THE LIBYAN JOURNAL OF AGRICULTURE
Volume (25): No. (1-2), 2020: 66 -74

Original Article

Effect of Humic Acid on Vegetative and Reproductive Growth of Squash (*Cucurbita pepo* L.) under Saline Stress Conditions

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ABSTRACT

In order to investigate the effect of Humic acid (HA) on growth and development of squash plants (*Cucurbita pepo* L.) cv. Alexandria F1 under salinity conditions, three concentrations of HA: 0, 200 and 400 ppm and four concentrations of salinity (NaCl): 500 (tap water), 1000, 2000 and 3000 ppm were used with irrigation. The number of leaves (NL), length (LP) and thickness (TP) of petioles, leaf area (LA), fresh weight (FWP), dry weight (DWP) and percentage of dry weight of plants (PDW) were measured at the end of the vegetative stage. The sex ratio of female flowers (SR), number of fruits (FN), fresh (FFW) and dry (FDW) weight of fruits were measured during the reproductive stage. The results indicate that treatment with HA led to a reduction in the severity of salinity effect in some of the vegetative parameters under salinity condition compared to the control treatment. In contrast, the reproductive parameters were decreased as the salinity increased without an apparent effect of applying the used HA concentrations. The results of this experiment suggest that the ability of HA treatment to ameliorate the vegetative growth process was not sufficiently reflected in the reproductive growth under salinity condition. Therefore, the lack of amelioration may be attributed to the inefficiency of the HA concentrations used in this experiment.

Keywords: Cucurbita pepo L.; Humic acid; NaCl; Salinity.

Introduction

Salt stress is one of the most important agricultural problems facing the world, especially in arid and semi-arid regions. International studies have estimated that more than 50% of the arable land would be salinized by the year 2050 (Jamil *et al.*, 2011). In many regions the lands were affected by salinity as a result of irrigation with high salinity groundwater wells (Brindha and Schneider, 2019; Brusseau, 2019). Salinity appears as a result of accumulation of dissolved salts such as sodium and chloride ions. Increasing the concentration of sodium ions in the soil solution leads to an increase in the exchange capacity of

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Received: 5/7/2020 **Accepted: 5 / 9/ 2020**

sodium, which causes a change in the characteristics and composition of the soil (Ashraf *et al.*, 2002), and the difficulty of applying the agricultural operations because of the dispersed colloids. Therefore any program to reduce the effect of salinity will necessarily lead to increased plant growth and productivity.

Squash (*Cucurbita pepo* L.) is one of the most important crops of the Cucurbitaceae family. It is a summer crop that can be grown most of the year. Plants are usually irrigated with freshwater, which is saline in large areas of Libya and leads to a negative impact on plants. Abd *et al.* (2013) found that salinity affected the squash plants and reduced the number and area of the leaves, whereas Elwan and El-shatoury (2014) found that saline stress caused a decrease in the number of leaves, fresh weight and reduced yield of plants. Abd *et al.* (2013) indicated that salinity led to a decrease in the early and total yield of quash, and decreased the weight, length and diameter of the fruits.

Humic acid (HA) is one of the organic acids that are produced naturally as a result of biological and chemical processes from the decomposition of plant and animal waste by microorganisms (Khaled and Fawy, 2011). HA helps to overcome the harmful effect of saline stress by its association with sodium, helping the plant to tolerate high concentrations of it and protecting it against the toxic effect and the osmotic problems associated with salt high concentrations (Turan *et al.*, 2011). HA increases the internal osmotic pressure of plant cells and increases plant tolerance to water shortages. In saline soils, the HA leads to a reaction in the soil as a result of its ability to ions exchange, as the cations are bonded and chelated by HA, such as calcium and magnesium as well as release of anions, thus reducing the osmotic pressure caused by the accumulation of salts on the plant roots (Türkmen *et al.*, 2005).

Hartwigsen and Evans (2000) found that adding HA to substrate increased the fresh weight of squash plant roots, and increased the length of the secondary roots. Jasim *et al.* (2015) also mentioned that adding HA increased the number of leaves for squash. Another study demonstrated that adding HA increased the fresh and dry weight of squash fruits and the number of feminine flowers (Esho and Saeed, 2017). Al-Zebari and Sarhan (2019) also indicated that adding HA with irrigation water increased the number of fruits per plant, the average weight and length of the fruit and the total yield of squash. Ozdamar Unlu *et al.* (2011) indicated that there were no differences in results obtained by applying humic acid either by spraying or with irrigation to produce cucumbers. Eshghi and Garazhian (2015) used different levels of HA up to 1200 mg per liter for strawberries and got good results, while Karakurt *et al.* (2009) used lower levels than that and obtained acceptable results. It appears that there are several factors determining the concentrations of HA that can be used to improve the productivity of many crops, such as the type of crop, the degree of soil fertility, etc.

