munns, 1980) in various plant species. Studies on *Brassica* have shown severe reduction in plant height due to salinity (Rameeh et al., 2012). In Brassica, increasing salinity levels from 0 to 12 dS m^{-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 (Rameeh et al., 2012). Similarly, significant variations of impact of increasing salinity were reported for rapeseed cultivars including interaction of salinity-cultivars for plant height (Bybordi, 2010; Rameeh, et al, 2012). The reduction in growth parameters

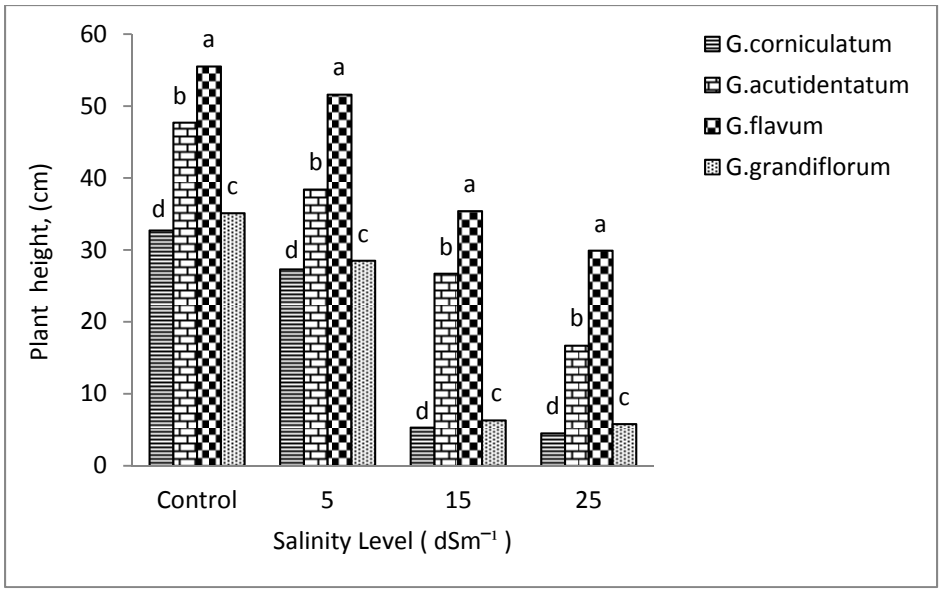

Figure 2. 3: Effect of different Salinity levels on the height of four *Glaucium spp*. Columns labeled with different letters are significantly different at P=0.05 within each salinity level.

such as height could be attributed to several effects such as the osmotic stress and /or ionic toxicity (Greenway and Munns, 1980) which is more harmful to plants during the succulent seedling stage in addition to the stressful effects of ion uptake (Dumbroff and Cooper, 1974; Chartzoulakis and Klapaki, 2000). Salinity stress favors the growth of roots rather than shoots which results in a decrease in plant height. Marcum (1999) 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 salt tolerant grasses as well (Dudeck et al., 1983; Peacock and Dudeck, 1985). Fu et al. (2005) 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}. Shahba (2010b) and Shahba et al. (2012) 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 Visser (1981) 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*.

Flowering characteristics:

Number of flower buds. Numbers of flower buds varied significantly among *Glaucium spp*., salinity levels and their interaction (Table 2-1). Increased salinity levels resulted in fewer flower buds (Fig. 2-4). 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 25 dS m^{-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 wih 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) (Fig.2-4).

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 2-1). 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* (Fig. 2-5). *G.flavum* produced the greatest number of flower under all salinity levels when compared to the other species (Fig. 2-5). *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 flowers at the salinity levels of 15 and 25 dS m^{-1} (Fig.2-5).

Flower area. Comparisons of flower area among species and among salinity levels and their interaction clearly showed significant differences (Table 2-1). Flower area decreased linearly with increasing salinity level. Regressions were strongly linear, with slope more negative with less tolerant species (Table 2-2). At the control treatment, flower area was the greatest in *G. flavum* (23 cm^2) followed by *G. acutidentatum* (17 cm^2), and *G. grandiflorum* (10.5 cm^2). *G. corniculatum* had the smallest total flower area at the control treatment (8.9 cm^2).

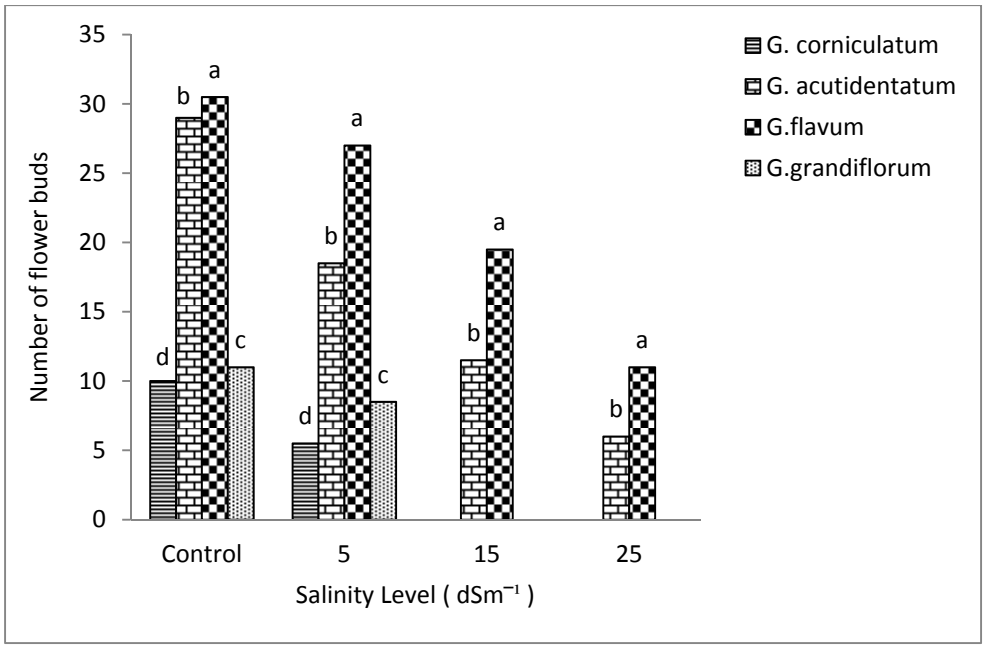

Figure 2. 4: Effect of different Salinity levels on number of flower buds of four *Glaucium spp*. Columns labeled with different letters are significantly different at P=0.05 within each salinity level.

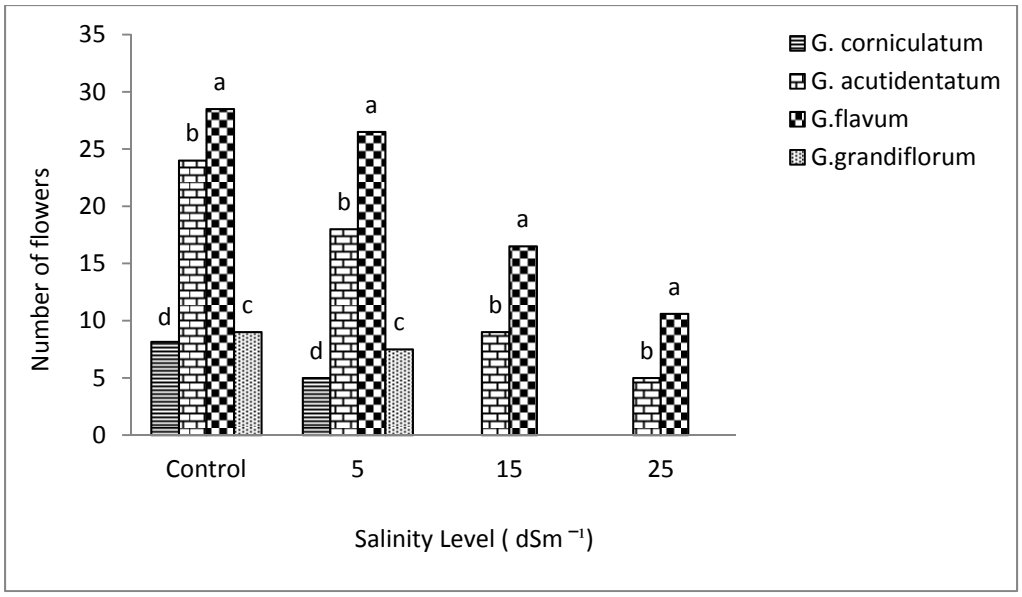

Figure 2. 5: Effect of different Salinity levels on the number of flowers of four *Glaucium spp.* Columns labeled within different letters are significantly different at P=0.05 within each salinity level.

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 salinitry and was moderate in *G.flavum* and *G. acutidentatum* (Fig. 2-6). *G.flavum* showed greater flower area under all salinity levels compared to other species (Fig.2-6).

