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Original Article

Evaluation of salt tolerance of Pepper plant (*Capsicum annuum* L., cv. Jalapino) cultivated in greenhouse

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ABSTRACT

Soil salinization is an increasing problem for many areas throughout the world especially in arid and semi-arid regions which reduces vegetables and crop production in general. The variability of crop salt tolerance under different environmental conditions requires species-specific and environment-specific field evaluations of salt tolerance. In this study, Pepper (*Capsicum annuum* L., cv. *Jalapino) p*lants were grown in greenhouse under six different irrigation treatments: a nonsalinized control (EC = 0.11 ds m^{-1}) and five concentrations of diluted sea water, corresponding to EC of 2.0, 4.0, 6.0, 8.0 and 10.0 ds m^{-1} in order to evaluate plant development as well as leaves and fruit content of some compounds related to salinity stress. Irrigation water with an EC of 2 ds m^{-1} or more resulted in progressive reduction in all parameters tested (plant height, branches number, leaves number, nodes number, node length, flowers number, fruit number, fruit length, fruit diameter, and fruit weight). For instance, increasing the electrical conductivity of the irrigation water to 2.0 ds m^{-1} caused reduction of (8%, 34%, 12%, 6%, 13%, 18%, 37%, 11%, 6%, 24%) of parameters tested, respectively. While increasing the electrical conductivity of the irrigation water to 6 ds m^{-1} caused reduction of (42%, 67%, 47%, 43%, 22%, 56%, 68%, 42%, 19%, 50%) of parameters tested, respectively, compared to control. The regulation of ascorbic acid content in leaves or fruit and chlorophyll content in leaves was most likely functionally associated with modifications of the plant, for instance, irrigation with 2 ds m^{-1} caused reduction of ascorbic content of 12% in leaves and 8% in fruit, and reduction of chlorophyll content of leaves of 21%, compared to control. While increasing the electrical conductivity of the irrigation water to 6 ds m^{-1} caused a reduction of ascorbic content to 23% in leaves and 26% in fruit, and reduce chlorophyll content in leaves to 24% compared to control. Irrigation water with 8 and 10 ds·m^{-1} highly affected all parameters tested specially fruit number where no fruit was obtained, at the same time flower number was highly diminished to 4% compared to control.

Keywords: Pepper (*Capsicum annuum* L, cv Jalapino), salinity stress, Chlorophyll, Ascorbic acid..

Introduction

The total agriculture land annually decreases, the available land is estimated to be 5 billion ha; of which about 40% arid and semi-arid, and about 30% of the arid and semi-arid land