This study, therefore, aimed to investigate the effects of HA application with saline irrigation water on the vegetative and reproductive growth of squash.

Materials and Methods

Plant material, growth conditions and treatments:

The experiment was carried out by cultivating the commercial variety "Alexandria F1" of the squash plant (*Cucurbita pepo* L.) in the research station (Faculty of Agriculture - University of Benghazi - Libya). Seeds were sowed in a silty clay soil mixed with sand and compost (1:1:1 vol.) in 30 liter plastic bags. Soil composition was 1.27% organic matter, 20% calcium carbonate, pH 7.56, EC 0.98 dSm^{-1}, available P 10.4 ppm and total N 0.11%. Throughout the experiment, the plants were fertilized by modified Hoagland Solution as a complete nutrient solution (NS) with irrigation. The full NS contains (in $mmol.L^{-1}$) 5 KNO_3, 5 $Ca(NO_3)_2·4H_2O$, 2 $MgSO_4·7H_2O$, 1 KH_2PO_4, mixture of 0.02 $FeSO_4·7\ H_2O$; 0.02 Na_2- EDTA; 2 H_2O; 0.045 H_3BO_3; 0.01 $MnCl_2·4\ H_2O$, and (in µmol/L) 0.8 $ZnSO_4·7\ H_2O$, 0.4 $Na_2MoO_4·2\ H_2O$, and 0.3 $CuSO_4·5\ H_2O$. Plants were grown under a plastic canopy for protection from rain at 14h photoperiod, photosynthetic active radiation reached a daytime peak value of 1200 $µmol.m^{-2}.s^{-1}$, the temperature and relative humidity were 30/18°C and 40/75% during day/night periods respectively.

Treatments were initiated when the plants reached the first leaf stage by adding different concentrations of mixed solutions of NaCl and humic acid (HA) with irrigation water. Plants were irrigated daily using 4 levels of salinity, tap water (the concentration of salts is about 500 ppm), 1000, 2000 and 3000 ppm, prepared by adding 0, 500, 1500 and 2500 $mg.L^{-1}$ of NaCl to tap water, and; 3 levels of humus solution, 0, 200 and 400 ppm of HA. Irrigation water was added according to the needs of the plants to reach field capacity. Treatments continued until the beginning of the fruit maturity stage.

Vegetative stage measurements:

Plants designated for the measurements were cut off at the end of the vegetative stage; the number of leaves (NL) of the plant was counted, the length (LP) and thickness (TP) of petioles were measured and leaf area (LA) was also estimated by the correlation between leaf area and leaf fresh weight (Watson, 1937). Fresh weight of plants (FWP) were measured, then plants were dried for three days in an oven at 65 °C (until there was no decrease in weight) for determination of dry weight (DWP) and percentage of dry weight (DW%).

Reproductive stage measurements:

At flowering stage; total number of flower buds (TFB), male flower buds (MFB), female flower buds (FFB), percentage of female flowers (PFF) and sex ratio of female flowers (SR) were calculated. SR was calculated by dividing the number of female flowers by the number of male flowers produced by a plant. Additionally, when the fruits reached maturity; the number of fruits (FN) was counted for each plant, and the fresh (FFW) and dry (FDW) weights of fruits were measured.

Experimental design and statistical analysis:

The data represent the mean values of two independent experiments performed during the summer seasons of 2018 and 2019. Each experimentation was conducted using four replicates for each treatment, using factorial experimental 4×3 in a completely randomized design, with the treatments of salinity and humic acid. Data were subjected to analysis of variance using a two-way ANOVA test and means were compared by Duncan's means test ($P<0.05$) using the SAS GLM procedure (SAS Institute, Cary, NC).

Results and discussion

Vegetative growth:

The results of some vegetative parameters are shown in Table 1. Salinity decreased both of plant fresh and dry weights (FWP and DWP) significantly. On the other hand, the HA treatment reduced the salinity effect on FWP (Table 1.), this result was consistent with those of other studies (Turan *et al.*, 2011; Türkmen *et al.*, 2005). Despite this positive effect of HA treatment on biomass, other studies confirm that salinity reduces the overall growth rate of the plant (Shannon *et al.*, 1987; Cuartero and Fernandez-Munoz, 1999; Maggio *et al.*, 2004; Hajer *et al.*, 2006). On the other hand, the highest salinity concentration increased the percentage of dry matter (PDW) in plant tissues regardless of the concentration of HA used (Table 1.). Similar results were also reported by Abdul Qados (2011) where the salinity increased the dry weight but reduced the fresh weight. Therefore, an important development occurred by accumulation of substances which responsible to increasing the osmotic potential such as sugars, where the dry matter forms the largest part of solutes in the cell. Thus, an osmotic adjustment may allow the cells to restore their ability to absorb water under conditions of saline stress (Fageria *et al.*, 2006).