The results are similar to several previous reports in other species. Soil salinity resulted in significant flower bud mortality in blueberry (Berkheimer and Hanson, 2006) while Falcon et al. (1986) reported a continuous yield reduction of rose as salinity increase. Küçükahmetler (2002) 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 (Munns, 2003). Forthermore, 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 (Cheesman, 1988; Razmjoo et al, 2008; Baas et al., 1995; Küçükahmetler, 2002). 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 (Fu et al., 2005), 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 (Chartzoulaki et al., 2002; Liu and Cooper, 2002, Qian and Fu, 2005, Shahba 2010b, Shahba et al., 2012). In creeping bentgrass, Liu and Cooper (2002) reported a 20% decrease in Pn when

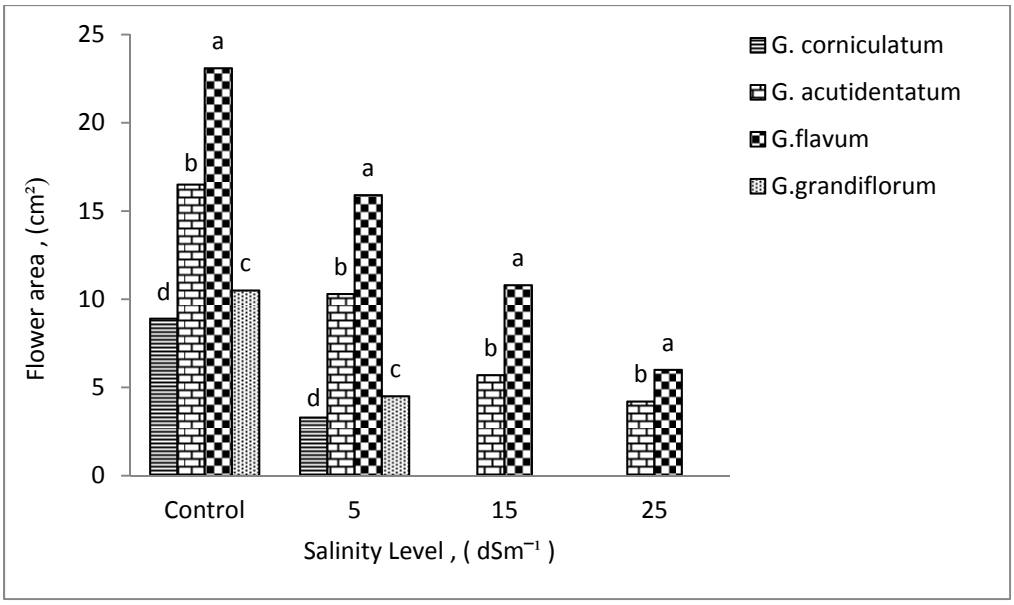

Figure 2. 6: Effect of different Salinity levels on flower area of four *Glaucium spp*. Columns labeled with different letters are significantly different at P=0.05 within each salinity level.

salinity increased from 0.0 to 16 dS m^{-1}, while Qian and Fu (2005) reported a 40% decrease for the same species when salinity increased from control (0.2 dS m^{-1}) to 15 dS m^{-1}. Increasing salinity and reduction in mowing heights of bermudgrass (Shahba, 2010b) cultivars and seashore paspalum cultivars (Shahba et al., 2012) 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 (Flowers et al., 1985; Lee et al., 2004c).

Razmjoo e al. (2008) has related the negative effects of salinity on flower number to its early effect on the growth and production of strong a shoot system. Pessarakli and Touchane(2006) 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 (Greenway and Munns, 1980). In addition, elevated salinity may adversely affect photosynthesis and as a result adversely affect plant biomass production through less accumulation of carbon products (Munns and Termatt, 1986).

The reduction in the number of flowers usually is more drastic than other growth parameters under high salinity as it is a cumulative effect (Razmjoo et al., 2008). High salinity in irrigation water has been reported to reduce flowering intensity, fruit set, number of fruits, and fruit growth (Cole and McLeod, 1985; Howie and Lloyd, 1989; Iglesias et al., 2007). 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 dS m^{-1} (Küçükahmetler, 2002).

Plant Quality (attractiveness):

Plant quality (attractiveness) varied significantly among species and salinity levels. The interaction between species and salinity levels was significant too (Table 2-1). 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 2-2). Increasing salinity decreased the attractiveness of all *Glaucium spp*. although to different degrees (Fig. 2-7). Under the control treatment, there was no difference between *G, flavum*, and G. *acutidenatum* and both did equally well and achieved the maximum quality (10, 10), while there was a significant difference between *G. grandflorum* (9.5) and *G. corniculatum* (9) (Fig. 2-7). The treatment of 5 dS m^{-1} did not have a significant effect on the quality of *G. flavum*, although it significantly reduced the quality of the other species (Fig. 2-7). All species were adversely affected at the salinity level of15 dS m^{-1}, where, *G.flavum* had a limited decline (9) followed by *G. acutidetutum* (8), *G. grandiflorum* (6.24) and *G. corniclatum* (5.5) (Fig. 2-7). 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 (Fig. 2-7). 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 (Ball 1988); In several species growth may be affected by either the absence of or excess of NaCl in the substrate (Downton 1982; Clough 1984; Burchett et al. 1989; Pezeshki et al. 1990; Ball and Pidsley 1995; N. Su´arez and E. Medina,2005). 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

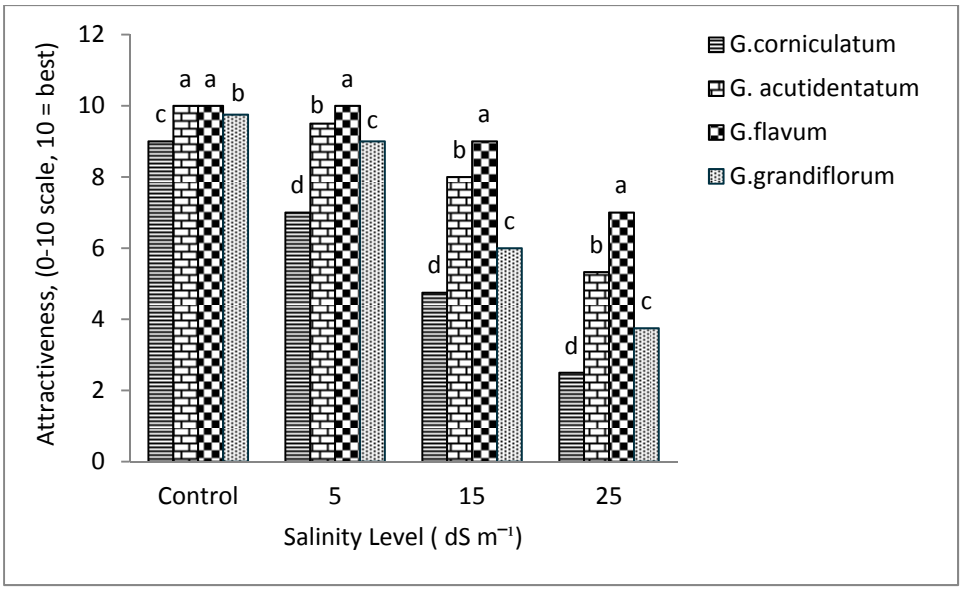

Figure 2. 7: Effect of different salinity levels on the attractiveness of four *Glaucium spp*. Columns labeled with different letters are significantly different at P=0.05 within each salinity level.

toxic effects of Na (Razmjoo et al., 2008).or examplr, The quality of lilies (plant height, flower bud length and flower diameter) decreased as salinity levels increased (Küçükahmetler, 2002).

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/browing of foliage, premature leaf fall, twig and branch die back, loss of vigor and stunted growth.(Aggarwal, et al.,2012). Several previous studies indicated similar results to the findings on Horny poppy. Fu et al. (2005) 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 cultivars (Shahba, 2010b) and in seashore paspalum cultivars (Shahba et al., 2012). Razmjoo et al. (2008) 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 (Pessarakli and Touchane, 2006). The decrease in plant biomass production due to salinity may be attributed to low or medium water potential, specific ion toxicity, or ion imbalance (Greenway and Munns, 1980). In addition, elevated salinity may adversely affect photosynthesis and as a result adversely affect plant biomass production through less accumulation of carbon products (Munns and Termaat, 1986). The reduction in the number of flowers usually is more drastic than other growth parameters under high salinity as it is a cumulative effect (Razmjoo et al., 2008). 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 2-1). Increasing salinity decreased shoot TNC of *Glaucium spp.* (Table 2-3). Regression analysis indicated a significant negative linear relationship between salinity levels and TNC contents (Table 2-3). 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. grandflorum* 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

Table 2. 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 dS m^{-1}.

Species	TNC (mg g^{-1} dry wt)				Regression	R^2
	Salinity level (dS m^{-1})					
	C	5	15	25		
G. acutidentatum	125.6b†	103.8b	79.2b	65.2b	Y = 131.1 – 1.1X	0.91**
G. corniculatum	103.8d	79.7d	53.8d	49.2d	Y = 119.1 – 1.2X	0.92**
G. flavum	133.2a	112.2a	95.5a	74.9a	Y = 116.6 – 0.9X	0.89**
G. grandiflorum	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 $P \leq 0.01$.

their interactions (Table 2-1). RSC response to different salinity treatments followed a different trend than TNC (Table 2-4). Reducing sugars in plants mainly consists of glucose and fructose

(Ball et al., 2002; Shahba et al., 2003). 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 (Popp and Smirnoff, 1995). Regression analysis indicated a significant positive association between salinity and RSC content in all species at all salinity levels (Table 2-4). 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. grandflorum* and 4, 23.3, and 61% in *G. corniculatum*, respectively. Similar results were observed by Qian and Fu (2005) who found a decrease in TNC and an increase in RSC with increasing salinity level in shoots of L-93 creeping bentgrass. Shahba also (2010b) 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

Table 2. 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 dS m^{-1}.

Species	RSC (mg g^{-1} dry wt) Salinity level (dS m^{-1})				Regression	R^2
	c	5	15	25		
G. acutidentatum	19.2	22.5b†	27.6b	39.6b	Y = 21.7 + 0.12 X	0.82**
G. corniculatum	15.9	16.6d	19.6d	25.6d	Y = 16.3 + 0.11 X	0.72*
G. flavum	18.9	25.9a	35.8a	46.8a	Y = 11.5 + 0.30 X	0.92**
G. grandiflorum	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 $P \leq 0.01$.

paspalum cultivars (Shahba et al., 2012). 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 (Popp and Smirnoff, 1995). In agreement with the horned poppy findings, Lee et al. (2008a, 2008b) reported a positive association between salinity tolerance and reducing sugars accumulation in seashore paspalums. Also, Qian and Fu (2005) 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 2-1). Increasing salinity increased shoot proline content of *Glaucium* species.(Table 2-5). 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. grandflorum*, 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 2-5). Although the role of proline accumulation in salinity tolerance is well documented in this study, it has been questioned by others (Ashraf and Harris, 2004). 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 (Shahba et al., 2012). Accumulation of proline in plant tissues in response to

salinity stress has been attributed to enzyme stabilization and/or osmoregulation (Flowers et al., 1977; Levitt, 1980). It likrly 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 (Ashraf and Foolad, 2007). Maggio et al. (2002) 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 2. 5. Proline content in shoots of *Glaucium spp.* measured at the end of the experiment vs. salinity levels, Control (c), 5, 15, 25dS m^{-1}.