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categorized as salt-affected (Ashraf, 1994). It is well recognized that salt affected soils are expanding. Consequently, searching for a solution of sustainable agriculture in salt-affected regions is important, and extensive research in this regard has been devoted worldwide. One strategy is to undertake a project of reclamation, transforming salt-affected lands into arable ones. This strategy is expansive and might be impractical due to the application of poor quality water in irrigating crops. Other strategies such as select and breed for cultivars with salt tolerance enhancement (Collins *et al.*, 1990). Salinity affects almost every aspect of the physiology and biochemistry of plants which in turn significantly reduces yield (Ashraf, 1994; Parida and Das, 2005; Munns and Tester, 2008). Salinity undesirably affects seeds and seedlings in most of the crops (Sivritepe *et al.*, 2003; Ashraf and Foolad, 2005) and greatly contributes in establishment of poor stand and eventually poor production of vegetable crops (Grassbaugh and Bennett, 1998). Hot pepper (*Capsicum annuum L.*), commonly known as chilli, is an important vegetable as well as spice crop in Libya. Pepper has important nutritional value, which are excellent source of natural colours and antioxidant compounds (Howard *et al.*, 2000). They have been recognized as being beneficial for prevention of widespread human diseases, including cancer and cardiovascular diseases, when taken daily in adequate amounts (BramleyIs, 2000). Phenolic compounds retard or inhibit lipid autoxidation by acting as radical scavengers and, consequently, are essential antioxidants that protect against propagation of the oxidative chain. It is also known that vitamin C (ascorbic acid), an important compound of pepper fruits, chelates heavy metal ions (Namiki, 1990), reacts with singlet oxygen and other free radicals, and suppresses peroxidation, reducing the risk of arteriosclerosis, cardiovascular diseases, and some forms of cancer (Harris, 1996).The pepper production in Libya is estimated to be 4.794 thousand tons from an area of 1858 hectares (FAOSTAT, 2013). The yield difference is high between Libya and other Pepper growing countries, mainly due to high salinity levels in many regions especially in western coastal areas, which is main Pepper producing area. In addition to its sensitivity to salinity (Haman, 2000), Capsicum genus cultivars do not possess the same sensitivity to salinity stresses (Lopez-Serrano *et al.*, 2017; Penella *et al.*, 2013). Therefore, the study and identification of the tolerance level and mechanisms of different pepper genotypes are enormously important to breed new cultivars that can overcome salinity stresses as well as to acquire more information about genotype variability in salinity stress. The variability of crop salt tolerance under different environmental conditions requires species-specific and environment-specific field evaluations of salt tolerance. Lopez-Serrano *et al.*, (2017) showed wide variability of seven pepper accessions in response to salinity stress. Photosynthesis, stomatal conductance and transpiration reduced mainly under salinity due to stomatal and non-stomatal (Na+ accumulation) constraints and display wide variability in tolerance/ sensitivity terms in response to salinity stress (Lopez-Serrano *et al.*, 2017; Penella *et al.*, 2013). For pepper, very rare information about genotype variability in terms of its behavior under salinity stress is available. In this study, our objective was to evaluate sensitivity to salinity stress of pepper (*Capsicum annuum L. cv., Jalapino)* under greenhouse condition. *Different* plant development traits were assessed, as well as; some physiological markers that have been suggested as key traits to determine salt sensitivity such as leaves and fruit content modification of ascorbic acid and chlorophyll.

Materials and Methods

The experiment was carried out in a greenhouse in the Horticulture unit, Center of Researches and Experiments, Faculty of Agriculture, University of Tripoli in 2016. Seeds of pepper (*Capsicum annuum L., cv. Jalapino)* were obtained from a commercial company. Seeds were planted directly into pots of 4 kg capacity, each pot contains one plant. The growing media used was a mixture of field soil and peat moss 3:2 v/v. Six different water of varying EC were utilized for irrigation (0.11 ds m^{-1}, 2.0 ds m^{-1}, 4.0 ds m^{-1}, 6.0 ds m^{-1}, 8.0 ds m^{-1}, and 10.0 ds m^{-1}), EC = 0.11 ds m^{-1} is desalinated water (control), while the higher salinity levels were achieved by sea water (EC = 58.2 ds m^{-1}) dilution. Electrical conductivity of water was determined by EC—meter as explained by Jackson (1967). The plants were irrigated according to the demand, the amount added was equal to all plants, 250 ml of water was added for each pot two to three times weekly during first month, water amount was increased subsequently and retained during the experiment according to plant demand.

Plants were harvested after 4 months from seedlings. Fruits were collected at green ripening stage and the number of fruits per plant, fruit weight, fruit length, fruit diameter, and fruit number, were determined. Other parameters tested were; plant length, number of branches, leaves number, nodes number, node length and total flowers number.

Measuring chlorophyll content:

Chlorophyll measurement was performed as described by Wintermans and Mots (1965). 200 mg of fruit or of fully expanded leaf was homogenized with a mortar and pestle in 2.8 mL acetone 95%. The mixture was then strongly agitated and filtered. Filtered solution was measured at 665 nm for chlorophyll a and at 649 nm for chlorophyll b. Total chlorophyll content was calculated as follows:

Chlorophyll (a+b) = 6.1 $OD_{665\ nm}$ + 20.04 OD_{649nm} (ug/mL).