The number of leaves (NL), thickness of petioles (TP) and leaf area (LA) were significantly affected by salinity, while HA treatments led to increasing the values of these parameters (Table 1.). Whereas the high concentrations of salt and HA decreased the length of the petioles (LP) (Table 1.). Although a previous study reported that NL did not always decrease under the influence of salt stress (Jasim *et al.*, 2015), these parameters may be an acceptable indicator reflecting the effects of salt stress. The results showed a relative improvement in the growth of vegetative parts with HA treatments under saline stress conditions.

Reproductive growth

Table 2. shows the effect of salinity and HA treatments on reproductive growth parameters of squash. Salinity decreased the total number of flower buds (TFB), the number of female buds (FFB) and the percentage of female flowers (PFF) regardless of the concentration of HA treatment (Table 2.). In contrast, the number of male flower buds (MFB) was slightly increased which decreased the sex ratio of female flowers (SR). parameters.

Table 1. Effect of salinity and humic acid (HA) treatments on fresh weight of plants (FWP), dry weight of plants (DWP), percentage of dry weight (PDW), number of leaves (NL), length of petioles (LP), thickness of petioles (TP) and leaf area (LA) of squash plants.

Treatments		Measurements						
Salinity (ppm)	HA (ppm)	FWP (g/plant)	DWP (g/plant)	PDW (%)	NL (Leaf)	LP (cm)	TP (cm)	LA (cm^2)
Tap Water	0	321^{a}	37^{a}	11.7^{cde}	14.8^{bc}	27.3^{ab}	$18.0b^{cd}$	4170^{abc}
	200	333^{a}	38^{a}	11.5^{ef}	15.0^{abc}	27.5^{a}	20.3^{a}	4334^{ab}
	400	338^{a}	39^{a}	11.6^{de}	14.5^{bc}	25.8^{b}	19.5^{ab}	4415^{a}
1000	0	293^{b}	37^{a}	12.5^{a}	13.3^{cd}	25.8^{b}	19.8^{ab}	3934^{bcd}
	200	318^{a}	36^{a}	11.3^{ef}	16.0^{ab}	27.8^{a}	17.0^{bcd}	4213^{abc}
	400	331^{a}	39^{a}	11.7^{cde}	16.5^{a}	25.8^{b}	18.8^{abc}	4336^{ab}
2000	0	267^{d}	30^{c}	11.5^{ef}	13.0^{d}	24.0^{cd}	18.5^{abc}	3778^{de}
	200	292^{b}	31^{c}	10.7^{cde}	14.3^{bc}	24.8^{bc}	17.5^{bcd}	4053^{bcd}
	400	280^{bc}	31^{c}	11.1^{fg}	14.0^{bc}	22.1^{e}	20.0^{a}	3722^{de}
3000	0	233^{e}	29^{c}	12.4^{ab}	12.8^{d}	20.4^{f}	16.8^{cd}	3432^{e}
	200	271^{c}	33^{b}	12.0^{bcd}	14.3^{bc}	23.1^{de}	18.8^{abc}	4030^{bcd}
	400	285^{b}	35^{b}	12.1^{abc}	14.3^{bc}	21.0^{ef}	18.5^{abc}	4100^{abc}

Each value represents the mean values of two independent experiments, four replicates for each experiment. Means followed by the same letter in each column are not significantly different by Duncan's multiple range test at 5% level. Salinity has been increased by NaCl addition. Salinity of tap water is about 500 ppm.

Salt stress decreased the number of fruits (FN) and the fresh and dry weight of fruits (FFW and FDW) without clear effect for HA on these Researches have indicated that the treatment of squash plants with HA under conditions of saline stress led to ameliorate of some vegetative and reproductive measurements (Hartwigsen and Evans, 2000; El-Masry *et al.*, 2014). Results of this experiment did not record improvements in reproductive measurements, despite the relative improvement in vegetative measurements, may be because of the variation in the used concentrations of HA.

Conclusions

Previous studies showed that HA reduces the harmful effect of saline stress on plants. This experiment demonstrated that the concentration of HA is a limiting factor and that its effect on the vegetative growth can be different from the reproductive ones.