Species	Proline content (µg g^{-1} fresh wt) Salinity level (dS m^{-1})				Regression	R^2
	c	5	15	25		
G. acutidentatum	250.0	646.0b†	990.0b	1527.0b	Y = 218.3 + 22.9 X	0.87**
G. corniculatum	233.7	418.0d	670.0d	1018.0d	Y = 150.7 + 19.7X	0.77*
G. flavum	268.7	855.0a	1255.0a	1712.0a	Y = 142.4 + 12.6 X	0.92**
G. grandiflorum	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 2-1). Increasing salinity decreased shoot K^+/Na^+ ratio (Table 2-6). 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 2-6). Wyn Jones et al. (1979) 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. acutidenatum*, *G. grandflorum* and *G. corniculatum* (Table 2-3). Similar results were reported by Qian and Fu (2005), who studied the response of creeping bentgrass, Shahba (2010b), who studied the response of bermudagrass cultivars to salinity and mowing heights, and Shahba et al. (2012), who studied the response of seashore paspalum

Table 2. 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 dS m^{-1}.

Species	K^+/Na^+ ratio Salinity level (dS m^{-1})				Regression	R^2
	C	5	15	25		
G. acutidentatum	2.8b	2.1b	1.1b	0.6b	Y = 128.0 – 0.91 X	0.77*
G. corniculatum	2.0d	1.3d	0.5d	0.2c	Y = 112.4 – 1.1 X	0.84**
G. flavum	3.2a	2.5a	1.5a	1.0a	Y = 121.1 – 1.66 X	0.82**
G. grandiflorum	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 $P \leq 0.05$.
** Significant at $P \leq 0.01$.

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 Jones (1979) 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. Shannon (1978) 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 (Torello and Rice, 1986; Qian et al., 2000 and 2001; Qian and Fu, 2005; Shahba 2010b, Shahba et al., 2012). Greive et al. (2004) 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. (2007) concluded that K^+/Na^+ ratio did not appear to be related to salinity tolerance of seashore pasplaum ecotypes.

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 (Ashraf and Harris, 2004; Tester and Davenport, 2003). 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^+-ATPase 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 (Qian and Fu, 2005; Shahba 2010b, Shahba et al., 2012).

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 (Carrow and Duncan, 1998). 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. grandflorum* 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.

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CHAPTER 3

SCREENING GLAUCIUM SPP. FOR DROUGHT RESISTANCE WITH EMPHASIS ON THE CONTRIBUTING PHYSIOLOGICAL CHARACTERS AND OVERALL PERFORMANCE

SUMMARY

With increasing population demands on the world's water supply, there is a greater need for drastic water conservation methods especially in arid and semiarid regions. Plant species, and cultivars within a species, vary in their salinity/drought tolerance. These variations are the result of variations in genes relating to drought tolerance mechanisms and their interaction with the environment. In order to reduce water usage, it is important to understand the mechanisms of plant adaptation to drought stress. Horned Poppies (*Glaucium spp*) are members of the Poppy family, Papaveraceae, that are native to the Mediterranean and Middle East regions. The objectives of this study were to 1) screen for drought tolerance of the common Horned Poppy species available through the Denver Botanic Gardens, *G. flavum, G. corniculatum, G. grandiflorum and G. acutidentatums;* 2) examine the effects of drought on plant characteristics as related to their aesthetics as well as the mechanisms associated with drought tolerance such as proline content, total non-structural carbohydrate content (TNC), shoot reducing sugar content (RSC) and evapotranspiration rate (ET) as an indication of water use efficiency among the tested species.

Lysimeter columns were used in this study which was replicated twice. All columns were placed in the Colorado State University plant science green house in Fort Collins, Co. *Glaucium spp.* were initiated from seeds sown in potting mix, (Pro-Mix , Mycorrhizae and Biofunglcide).

Fifty seedlings, at the 3 leaf stage, of each species were transplanted, each per PVS tubes (15 cm diameter and 50 cm long) containing commercial potting mix The potting mix was mixed with sand 2:1 to increase pore space. Water regimes applied included control (100% of the total ET), as well as 75%, 50% and 25% of the total ET. With lower water regimes, leaf color declined over time to unacceptable ratings (below 6) in both *G. grandiflorun* and *G. corniculatum*. In *G.flavum*, and *G. acutidentatum*, leaf color was not adversely affected under all water regimes. The effect of water stress on leaf color among all species was highly significant. The decline in leaf color was high for all species at 50% and 25% of ET. Leaf area decreased linearly in all species with increasing drought with a sharp drop at 25% of the total ET. *G.flavum* achieved the highest leaf area at all water regimes followed by *G. acutidentatum*, *G.grandiflorum* and *G. corniculatum*. *G.flavum* acheived an average leaf area of 24.3 cm^2, while *G. acutidentatum* leaf area was 22.2 cm^2 at 100% ET. *G.flavum* achieved an average height of 45.8 cm while *G. acutidentatum* was 40.5 cm and *G.grandiflorum* was 30.0 cm at 200% ET. *G. corniculatum* had the lowest height of 27.8 cm in the control treatment. Increased water stress resulted in fewer flower buds, reduced flower number, and smaller flowers in all tested species. Also, increasing drought decreased the attractiveness of all *Glaucium spp*. although at different degrees. *G. flavum* showed greater transpiration efficiency (TE) since it was able to maintain its ET at lower rates while maintaining higher attractiveness when compared with *G. acutidentatum*, *G. grandiflorum* and *G. corniculatum* in order of attractiveness, respectively. In *G. flavum*, as water regimes decreased from control to 75, 50 and 25 % of the total ET, average TNC decreased by 15.1, 30.3 and 48.0% and the average TNC decrease in *G. acutidentatum* shoots was 21.6, 40.1, and 53.7%. RSC response to drought treatments followed a different trend than TNC. As water stress increased from control to 75, 50 and 25%, average RSC increased by 40.7, 101.8 and

166.5 % in *G. flavum* and by 17.4, 40.0 and 103.4% in *G. acutidentatum.* The increase was 122.2, 39.6, and 90.6% in *G. grandiflorum* and 4.4, 26.5, and 62.5% in *G. corniculatum*, respectively. As water regimes decreased from control to 75, 50 and 25%, average proline content in shoots increased by 186, 325, and 472% in *G. flavum*; 163, 303 and 517% in *G. acutidentatum*; 160, 280 and 418% in *G. grandiflorum*, and 80, 190, and 340% in *G. corniculatum*, respectively. On the basis of the number of times in the best statistical category for leaf characteristics, plant height, flowering characteristics, overall plant quality (attractiveness), water use efficiency, TNC, RSC, and Proline, *G. flavum* was found to have higher drought tolerance as compared to *G. acutidentatum*, *G. grandiflorum* and *G. corniculatum*. In summary, as drought increased, *Glaucium spp*. exhibited reduction in leaf characteristics, plant height, flowering characteristics, overall plant quality (attractiveness), TNC, and ET rate, and increased shoot total reducing sugars and proline content. *G. flavum* showed higher drought tolerance at all water regimes when compared to the other tested species. Since proline accumulation increased with drought stress it is likely that it aided drought tolerance through osmoregulation or by acting as a carbon and nitrogen sink for stress recovery.

INTRODUCTION

The demand for water has increased more than 300% during the past five decades (Huffman, 2004). With increasing population demands on the world's water supply, there is a greater need for drastic water conservation methods especially in arid and semiarid regions. Because of this immense water usage and diminishing water resources, many arid states have implemented water conservation programs (Soeder and Kappel, 2009). The demand for water has led to an inadequate water supply for landscapes and as a result negative impacts on the

aesthetics and functionality. Therefore, the development of efficient irrigation management programs as well as the selection and improvement of drought tolerant landscape plants has become extremely important in order to maintain quality landscapes.

Plant species, and cultivars within a species, vary in their salinity/drought tolerance. These variations are the result of genes relating to drought tolerance mechanisms and their interaction with the environment (Duncan and Carrow, 1999). Usually evaluations for drought and salt tolerance of plants depend on shoot (above ground) growth, as reported in crop yield response curves proposed by Maas and Hoffman (Igartua, 1995; Maas and Hoffman, 1977).

Horned Poppies (*Glaucium spp*) are members of the Poppy family, Papaveraceae. *Glaucium* are species that have originated in 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, and are best grown by seeding in the fall where they are to bloom and thinning to the desired spacing as they germinate in the spring. For earlier bloom, sow seed indoors 8 to 10 weeks prior to planting and then transplant them into the garden after danger of frost has passed. Germination takes 8 to 15 days at 15 to 18oC. 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. Stems of horned poppy branch and grow to from a rosette of leaves. The crinkly, gray-green leaves also appear on the stems and below each flower. The golden-yellow flowers may be up to 5 cm in diameter. There are also orange or red flowers. The roots of the horned poppy are considered poisonous.

All horned poppies have blue-green foliage that is deeply pinnatified 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 sublyrate 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 completely 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 were *G. flavum*, *G. grandiflorum*, *G. acutidentatum* and *G. corniculatum*.

G. flavum Crantz is the most widely spread species in the genus. It is found from the coasts of Britain and the Atlantic Islands to the coasts of the Mediterranean Basin and the Black Sea (Grey-Wilson, 2000). 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 as it grows along the sea. According to Davis (1965), *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 (Davis, 1965).

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 (Grey-Wilson, 2000). 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 great differences in climate changes with harsh winters and drier conditions with 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 (Davis, 1965). 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 & Bornm is endemic to Turkey where it is found on dry hillslopes and rocky places (Grey-Wilson, 2000). *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 color. *G. acutidentatum* is found at elevations of 950-1400 m on dry hills (Davis, 1965).

G.corniculatum (L.) J.H. Rudolph is native to the Mediterranean basin, Atlantic islands, Caucasus Mountains, Bulgaria, Romania, northern Iraq and northwestern Iran (Grey-Wilson, 2000; Davis, 1965). *G. corniculatum* also has some unique characteristics. Its leaves have a soft, villous texture and its sepals are scabrous to hirsute. The petals are yellow, orange or red (Davis, 1965) with a black basal spot (Grey-Wilson, 2000).