Ascorbic acid assay:

One g of samples of either fresh fully expanded leaf or fruit were cleaned, chopped into small pieces mixed with 15 mL of Metaphosphoric acid and passed through a juice extractor, the juice was filtered. The dye-titration method used was as described by Tee *et al.,* (1988), pH of Metaphosphoric acid extracts of the samples were about 1.2. The reducing capacity of the

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extracts was then measured by titrating with 2, 6-dichlorophenolindophenol (DCIP). In this oxidation-reduction reaction, ascorbic acid in the extract was oxidised to DHAA and the indophenol dye reduced to a colourless compound. End point of the titration was detected when excess of the unreduced dye gave a rose pink colour in acid solution. Ascorbic acid solution was prepared by dissolving 44 mg high-purity ascorbic acid (Sigma Chemical Company) in 250 mL deionized water. Less concentrated solutions were made by diluting the stock solution.

Experimental design:

The experimental design used was a completely randomized design. Four replicates per treatment were used, and every replicate contained four plants. Data were analyzed statistically by ANOVA, mean separation was analyzed by Duncan multiple range test at 5% level of significance.

Results and discussion

Pepper plants exposed to salt stress exhibited stunted growth and chlorotic leaves accompanied by necrotic tips. The lower leaves were shed prematurely. In general, the plants seemed to be wilting. These effects increased as the salt concentration augmented in irrigation water. Sever effects on growth parameters were recorded as salinity level increased. Visual observations indicated that Jalapino cultivar is sensitive to salinity stress.

Effect of irrigation water salinity on growth parameters:

As shown in Table 1, increased salinity caused diminutions in all parameters tested including; Plant height, branches number, leaves number, nodes number, node length, fruit length, fruit diameter, and fruit weight. The highest plant height (31.6 cm) was measured from control (0.11 ds m) whereas the lowest values from 10 ds m treatment (12.3 cm). Compared to control, a reduction in plant heights for 2 ds m^{-1}, 4 ds m^{-1}, 6 ds m^{-1}, 8 ds m^{-1}, and 10 ds m^{-1} were 8%, 21%, 42%, 54%, and 61%, respectively. Concomitant with plant heights, similar results with the same manner were obtained for branches number, leaves number, nodes number, node length, fruit length, fruit diameter, and fruit weight (table 1). Flowers number showed the highest which was recorded from control. As salinity augmented flowers number diminished, the reduction in flowers number of plants irrigated with 2 ds m^{-1}, 4 ds m^{-1}, 6 ds m^{-1}, 8 ds m^{-1}, and 10 ds m^{-1} were 18%, 31%, 56%, 96%, and 96%, respectively (Fig. 1). Jalapino pepper fruit yield per plant declined 36% when irrigating water salinity raised to 2 ds m^{-1} (Fig. 2). Navarro *et al.* (2010) reported that pepper cv. Bell fruit yield decreased 10.9% per unit increase of soil salinity after 1.2 ds m1 threshold value. A 1.5 ds m^{-1} threshold and a 14% slope value by Hoffman *et al.*, (1992) and a 1.7 ds m^{-1} threshold and a 12% slope value by Rhoades *et al.*, (1992) were reported for the same crop (pepper); however, the varieties of pepper plants have not been indicated in their studies.

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Table 1. Plant height (PlH), branches number (BN), leaves number (LN),nodes number (NN), node length (NL), fruit length (FL), fruit diameter (FD), and fruit weight (FW) of pepper (*Capsicum annuum* L., cv. Jalapino) harvested in response to irrigation treatments (nonsalinized control; EC = 0.11).