Table 2. Effect of salinity and humic acid (HA) treatments on total number of flower buds (TFB), male flower buds (MFB), female flower buds (FFB), percentage of female flowers (PFF), sex ratio of female flowers (SR), fruit number (FN), fruit fresh weight (FFW) and fruit dry weight (FDW) of squash plants.

Treatments		Measurements							
Salinity (ppm)	HA (ppm)	TFB (No.)	MFB (No.)	FFB (No.)	PFF (%)	SR (f:1m)*	FN (No.)	FFW (g/plant)	FDW (g/plant)
Tap Water	0	19.2[a]	6.2[b]	13.0[ab]	68[a]	2.01[a]	6.2[a]	233[a]	15.0[a]
	200	20.5[a]	6.0[b]	14.5[ab]	71[a]	2.40[a]	3.7[b]	193[a]	11.6[b]
	400	20.8[a]	5.2[b]	15.5[a]	75[a]	2.90[a]	6.2[a]	218[a]	14.0[a]
1000	0	19.2[a]	6.5[b]	12.7[b]	66[ab]	1.92[a]	3.5[bc]	126[b]	8.0[c]
	200	20.2[a]	6.2[b]	14.0[ab]	70[a]	2.21[a]	2.0[cde]	140[b]	9.5[c]
	400	20.2[a]	7.0[b]	13.2[ab]	66[ab]	1.85[a]	2.7[bcd]	134[b]	9.2[c]
2000	0	16.2[bc]	7.2[a]	9.0[c]	56[b]	1.23[b]	1.2[e]	33[d]	2.1[e]
	200	18.2[b]	9.2[a]	9.0[c]	49[b]	1.00[b]	1.7[de]	73[c]	5.3[d]
	400	17.5[bc]	8.2[ab]	9.2[c]	53[b]	1.10[b]	1.2[e]	36[cd]	2.1[e]
3000	0	16.0[c]	7.0[b]	9.0[c]	57[b]	1.24[b]	1.0[e]	26[d]	1.8[e]
	200	15.8[c]	6.7[b]	9.0[c]	58[b]	1.31[b]	1.7[de]	43[cd]	2.3[e]
	400	16.8[bc]	7.2[a]	9.5[c]	57[b]	1.29[b]	1.2[e]	38[cd]	2.6[e]

Each value represents the mean values of two independent experiments, four replicates for each experiment. Means followed by the same letter in each column are not significantly different by Duncan's multiple range test at 5% level. Salinity has been increased by NaCl addition. Salinity of tap water is about 500 ppm. * Number of female:one male flower.

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تأثير حمض الهيومك على النمو الخضري والثمري للكوسة (*Cucurbita pepo* L.) تحت ظروف الإجهاد الملحي

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المستخلص

لدراسة تأثير حمض الهيوميك (HA) على نمو وتطور نباتات الكوسة (*Cucurbita pepo* L.) صنف Alexandria (F1) تحت ظروف الملوحة، استخدام ثلاثة تركيزات من HA: 0، 200 و 400 جزء في المليون وأربعة تركيزات للملوحة NaCl: 500 (ماء الصنبور)، 1000، 2000 و 3000 جزء في المليون، مع الري. تم قياس عدد الأوراق (NL)، طول (LP) وسماكة (TP) أعناق الأوراق ، مساحة الورقة (LA)، وزن النبات الرطب (FWP)، وزن النبات الجاف (DWP) والنسبة المئوية للمادة الجافة للنبات (PDW) عند نهاية مرحلة النمو الخضري. تم قياس النسبة الجنسية للأزهار الأنثوية (SR)، عدد الثمار (FN)، وزن الثمرة الطازج (FFW) ووزن الثمرة الجاف (FDW) خلال مرحلة الإثمار. أشارت النتائج إلى أن المعاملة بالـ HA أدت إلى تقليل شدة تأثير الملوحة في بعض القياسات الخضرية تحت ظروف الملوحة مقارنة بمعاملة المقارنة. بالمقابل، انخفضت القياسات الإنتاجية مع زيادة الملوحة دون وجود تأثير واضح لتطبيق تركيزات HA المستخدمة. تشير نتائج هذه التجربة إلى أن قدرة HA على تحسين عملية النمو الخضري لم تنعكس بشكل كافٍ في النمو الثمري تحت ظروف الملوحة. لذلك، قد يعزى نقص التحسين إلى عدم كفاءة تركيزات HA المستخدمة في هذه التجربة.

الكلمات الدالة: Cucurbita pepo L.، حمض الهيومك، NaCl، الملوحة.

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استلمت بتاريخ: 2020/7/5 **أجيزت بتاريخ: 2020/9/5**