In order to reduce water usage, it is important to understand the mechanisms of plant adaptation to drought stress. Drought resistance includes a range of mechanisms employed by plants to withstand periods of drought (Beard, 1989). Strategic mechanisms include drought escape, drought avoidance, and drought tolerance (Turner, 1986). The significance of each of these strategies is related to drought duration and severity in addition to the plant species. These mechanisms are associated with anatomical, morphological, physiological, and biochemical

changes. The reduction in the evapotranspiration (ET) rate and the ability of a species to maintain transpiration as the soil dries are example of drought tolerance mechanisms as the reduction in ET indicates a better water use efficiency. Changes in leaves that facilitate drought tolerance include reduced leaf growth and area, increased pubescence, rolling or folding, and fewer stomates (Duncan and Carrow, 1999). The balance between carbohydrate production and consumption will impact the ability of plant species to cope with stresses (Huang and Fry, 1999; Lee et al., 2008a, 2008b; Shahba, 2010b, Shahba et al., 2012). Amino acids, especially proline, accumulate in larger amounts to cope with increasing stress in plants (Lee et al., 2008b). Proline accumulation is one of the first responses of plants exposed to water-deficit stress and serves to reduce injury to cells (Ashraf and Foolad 2007). Rapid accumulation of proline in tissues of many plant species in response to drought, salt or temperature stresses has been attributed to enzyme stabilization and/or osmoregulation (Flowers et al., 1977; Levitt, 1980). However, because of contrasting reports related to proline accumulation effect on stress tolerance (Marcum, 2002; Torello and Rice, 1986), its use as selection criterion for stress tolerance has been questioned (Ashraf and Harris, 2004). Thus it is critical that tests be made before making any conclusion regarding the role of proline in stress tolerance of any specific species.

In the previous chapter, it was shown that drought tolerance of *Glaucium spp.* is dependent on the internal osmoregulator content. There is no published information that addresses the mechanisms of *Glaucium spp.* drought tolerance. The objectives of this study were to 1) screen for drought tolerance of the common Horned Poppy species that were available from Denver Botanic Gardens, *G. flavum, G. corniculatum, G. grandiflorum and G. acutidentatums;* 2) examine the effects of drought on plant characteristics associated with aesthetics and the mechanisms associated with drought tolerance such as proline content, total non-structural

carbohydrate content (TNC), shoot reducing sugar content (RSC) and ET rates (water use efficiency) among the tested species.

MATERIALS AND METHODS

Lysimeter columns were used in this study which was replicated twice. All columns were placed in the plant science greenhouse at Colorado State University, Fort Collins, Co. *Glaucium spp.* were initiated from seeds and transplanted into potting mix, (Pro-Mix, Mycorrhizae and Biofunglcide). Fifty seedlings of each species at the 3 leaf stage were transplanted, 1 per PVS tubes (15 cm diameter and 50 cm long) containing commercial potting mix, (Pro-Mix, Mycorrhizae and Biofunglcide). The potting mix was mixed with sand 2:1 to increase pore space. The plants were maintained in the greenhouse until full establishment and recovery from transplanting. Those seedlings that survived were used as experimental units in the drought study. The experimental design was randomized complete Block (RCB). Each block contained one of the studied species with 16 tubes. Chosen seedlings had the same size and same number of leaves.

Water regimes applied included control (100% of the total evapotranspiration), as well as 75%, 50% and 25% of the total ET. ET was measured weekly. Two representative pots for each of the species were used as lysimeters and were watered with enough water and left to drain for 2 h, after which the weight of each pot was recorded. Each pot was re-weighed every 24 hours. The daily changes in weight represented the daily ET for each species. Treatments were replicated four times. Seedling ET was the average of four lysimeters for each species. Treatments continued until plants reached the flowering stage. ET was updated weekly and treatments were adjusted accordingly. 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, as well as quality and general attractiveness of the plant using a scale of 0 (not attractive) to 10 (optimum attractiveness). Samples were collected for TNC, RSC, and proline.

ET measurements were collected every 2 to 3 days during the four month growth period. Five weight readings per pot were made during each measurement and the average value was used for ET calculation. ET was calculated by mass difference and expressed as mm d-1.

TNC, RSC, and proline content were determined at the termination of the experiment. Shoot tissue was harvested and washed with cold distilled water to remove plant debris for carbohydrate analysis. Then, approximately 5 g of samples were freeze-dried (Genesis 25 LL Lyophilizer, Virtis, and Gardiner, NY). After freeze-drying, samples were ground with a Wiley mill, sieved thought a screen with 425 μm openings, and kept in airtight vials at –20 ^{o}C. Total nonstructural carbohydrate content was measured using the method described by Chatterton et al. (1987). In brief, 25 mg of freeze-dried samples were transferred to 5 mL 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 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.

Actual proline tissue accumulation levels were determined according to the method of Bates et al. (1973) as modified by Torello and Rice (1986) 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 ninhydrin in 30 mL of glacial acetic acid and 20 mL of 6 M H3PO4) and 2ml of glacial acetic acid followed by 1 h of heating at 100 ^{o}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 drought treatments 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 drought, species and their interactions using ANOVA (SAS Institute, 2006). Leaf characteristics (color and area), number of flower buds, and flower characteristics (number and size) were analyzed on individual measurement dates to examine drought, 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 water regiems (independent variable).

RESULTS AND DISCUSSION

Leaf characteristics:

Leaf color. Comparisons of leaf color among species and water regimes indicated significant differences (Table 3-1). At all lower water regimes, leaf color declined over time to unacceptable ratings (below 6) in *G. grandiflorun* and *G. corniculatum*. In *G.flavum*, and *G. acutidentatum*, leaf color was not adversely affected at 75% regimes (Fig. 3-1). The effect of water stress on leaf color among all species was highly significant. The decline in leaf color was high for all species at 50% and 25% of irrigation (Fig.3-1). *G.flavum* had the highest leaf color level under all treatments. Under the control treatment, there was no difference among *G. flavum, G. acutidentatum,* and *G. grandiflorum* in leaf color (full rating of 10). They showed 100% full green leaf while *G. corniculatum* showed a rating of 9.5. Leaf color decreased as water regimes decreased. At the water regime of 75% of the total ET, *G. flavum* and *G. acutidentatum* did equally well and leaf color rating did not change (rating of 10). *G. grandiflorum* had a reduced rating of (9) while *G. corniculatum* rating was 8.6 at 75% ET (Fig.3-1). Under the lowest water regime (25% of the total ET), the leaf color of all species were adversely affected, however, only *G. grandiflorum* and *G.corniculatum* leaf color ratings were below the accepted levels (4.8 and 4.5 respectively) (Fig. 3-1). Similarity, leaf greenness decreased under severe water stress in all almond genotypes studied by Yadollahia, 2011. Flexas and Medrano (2002) reported a reduction in leaf greenness in C3 plant leaves under water stress and associated that to degradation in chlorophyll content. The retention of leaves or the observation of 'stay green' under water stress conditions has been reported in cassava lines MH96/0686 and has correlated well with drought tolerance and improved yields in cassava (Lenis et al. 2006). The decrease in relative greenness

Table 3. 1. Analysis of variances with mean square and treatment significance of leaf color, leaf area, plant height, number of flower buds, number of flowers, flower area, plant quality (attractiveness), total non-structure carbohydrate content (TNC), shoot reducing sugar content (RSC), proline content and total evapotranspiration in *Glaucium spp.*

Parameters	Source		
	Species (S)	Water regimes (W)	S X W
Leaf color (0-10 scale)	8.5**	65.1**	59.2*
Leaf area (cm^2)	3.5**	4.11**	3.2*
Plant height (cm)	2.22**	2.66**	2.33*
Number of buds	29.5**	67.0**	20.6*
Number of flowers	3.2**	6.1**	4.9*
Flower area (cm^2)	8.8**	9.7**	1.7*
Plant quality (0-10 scale)	8.5**	9.6**	6.9*
TNC (mg g^{-1} dry wt)	8800**	711.0**	895.0*
RSC (mg g^{-1} dry wt)	56.0**	92.0**	21.0*
Proline content (µg g^{-1} fresh wt.)	1270**	1337**	1227*
Total ET (mm d^{-1})	1.9.0**	5.1**	2.9*

* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.

Table 3. 2. Linear regression of different parameters of *Glaucium spp.* measured at the end of the experiment vs. water regimes of control (C), 75, 50, and 25% of the total evapotranspiration.

Species	Parameter					
	Plant quality (0-10 scale)		Leaf area (cm^2)		Flower area (cm^2)	
	Regression	R^2	Regression	R^2	Regression	R^2
G. acutidentatum	Y = 4.2 – 0.2 X	0.80**	Y = 102.5 – 2.2 X	0.82**	Y = 210.5 - 6.3 X	0.80**
G. corniculatum	Y = 6.6 – 0.3 X	0.71*	Y = 125.2 – 2.1 X	0.69*	Y = 202.6 - 8.2 X	0.65*
G. flavum	Y = 7.8 – 0.5 X	0.82**	Y = 116.8 – 2.3 X	0.85**	Y = 113.3 - 8.8 X	0.90**
G. *grandiflorum*	Y = 3.9 – 0.3 X	0.65*	Y = 121.2 – 2.5 X	0.65*	Y = 199.0 - 6.8 X	0.67*

* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.

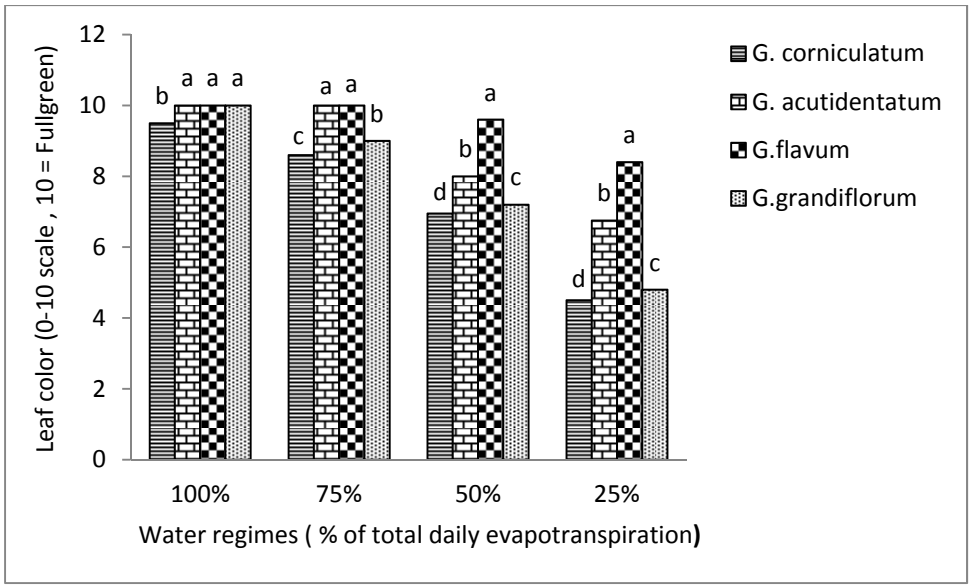

Figure 3. 1: Effect of four different water regimes on leaf color of four *Glaucium spp*. Columns labeled with different letters are significantly different at P=0.05 within each water regime.

of the leaf under water stress treatment as compared to the well- watered treatment is likely due to a decrease in chlorophyll content as reported in rapeseed plants (Gibon et al., 2000). There was a 38% reduction in chlorophyll content when compared to full irrigation of plants (Din et al., 2011). Increasing water stress reduced the (Chl a) and the (Chl a: b) significantly (Liu, et al, 2011). The pigment content generally decreased due to low synthesis rate and rapid degradation under water stress (Mihailovie et al., 1997; Lei et al., 2006, and Yadollahia, 2011).