Treatment EC= ds m^{-1}	(PlH) (cm)	(BN)/ plant	(LN)/ plant	(NN)/ Plant	(NL) (cm)	(FL) (cm)	(FD) (cm)	(FW) (g)
0.11	31.6 a	7.0 a	69.6 a	12.3 a	4.0 a	3.6 a	1.6 a	5.4 a
2.0	29 b	4.6 b	61 b	11.6 a	3.5 ab	3.2 ab	1.5 a	4.1 ab
4.0	25 c	4.0 bc	61 b	9.0 b	3.2 bc	3.0 ab	1.4 a	3.4 ab
6.0	18.3 d	2.3 cd	37 c	7.0 c	3.1 bc	2.1 b	1.3 b	2.7 b
8.0	14.6 e	1,3 d	9.0 d	6.3 c	2.7 c	-	-	-
10.0	12.3 e	1.3 d	8.3 d	5.6 c	2.7 c	-	-	-

Mean separation in columns by duncan's multiple range test at $P \le 0.05$. Columns with same letter are not significantly different.

Gurjinder *et al.,* (2020) found linear function resulted in a threshold soil electrical conductivity (EC) ranging between 1.0–1.3 ds m^{-1} for five pepper cultivars , they reported also saline water with an EC ≤ 3 ds m^{-1} could be used for irrigation in rare freshwater areas. In areas where saline water is the only water available for irrigation, it is expected that a recurrent salinization of the soil will eventually modify its physicochemical properties, and subsequently affect plant production. Irrigation with an EC of 2 ds m^{-1}, which caused a reduction of 18% in plant flower and 36% in plant fruit number of Pepper cv. Jalapino (fig. 1, 2), may still be considered economically acceptable in certain Libyan areas. Doubling the EC of the irrigation water (4 ds m^{-1}) resulted in 31% and 68% reductions in plant flower number and plant fruit number respectively (fig. 1, 2), which is not considered to be economically acceptable. To compare our results with available salt tolerance data for pepper, we expressed the relative yield per plant, a yield reduction of about 36% at an EC of 2 ds m^{-1} was to some extent consistent with the one calculated by Maas and Hoffman (1977) model, based on the pepper threshold (1.5 ds m^{-1}) and slope [14% × ds m^{-1})–1]. In spite of the general use of the Maas-Hoffmann model to assess plant salt tolerance, the relationship between yield and root-zone salinity, environmental and cultural variables may affect plant response to salinity (Parida *et al*., 2005; Rhoades *et al*., 1992). Soil aeration and efficient drainage, for instance, are factors that may substantially contribute to the maintenance of tolerable salinity levels in soil for pepper plants (Dalton *et al*. 2000). The complexity of salt stress acclimatization on a whole-plant basis was discussed in detail.by Lecerda *et al*., (2005), they reported that under salt stress, severe disorder in mineral nutrients uptake and imbalances between Na, K and Ca at cellular level, and these damages play a critical role in the extent of salt tolerance of plants. They also observed that when Na absorbed and accumulated at large amounts in plants, it becomes highly toxic. Physiological damages caused by Na toxicity include disruption of K and Ca nutrition, development of water stress and induction of oxidative cell damage (Lecerda *et al.*, 2005).

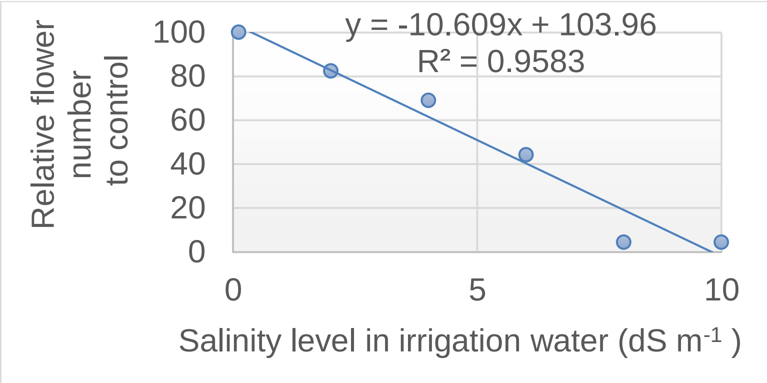

Fig. 1. Relative flower number of pepper (*Capsicum annuum* L. cv., Jalapino) irrigated with different salinity levels. The solid line represents regression and the scattered points are experimental.