Leaf area. Analysis of variance indicated significant differences among species and among water regimes and their interactions (Table 3-1). Linear regression indicated a significant negative association between leaf area and water regimes (Table 3-2). Leaf area decreased linearly in all species with increasing drought with a sharp drop at the water regime of 25% of the total evapotranspiration. *G.flavum* achieved the highest leaf area at all water regimes followed by *G.acutidentatum*, *G.grandiflorum* and *G. corniculatum*. *G.flavum* acheived an average leaf area of 24.3 cm^2, while G. *acutidentatum* achieved leaf area of 22.*2* cm^2 at 100% ET. *G.grandiflorum* was ranked third with an average leaf area of 22.0 cm^2 while *G. corniculatum* had the lowest leaf area of 19.9 cm^2 with control treatment (Fig. 3-2). Leaf area decreased from 23 to 21.8, 18.1 and 12.5 cm^2 in *G. flavum*; from 22.2 to 18.2, 12.5, and 10.2 cm^2 in *G. acutidentatum*, from 22.0 to17.7, 10.6, and 7.8 cm^2 in *G.grandiflorum*, and from 19.9 to 15.4, 10.3, and 7.0 cm^2 in *G. corniculatum* with increased drought from the control to 75, 50 and 25% ET, respectively (Fig. 3-2). It is logic that the leaf area followed the trend of leaf color since healthy leaves should have a greater leaf area. Although there was considerable decrease in overall leaf area in *G.flavum*, it appeared to be the most drought tolerant species. Water stress is one of the most common environmental factors affecting plant growth and productivity. Reduced water availability induces numerous physiological and biochemical changes in all plant organs.

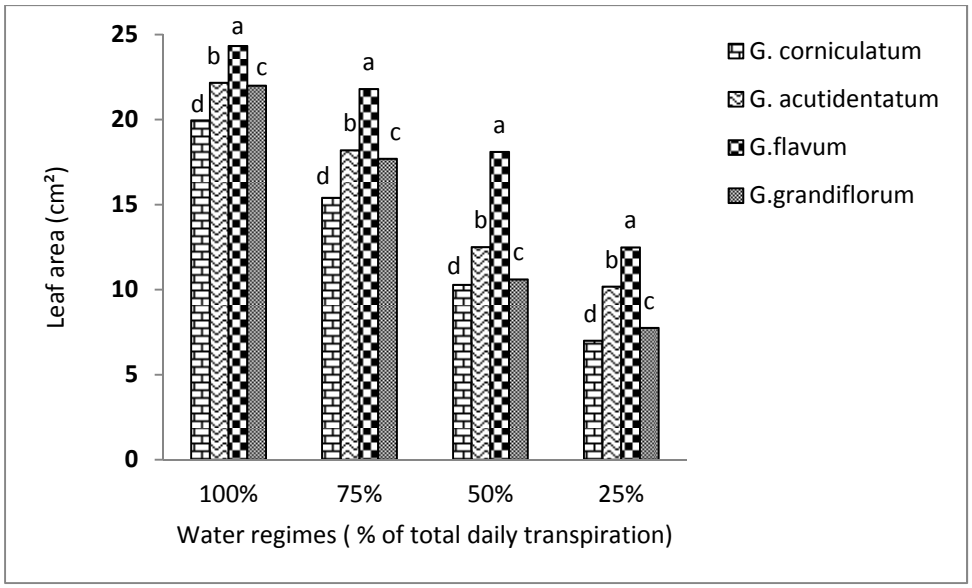

Figure 3. 2: Effect of four different water regimes on leaf area of four *Glaucium spp*. Columns labeled with different letters are significantly different at P=0.05 within each water regim

Gas exchange in leaves is limited, which in turn reduces carbon assimilation. Changes in the distribution of photo- assimilates can reduce vegetative growth (Boyer, 1970; Gehrmann, 1985; Singer et al., 2003) as well. The reduction of leaf area is principally explained by a lower leaf unfolding rate which results in smaller leaf size (Lecoeur et al., 1995; Lecoeur and Guilioni, 1998). The reduction in leaf area could be an adapting mechanism to water stress. Water stress induced a significant reduction in the leaf area which is a benefit in reducing leaf transpiration (Hessini, 2009). Similar results were found by Grant (2010) who studied ten strawberry cultivars under different water regimes. Furthermore, it was concluded that strawberry genotypes differed in their response to water deficiency although drought stress reduced leaf area in all of them (Klamkowski and Treder, 2008). In another study, it was found that total leaf area and leaf blade area decreased with the increase in water stress in *Campylotropis polyantha* seedlings, while total leaf area was reduced sharply in response to progressive water stress (Liu, et al., 2011). Similar results were found in eggplants (Madramootoo and Rigby, 1991), different almond genotypes (Yadollahia, 2011) and wheat cultivars (Tahar Boutraa, 2010; Kameli and Lösel, 1996). The common cause of the reduced vegetative growth of vegetables under water deficit conditions has been understood to be accelerated leaf senescence in eggplant (Kirnak et al., 2002).

However, the leaf area in bell pepper was not affected by drought (Alexieva, 2001). Specific leaf area (SLA), an indicator of leaf thickness, has often been observed to be reduced under drought conditions (Marcelis et al., 1998). Decrease in SLA in plants under drought stress may be due to the different sensitivity of photosynthesis and leaf area expansion to soil drying. Drought stress affects leaf expansion earlier than photosynthesis (Jensen et al., 1996; Tardieu et al., 1999). Reduction of SLA is assumed to be a way to improve water use efficiency (WUE) (Wright et al.,

1994; Craufurd et al., 1999; Passioura, 2002). This is because thicker leaves usually have a higher density of chlorophyll and proteins per unit leaf area and, hence, have a greater photosynthetic capacity than thinner leaves. The mechanism, by which plant leaf area is reduced under water stress, is thought to be the reduction of cell elongation, which leads to reduction of cell size and therefore a reduction of leaf area (Schuppler et al., 1998).

Plant Height:

Generally, there was a significant decrease in plant height as drought stress increased. (Table 3-1). G.flavum achieved an average height of 45.8 cm while G. acutidentatum averaged 40.5 cm; G.grandiflorum averaged 30.0 cm, and G. corniculatum the lowest at 27.8 cm in the control treatment (Fig. 3-3). Plant height decreased from 45.8 to 42.0, 30.0 and 21.3 in G. flavum, from 40.5 to 33.0, 22.5, and 14.3 cm in G. acutidentatum, from 30.0 to 24.0, 13.0, and 5.3 cm in G.grandiflorum, and from 27.8 to 20.3, 9.0, and 4.5 cm in G. corniculatum as the drought increased from the control to 75, 50 and 25% of the total ET, respectively (Fig. 3-3). Several reports have reported similar negative effects of drought on plant height (Alexieva, 2001; Baher et al., 2002; Colom and Vazzana, 2002; Li,et al., 2011; Razmjoo, 2008; Shihab Udden, et al., 2013). Previous studies indicated a significant reduction in plant height in mungbean (*Vigna radiate* L.) (Shihab Udden, et al., 2013), in *Satureja hortensis* (Baher et al., 2002), and in *Eragrostis curvula* (Colom and Vazzana, 2002). However, Alexieva (2001) reported no effect on pea and wheat height due to drought stress. The reduction in growth parameters such as height could be attributed to several effects such as the osmotic stress and /or ionic toxicity (Greenway and Munns, 1980) which is more harmful to plants during the succulent seedling stage in addition to the stressful effects of ion uptake (Dumbroff and Cooper, 1974; Chartzoulakis and

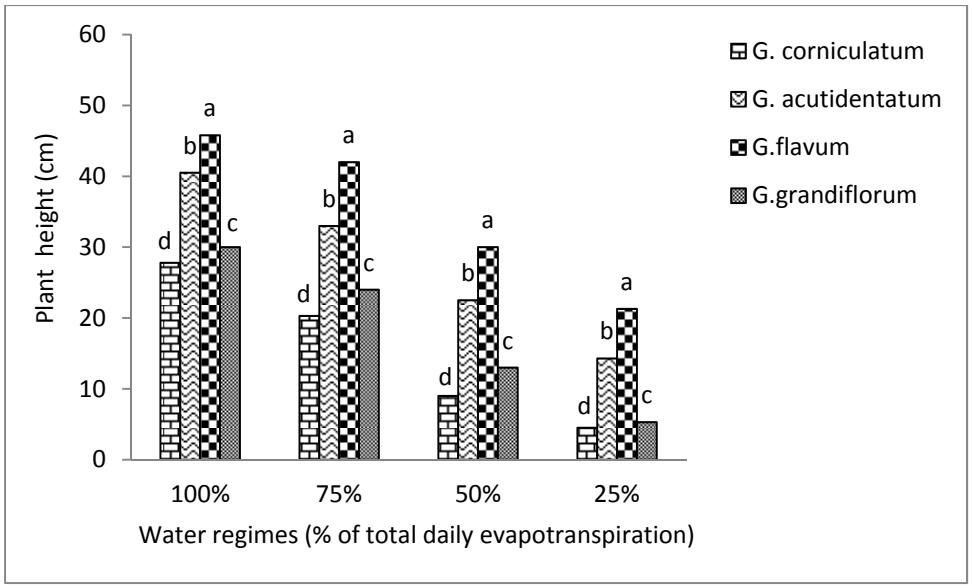

Figure 3. 3: Effect of four different water regimes on plant height of four *Glaucium spp*. Columns labeled with different letters are significantly different at P=0.05 within each water regime.