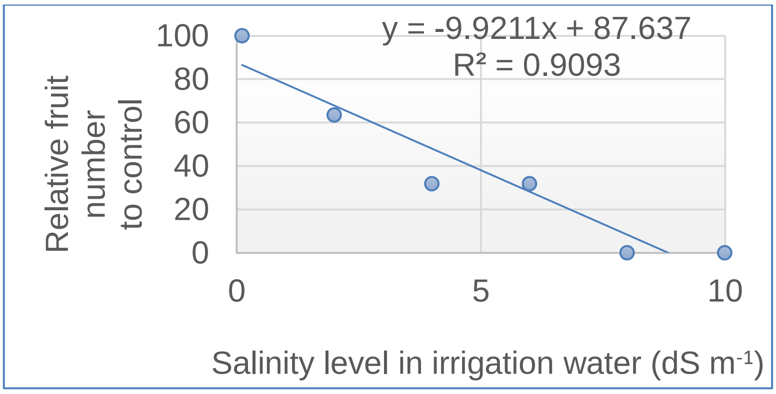

Fig. 2. Relative fruit number of pepper (*Capsicum annuum* L. cv., Jalapino) irrigated with different salinity levels. The solid line represents regression and the scattered points are experiment

In our experiment, ascorbic acid content in both leaves and fruits was affected, as salinity increased in irrigation water ascorbic acid decreased linearly (Fig.3, 4). Plants irrigated with 2 ds m $^{-1}$, 4 ds m $^{-1}$, and 6 ds m $^{-1}$ showed reduction in ascorbic acid content of 12%, 22%, and 23% in leaves (Fig. 3), and reduction of 8% 13%, and 26% in fruits, respectively (Fig. 4). Chlorophyll content in leaves was highly affected by saline irrigation water (Fig. 5), especially at high salinity (6 ds m $^{-1}$). Fully expanded leaves of control plants documented 1075 ug/ g fresh weight. Increasing salinity was linear with reduction in chlorophyll content, plants irrigated with 2 ds m $^{-1}$, 4 ds m $^{-1}$, and 6 ds m $^{-1}$ monitored reduction of 21%, 29%, and 79% of chlorophyll content respectively (Fig. 5). Salinity induced decrease in Chlorophyll has been reported earlier. Srivastava *et al.*, (1988) reported chlorophyll content as one of the parameters of salt tolerance in crop plants, salinity sensitive of wheat genotype HD 2687 showed significantly higher decline in Chlorophyll than tolerant genotypes Kharchia

65 and KRL 19 under salt stress that reflects their tolerant nature (Sairam *et al.*, 2005). Hernandez *et al.*, (1995) observed higher chlorophyll degradation in salinity sensitive pea cultivar as compared to tolerant one. Sairam *et al.*, (2005) reported that tolerant wheat cv. Kharchia 65 has much better hydrogen peroxide scavenging mechanism as revealed by continuous increase in ascorbate peroxidase activities up to highest salinity level, resulting in lower H_2O_2 content and higher chlorophyll content. Consequently salt stress tolerance of Kharchia 65 as designated by lower H_2O_2 content and chlorophyll content under salt stress was mainly due to constitutively higher activity as well as salinity induced increase in some enzymes related to ascorbic acid recycling. In general, sensitive genotypes scavenging systems are very limited, and the genotype is hence weakly equipped to face salt stress as it fails to respond in a manner similar to tolerant ones, resulting in higher H_2O_2 content, and lower membrane stability and chlorophyll content under salt stress. It can be assumed that both constitutive as well as salt induced increase in antioxidant enzymes activities are important for providing protection against reactive oxidative stress. While constitutive levels provide protection from oxidative stress arising from normal oxidative metabolism, the salinity encouraged increase in antioxidant activity in response to increase in oxidative stress actually decide the level of tolerance of a plant (Sairam *et al.*, 2005).