Klapaki, 2000). Drought stress favors the growth of roots as an adaptive mechanism rather than shoots which results in a decrease in plant height. Marcum (1999) reported root mass increased under stress conditions of several grasses. Also, root growth stimulation under stress conditions has been reported in stress tolerant grasses by others as well (Dudeck et al., 1983; Peacock and Dudeck, 1985). Shahba (2010b), Shahba et al. (2012) and Shahba et al., (2013 *in press*) reported an increase in root mass of bermuda grass cultivars and seashore paspalum cultivars under salinity and drought condition. The reduction in plant height might be due to inhibition of cell division or cell enlargement with less soil moisture availability (Ranawake et al., 2011; Shihab Udden et al., 2013). Rozema and Visser (1981) indicated that increased rooting and the associated increase in root absorbing area is an adaptive mechanism to the osmotic and nutrient deficiency stresses occurring under stress conditions which in turn results in a reduction in shoot system and plant height. Unfortunately, we were not able to measure the change in root mass in this study to support this argument in *Glaucium spp*.

Flowering characteristics:

Number of flower buds. Numbers of flower buds are varied significantly among *Glaucium spp*., water regimes and their interaction (Table 3-1). Increased water stress resulted in fewer flower buds (Fig. 3-4). In *G.flavum*, as water regimes decreased from control to 75, 50 and 25 % ET, average bud number decreased by 7.8, 37.5 and 54.7 % respectively. The decrease was similar in *G. acutidentatum* where the average number of flower buds decreased by 11, 33 and 55.6 % when drought increased from control to 75, 50 and 25%, respectively. *G.grandiflorum* and *G. corniculatum* did not produce any flower buds at an ET of 25%. This study demonstrated that drought significantly affected the production of flower buds. At the control treatment, all species

produced flower buds with the highest number produced by *G. flavum* (32.0), followed by *G. acutidentatum* (22.5) , *G.grandiflorum* (9.9) and the lowest number by *G. corniculatum* (8.5) (Fig. 3-4).

Number of flowers. Flower number is another indicator of plant vigor. Number of flowers varied significantly ($P < 0.05$) among species, water regimes and their interaction (Table 3-1). The number of flowers declined with increased drought levels. The decline in flower number under higher drought stress was more severe and more rapid in the two less drought tolerant species (*G. grandiflorum* and *G. corniculatum*) while more moderate in *G. flavum* and *G. acutidentatum* (Fig. 3-5). *G. flavum* had higher flower number under all water regimes when compared to other species (Fig. 3-5). *G. corniculatum* had the lowest number of flowers at all water regimes. At the control treatment, the highest number of flowers produced was by *G. flavum* (26.5) followed by *acutidentatum* (20.5), *G. grandiflorum* (10) and *G. corniculatum* (8). Only *G. flavum* and *G. acutidentatum* produced flowers at the water regime of 25% of ET, (Fig. 3-5).

Flower area. Comparisons of flower area among species and among water regimes and their interaction clearly showed significant differences (Table 3-1). Flower area decreased linearly with increasing drought. Regressions were strongly linear, with slope more negative with less tolerant species (Table 3-2). As water regime decreased, the flower area decreased. At the control treatment, flower area was the greatest in *G. flavum* (22.4 cm^2) followed by *G. acutidentatum* (19.3 cm^2), *G. grandiflorum* (10.5 cm^2), and *G. corniculatum* had the smallest flower area (9.5 cm^2) (Fig. 3-6). The decline in flower area under substantial drought stress was more severe and more rapid in the less drought tolerant species (*G.grandiflorum* and *G. corniculatum*) while more moderate in *G.flavum* and *G. acutidentatum* (Fig. 3-6). *G.flavum* had

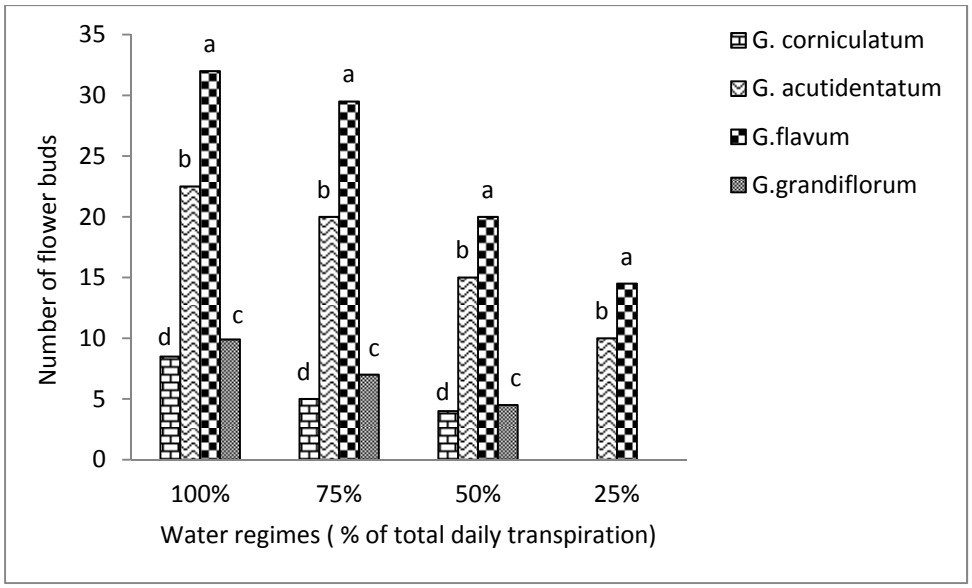

Figure 3. 4: Effect of four different water regimes on number of flower buds of four *Glaucium spp*. Columns labeled with different letters are significantly different at P=0.05 within each water regime.

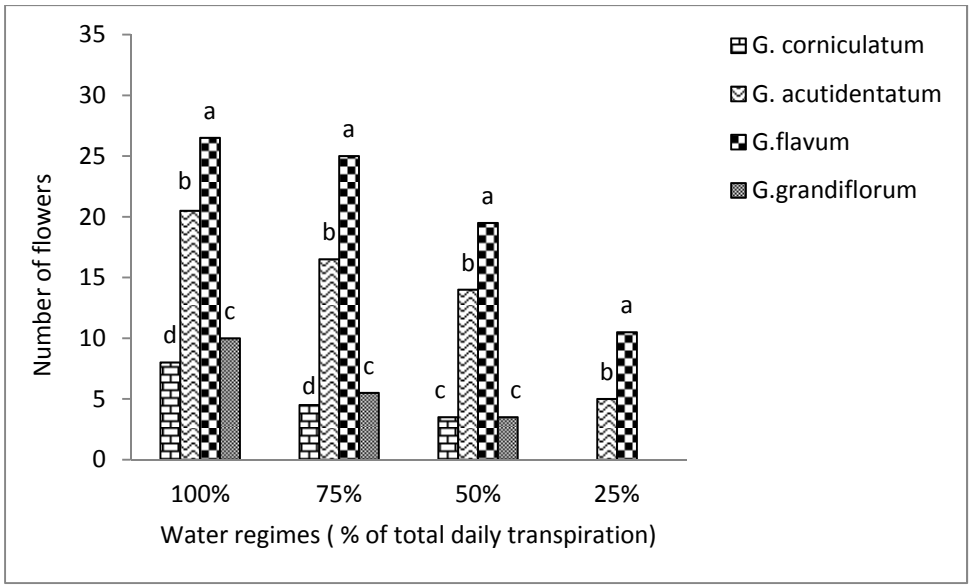

Figure 3. 5: Effect of four different water regimes on number of number of flowers of four *Glaucium spp*. Columns labeled with different letters are significantly different at P=0.05 within each water regime.

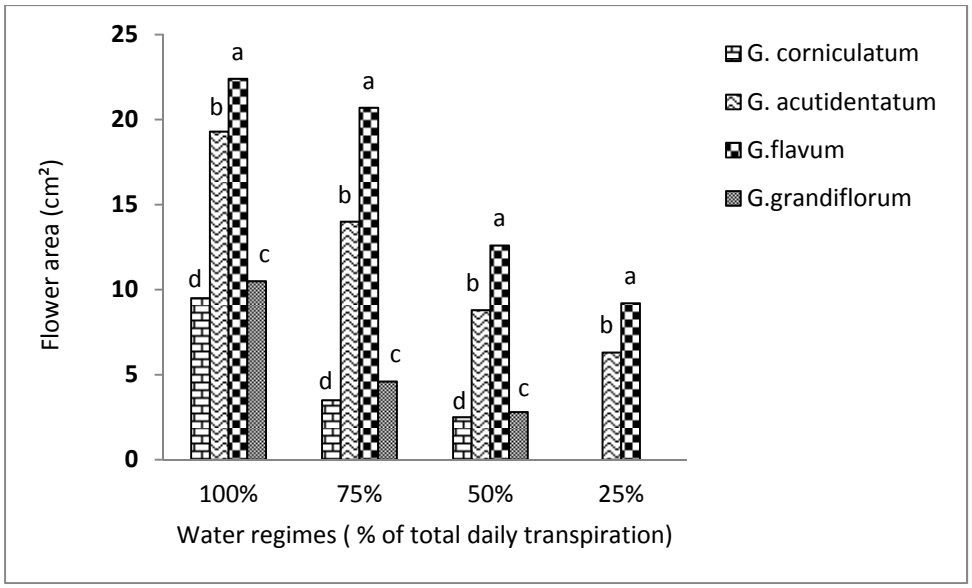

Figure 3. 6: Effect of four different water regimes on flower area of four *Glaucium spp*. Columns labeled with different letters are significantly different at P=0.05 within each water regime.

the greatest flower area under all water regimes compared to other species (Fig. 3-6).