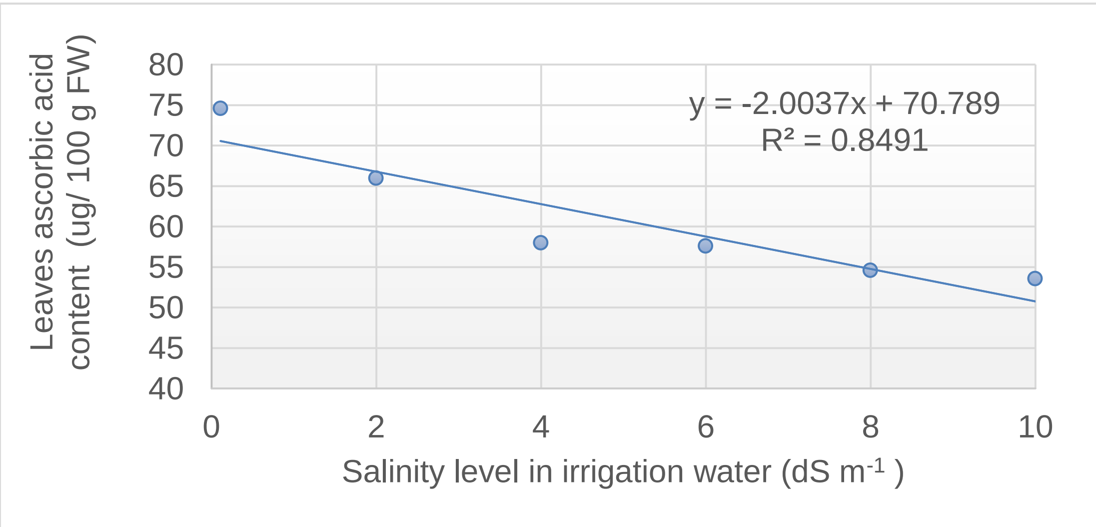

Fig. 3. Ascorbic acid content in leaves (ug/100g FW) of pepper (*Capsicum annuum* L. cv., Jalapino) irrigated with different salinity levels. The solid line represents regression and the scattered points are experimental.

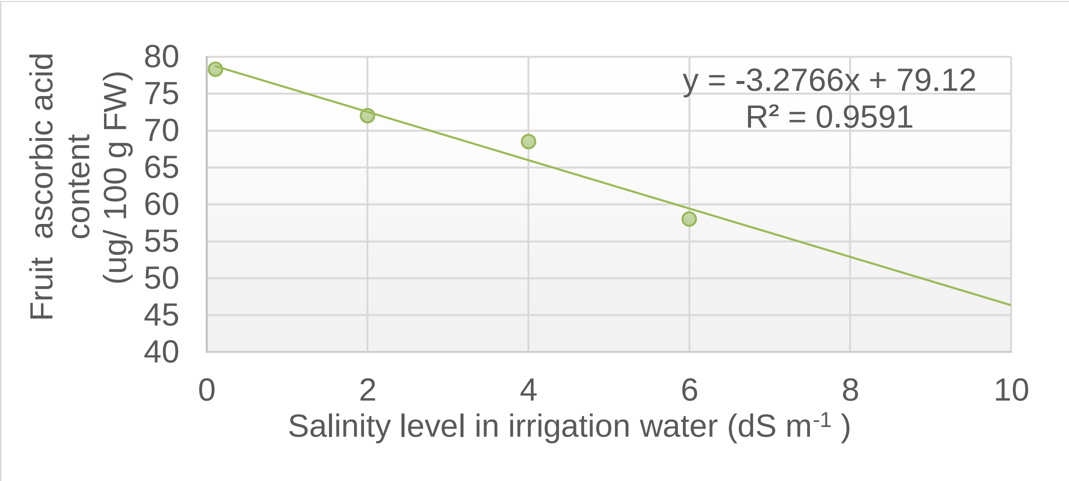

Fig. 4. Ascorbic acid content in fruits (ug/100g FW) of pepper (*Capsicum annuum* L. cv., Jalapino) irrigated with different salinity levels. The solid line represents regression and the scattered points are experimental.