Previous reports have indicated similar results in other species. Water stress affected flower induction in rice (*Oryza sativa* L.), (Sheoran and Saini1996), and in satsuma mandarin *Citrus unshiu* Marc. (Koshita and Takahara, 2004; Southwick and Davenport, 1986). Fewer flowers were often observed in cultivated satsuma mandarin under drought conditions (Katayama et al., 1989). Oilseed rape was also significantly affected by water shortage during the most sensitive flowering stage (Istanbulluoglu et al., 2010). Koshita and Takahara (2004) reported negative effects on flower-bud formation in citrus as a result of drought as well. Southwick and Davenport (1986) indicated that both continuous and cyclical water-stress treatments reduced flowering of *Citrus latifolia* Tan. However, cotton flower buds have been shown to be relatively insensitive to water deficits (Loka and Oosterhui, 2009). Flower bud induction under water stress treatments is likely due to the influence on hormonal metabolism roles. For example, plant growth regulators have been applied exogenously to elucidate the roles of plant hormones in flower-bud induction of citrus. The conclusion was that exogenously applied GA reduces the number of flowers in the following spring (Hirose, 1968). The suppression of plant growth under drought conditions may be due to decreased availability of water that leads to the toxicity of sodium chloride (Munns, 2003). Also, the hydrolysis of reserved foods to produce energy necessary for biological functions and survival reduces the amount of resources available for flower formation. Drought stress imposes additional energy requirements on plant cells and less carbon is available for growth and flower primordial initiation (Cheesman, 1988; Razmjoo et al., 2008; Baas et al., 1995; Küçükahmetler, 2002). Drought effect on flower formation can be an indirect result of its effect on photosynthesis (Pn) efficiency. Pn is less sensitive to drought as compared to other growth parameters (Fu et al., 2005), but photosynthetic capacity can be reduced in the presence

of great drought levels due to stomata closure, damage to photosynthetic systems by excessive energy, structural disorganization or reduction in photochemical quenching (Flowers et al., 1985; Lee et al., 2004c). On the other hand, Razmjoo e al. (2008) related the negative effects of drought on flower number to its early effect on the growth and production of a strong shoot system. Pessarakli and Touchane (2006) found that the reduction in biomass production due to drought stress is more obvious than the reduction in shoot lengths in bermudagrass. The decrease in plant biomass production due to drought may be attributed to low or medium water potential, specific ion toxicity, or ion imbalance that resulted from insufficient water for osmotic balance (Greenway and Munns, 1980). In addition, elevated drought may adversely affect photosynthesis and as a result adversely affect plant biomass production through reduced accumulation of carbon products (Munns and Termatt, 1986). The reduction in the number of flowers usually is more drastic than other growth parameters under high drought as it is a cumulative effect (Razmjoo et al., 2008).

Three contrasting faba bean genotypes (*Vicia faba* L.) were tested under drought stress. A reduction in the number of flowers was recorded. Saxena et al., (1981) concluded that the reduced flowering was a mean for maintaining stable and high seed yields under water stress. Also, reproductive development at the time of flowering is especially sensitive to drought stress (Zinselmeier et al. 1995, 1999; Samarah et al., 2009 a,b). Drought stress interferes not only with flowering but also flower opening, nectar production, and turgor maintenance of floral organs as well (Mohn Ram and Rao, 1984). Water stress during flower induction and inflorescence development may lead to a delay in flowering (anthesis) or even complete inhibition of flowers (Wopereis et al., 1996; Winkel et al., 1997). This confirms the differences in sensitivity to drought among different species and/or cultivars (Zollinger et al., 2006, Clary et al., 2004 and

Savé et al., 2000;Shahba 2010b; Shahba et al., 2012; Shahba et al., 2013 *in press*) and even between growth stages for many plants (Sionit et al., 1987). Water limitation has an impact on plant growth (Franco et al., 2006), although the exact effect may vary depending on the intensity of the water stress imposed (Cameron et al., 1999). A reduction in flower size is one of the consequences of exposing plants to water stress (Cameron et al., 1999). Carroll et al., (2001) reported that drought led to a 33% decrease in flower size relative to controls. Reduction of flower size under drought stress was recorded in populations of *Clarkia unguiculata* distributed along a natural moisture gradient (Jonas and Geber, 1999). The water stress, which decreased the water potential in the soil, reduced the flower head diameter (Lin et al., 2011). The effect on flower area as related to the decrease of water availability can be explained by the decrease in the influx from the vegetative portions of the plant to the reproductive organs (Mohan Ram and Rao, 1984) and the biochemical limitation which prevails under drought stress (Jones, 1992; Cornic and Massacci, 1996).

Plant Quality (attractiveness):

Plant quality (attractiveness) varied significantly among species and water regimes. The interaction between species and water regimes was significant as well (Table 3-1). Plant quality decreased linearly with increasing drought in all species. Regressions were strongly linear with larger slopes for less tolerant species (Table 3-2). Increasing drought decreased the attractiveness of all *Glaucium spp*. to different degrees (Fig. 3-7). Under the control treatment, there was no difference between *G. flavum*, and *G. acutidentatum* since both of them do equally well and achieved the maximum quality (10), while there was a slightly significant difference between *G. grandiflorum* (9.7) and *G. corniculatum* (9.5) (Fig. 3-7). The treatment of 75% ET did not have

a significant effect on the quality of *G. flavum* or *G. acutidentatum* while it significantly reduced the quality of the other two species (Fig. 3-7). All species were adversely affected at the water regimes of 50 and 25% ET, although *G.flavum* had less decline followed by *G. acutidetutum*, *G. grandiflorum* and *G. corniculatum* (Fig. 3-7).

Plants express various responses to drought and develop a wide range of tolerance strategies that affect both morphological and physiological traits (Blum, 1996). These responses may be reflected in plant leaf greenness, leaf size, plant height and flowering quality. Water stress has been shown to significantly reduce plant size (Champolivier and Merrien, 1996). Studies have also shown that drought stress can affect the growth of plant organs differently (Spollen et al., 1993) which may result in the alteration of morphology (French and Turner, 1991). Putievsky et al. (1990) reported that water stress had a negative impact on green tissue yield of geranium. Drought caused reduction in all growth parameters of *Matricaria chamomile* (Razmjoo, 2008). Furthermore, a study by Flexas and Medrano (2002) showed that moisture deficiency affects various physiological and metabolic responses such as stomatal closure, decline in growth rate and photosynthesis. Also, Baher et al. (2002) showed that greater soil water stress decreased plant height and total fresh and dry weight of *Satureja hortensis*. Colom and Vazzana (2002) showed that the number of branches per plant and total plant dry weight was negatively affected by water stress in *Eragrostis curvula*. The range of drought in which the plant is able to survive varies according to the species (Ball 1988). 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+ (Razmjoo et al., 2008) which is a process that requires sufficient water in plant cells. The quality of lilies (plant height, flower

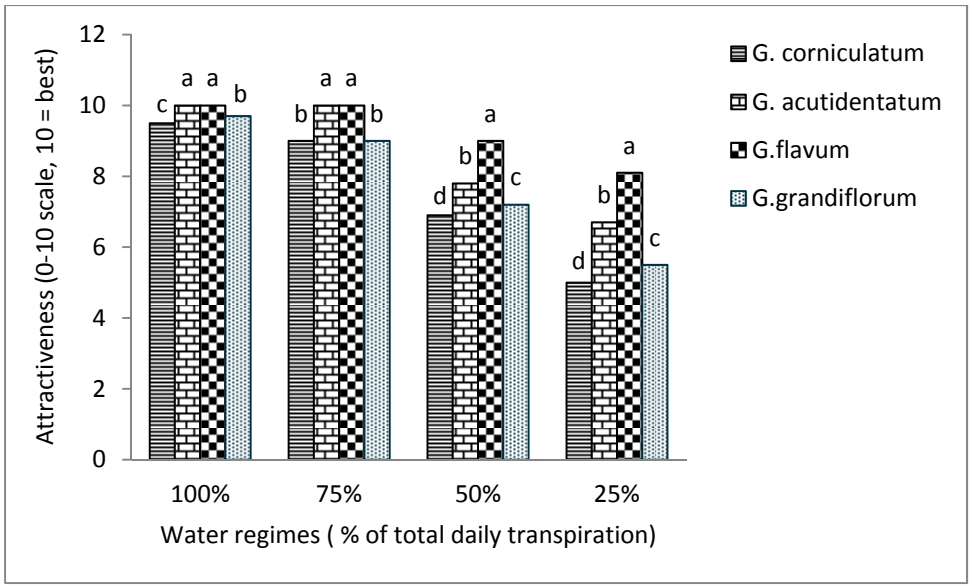

Figure 3. 7: Effect of four different water regimes on the attractiveness of four *Glaucium spp*. Columns labeled with different letters are significantly different at P=0.05 within each water regime.

bud length and flower diameter) decreased as water relations changed because of osmotic imbalances (Küçükahmetler, 2002). Also, drought 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 fall of leaves, twig and branch die back, loss of vigor and stunted growth. Several previous studies have found similar results to our findings. Drought caused a decline in the quality of bermudagrass cultivars (Shahba, 2010b) and seashore paspalum cultivars (Shahba et al., 2013 *in press*). In addition, elevated drought may adversely affect photosynthesis and as a result adversely affect plant biomass production through reduced accumulation of carbon products (Munns and Termaat, 1986). The reduction in the number of flowers usually is more drastic than other growth parameters under high drought as it is a cumulative effect (Razmjoo et al., 2008). Fewer flowers and reduced size of flowers adversely affect the attractiveness of landscape plants.

Water use efficiency:

Drought avoidance is an important drought resistance strategy. Drought avoidance can be achieved through the reduction in water use or water loss through the canopy and increasing water uptake of roots from deeper soils. ET is a measure of water use efficiency and is an indicator of plant vigor. ET varied significantly ($P < 0.05$) among species under different water regimes, among water regimes and their interaction (Table 3-1). Regression analysis indicated a significant negative linear relationship between water regimes and ET rates (Table 3-3). ET rate declined with the reduction in irrigation water. The decline in ET rate under lower water regimes was more severe and more rapid (Table 3-3). *G. flavum* showed lower ET rates under all water

regimes when compared to *G. acutidentatum*, *G. grandiflorum* and *G. corniculatum*. *G. corniculatum* had the highest ET rates at all water regimes (Fig. 3-3). Transpiration efficiency (TE) has been identified as one of the important physiological traits for improving drought adaptation of plants. The variation in TE is associated with variation in photosynthetic capacity per unit leaf area because thicker leaves usually have a higher density of chlorophyll per unit leaf area and hence have a greater photosynthetic capacity when compared with thinner leaves. Leaf thickness may also affect plant quality. *G. flavum* showed greater TE since it was able to maintain its ET at lower rates while maintaining higher attractiveness when compared with *G. acutidentatum* which was next in TE with *G. grandiflorum* and *G. corniculatum* which had the lowest TE (Table 3-3). Many species have shown considerable interspecific diversity for various environmental stresses, including drought (Duncan and Carrow, 1999; Trenholm et al., 1999; Lee et al., 2004c). Kim and Beard (1988) found that species/cultivar differences in ET rates under non-limiting soil moisture conditions were associated with canopy resistance and total leaf area. High canopy resistance and/or a low leaf area resulted in lower ET. Arunyanark et al. (2008) reported a reduction in transpiration rate as a result of drought while the transpiration efficiency, as indicated by total dry matter production, was increased in peanut (*Arachis hypogaea* L.).

Osmotic adjustment:

Osmotic adjustment facilitates water uptake and limits water loss from cells. Thus tissues may sustain metabolic and physiological functions under drought stress in addition to the

Table 3. 3. Effect of different water regimes on daily ET (mm d^{-1}) of *Glaucium spp.* linear regression of different ET rates vs. water regimes of control (C), 75, 50, and 25% of the total evapotranspiration.

Species	ET rate (mmd^{-1}) Water regimes (% of total ET)				Regression	R^2
	C	75	50	25		
G. acutidentatum	4.0c†	3.4c	2.4c	1.7c	Y = 22.0 – 0.9 X	0.79**
G. corniculatum	5.2a	4.5a	3.0a	2.5a	Y = 12.6 – 1.2 X	0.64*
G. flavum	4.0c	3.3c	2.2c	1.2d	Y = 11.8 – 1.6 X	0.80**
G. grandiflorum	4.4b	3.9b	2.7b	2.2b	Y = 10.7 – 0.8 X	0.72*

† 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 $P \leq 0.01$.

stability of cell membrane. Tested osmotic adjustment parameters included shoot total nonstructural carbohydrates, total reducing sugar content and shoot proline content.

Shoot Total Nonstructural Carbohydrates and Total Reducing Sugar Content:

Shoot TNC varied significantly among species, water regimes and their interaction (Table 1). Increasing drought decreased shoot TNC of *Glaucium spp.* (Table 3-4). Regression analysis indicated a significant negative linear relationship between water regimes and TNC content (Table 3-4). In *G. flavum*, as water regimes increased from control to 75, 50 and 25 % of the total ET, average TNC decreased by 15.1, 30.3 and 48.0% while the average TNC decrease in *G. acutidentatum* shoots was 21.6, 40.1, and 53.7%. The decrease in *G. grandiflorum* was 21.4, 42.7 and 54.8% while the decrease in *G. corniculatum* was 27.0, 53.7 and 59.4%, respectively. A decline in TNC was most likely due to the decline in photosynthesis because of stomatal closure as a water saving mechanism. Shoot RSC varied significantly among species, water regimes

Table 3. 4. Total nonstructural carbohydrates (TNC) in shoots of *Glaucium spp.* measured at the end of the experiment vs. water regimes of control (C), 75, 50, and 25% ET.

Species	TNC (mg g^{-1} dry wt) Water regimes (%)				Regression	R^2
	C	75	50	25		
G. acutidentatum	120.5b†	94.5b	72.2b	55.8b	Y = 122.5 – 2.1X	0.82**
G. corniculatum	98.3d	71.8d	45.5d	39.9d	Y = 108.6 – 2.0X	0.79*
G. flavum	126.6a	107.5a	88.2a	65.8a	Y = 107.2 – 1.9X	0.86**
G. grandiflorum	103.8c	81.6c	59.5c	46.9c	Y = 115.3 – 1.8X	0.76*

† 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 $P \leq 0.01$.

and their interactions (Table 3-1). RSC response to different drought treatments followed a different trend than TNC (Table 3-5). Reducing sugars in plants mainly consists of glucose and fructose (Ball et al., 2002; Shahba et al., 2003). While nonstructural carbohydrates are energy reserves in plants, soluble reducing sugars are thought to play an important role in drought, salinity and freezing tolerance as osmoregulators and as protectants as they prevent cell desiccation (Popp and Smirnoff, 1995). Regression analysis indicated a significant positive association between drought and RSC content in all species at all water regimes (Table 3-5). As water regimes increased from control to 75, 50 and 25% ET, average RSC increased by 40.7, 101.8 and 166.5 % in *G. flavum* and by 17.4, 40.0 and 103.4% in *G. acutidentatum.* The increase was 122.2, 39.6, and 90.6% in *G. grandiflorum* and 4.4, 26.5, and 62.5% in *G. corniculatum*, respectively. Carbon reduction could be related to the drought resistance mechanisms that are energy dependent. The results suggested that carbohydrate availability was a limiting factor for shoot growth under high drought stress. Shahba (2010b) found an increase in RSC and a

decrease in TNC with drought increase in bermudagrass species (Tifgreen, Tifdwarf and Tifway) and seashore paspalum cultivars (Shahba et al., 2012, 2013 *in press*).

Table 3. 5. Total reducing sugar content (RSC) in shoots of *Glaucium spp.* measured at the end of the experiment vs. water regimes of control (C), 75, 50, and 25% ET.

Species	RSC (mg g^{-1} dry wt) Water regimes (%)				Regression	R^2
	C	75	50	25		
G. acutidentatum	17.8	20.9b†	24.2b	36.2b	Y = 20.5 + 0.14 X	0.78**
G. corniculatum	13.6	14.2d	17.2d	22.1d	Y = 15.2 + 0.13 X	0.70*
G. flavum	16.7	23.5a	33.7a	44.5a	Y = 10.9 + 0.25 X	0.88**
G. grandiflorum	14.9	18.2c	20.8cb	28.4c	Y = 14.5 + 0.14 X	0.75*

† 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 $P \leq 0.01$.

Soluble carbohydrates may interact with membrane phospholipids and proteins to stabilize their structures and prevent desiccation under drought stress (Popp and Smirnoff, 1995). TNC serves as the resource for the increased RSC under drought conditions. The balance between carbohydrate production and consumption impacts the ability of plants to cope with stresses (Huang and Fry, 1999; Lee et al., 2008a, 2008b; Shahba, 2010b, Shahba et al., 2012).

Shoot Proline Content:

Shoot proline content varied significantly among species, water regimes and their interaction (Table 3-1). Increasing drought increased shoot proline content of *Glaucium* species. The increase in proline content was more obvious with increasing drought (Table 3-6). As water regimes decreased from control to 75, 50 and 25% average proline content in shoots increased by 186, 325, and 472% in *G. flavum,* 163, 303 and 517% in *G. acutidentatum,* 160, 280 and 418%

in *G. grandiflorum* and 80, 190, and 340% in *G. corniculatum,* respectively. Regression analysis indicated a significant positive association between drought and proline content in all species (Table 3-6). Although the role of proline accumulation in drought tolerance is well documented in this study, it has been questioned by others (Ashraf and Harris, 2004). Our results suggest a positive role for proline in *Glaucium* species drought tolerance. A positive effect of proline accumulation in drought tolerance was also reported in seashore paspalum cultivars (Shahba et al., 2013 in press). Accumulation of proline in plant tissues in response to drought stress has been attributed to enzyme stabilization and/or osmoregulation (Flowers et al., 1977; Levitt, 1980). It could act as a sink for carbon and nitrogen for stress recovery, and may buffer cellular redox potential under drought stress (Ashraf and Foolad, 2007). Maggio et al. (2002) suggested that proline may act as a signaling/regulatory molecule able to activate multiple responses that participate in the adaptation process to environmental stresses. Little is known of metabolic factors controlling root survival in drying soils and the proteins or genes associated with the accumulation of osmolytes (Huang, 2008). The accumulation of solutes in leaves, such as soluble sugars, inorganic ions, and proline has been associated with osmotic adjustment and increased drought tolerance in Kentucky bluegrass (Jiang and Huang, 2001), tall fescue (Richardson et al.,

Table 3. 6. Proline content in shoots of *Glaucium spp.* measured at the end of the experiment vs. water regimes of control (C), 75, 50, and 25% ET.

Species	Proline content (µg g^{-1} fresh wt) Water regimes (%)				Regression	R^2
	C	75	50	25		
G. acutidentatum	243.0	639.0b†	980.0b	1499.0b	Y = 218.3 + 22.9 X	0.82**
G. corniculatum	226.9	408.0d	659.0d	998.0d	Y = 144.5 + 14.5X	0.72*
G. flavum	281.5	805.0a	1195.0a	1610.0a	Y = 139.6 + 11.4 X	0.90**
G. grandiflorum	223.2	580.0c	849.0c	1155.0c	Y = 172.2 + 18.4 X	0.74*

† 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 $P \leq 0.01$.

1992), perennial ryegrass (Thomas, 1990), and zoysiagrass (Qian et al., 1997). Osmotic adjustment has also been observed in roots of crops which contribute to the maintenance of root turgor and elongation in dry soils (Sharp et al., 1990). A positive correlation between the capacity of osmotic adjustment and recovery from prolonged drought has been reported in several species, where species with the greatest osmotic adjustment regrew faster after watering (DaCosta and Huang, 2006). Any cultural practice that promotes accumulation of osmotic solutes during drought stress should be helpful in landscape plants for rapid recovery from that stress. On the basis of best results relative to categories for leaf characteristics, plant height, flowering characteristics, overall plant quality (attractiveness), water use efficiency, TNC, RSC, and proline, *G. flavum* was found to have greater drought tolerance when compared to *G. acutidentatum, G. grandiflorum* and *G. corniculatum.* In summary, as drought increased, *Glaucium* spp. exhibited reduction in leaf characteristics, plant height, flowering characteristics, overall plant quality (attractiveness), TNC, and ET rate, and increased shoot total reducing sugars and proline content. *G. flavum* showed greater drought tolerance at all water regimes compared to the other tested species. Proline accumulation could add to the drought tolerance through osmoregulation or by acting as carbon and nitrogen sink for stress recovery.

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