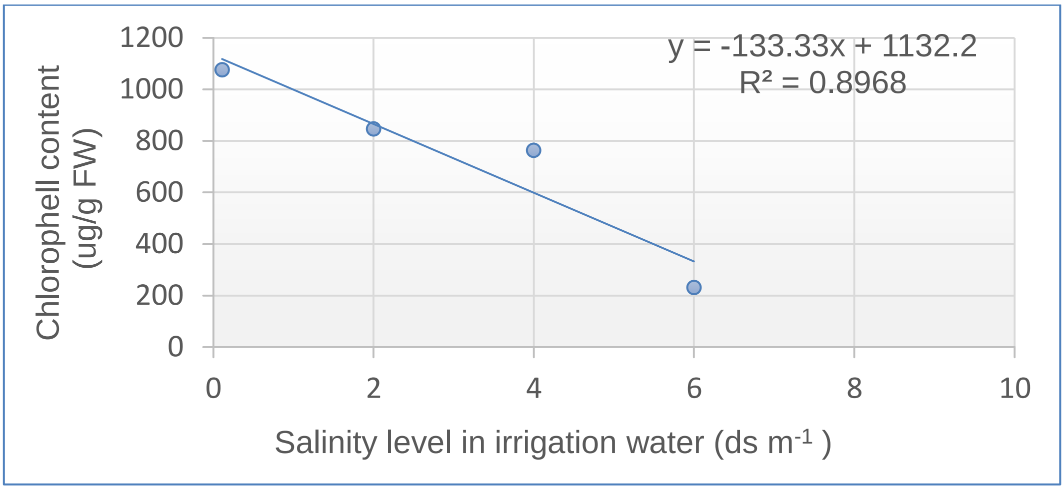

Fig. 5. Chlorophyll content in leaves (ug/g FW) of pepper (*Capsicum annuum* L. cv., Jalapino) irrigated with different salinity levels. The solid line represents regression and the scattered points are experimental.

Conclusions

We can conclude that the growth of the pepper (*Capsicum annuum* L cv., Jalapino) irrigated by saline water grown in greenhouse, showed high decrease in all parameter tested, this reduction was linear with salinity level. Yield decline was significantly, mainly due to the high incidence of both fruit number and fruit weight. The use of waters of moderate salinity (2 ds m^{-1}) in these conditions increased the incidence of this affliction. Moderate salinity (2 ds m^{-1}) caused a 36% reduction in fruit number that could be acceptable economically in certain local areas where only water of low quality is available. Doubling the salinity of the irrigation water, to 4 ds m^{-1} resulted in a 68% reduction in marketable fruit number, which is considered economically unacceptable. It is well accepted that the variability of crop salt tolerance under different environmental conditions requires species-specific and environment-specific field evaluations of salt tolerance, thus assessment of salt sensitivity under greenhouse conditions only seems to be insufficient, field investigation to assess tolerance level of pepper cv. Jalapino is highly recommended.

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ورقة أصيلة

تقييم تحمل الملوحة لنبات الفلفل .(*Capsicum annum* L) صنف Jalapino المزروعة بالصوبة

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المستخلص

ملوحة التربة مشكلة متزايدة للكثير من المناطق بالعالم خاصة المناطق الجافة والشبه جافة والتي تسبب في انخفاض في إنتاج الخضروات والمحاصيل عامة. اختلاف تحمل المحصول للملوحة تحت ظروف بيئية مختلفة تتطلب تقييم حقلي خاص لأنواع المحاصيل والبيئة. في هذه الدراسة نبات الفلفل (*Capsicum annuum* L) صنف Jalapino نمى بالصوبة تحت ستة معاملات ري مختلفة، غير ملحية الشاهد (EC= 0.11 ds m^{-1}) وخمسة تركيزات أخرى تحصل عليها من تخفيف ماء البحر ذات توصيل كهربائي [2، 4، 6، 8، 10(ds ml^{-1})] لغرض تقييم تطور النبات ومحتوى الأوراق والثمار من المركبات ذات العلاقة بالإجهاد الملحي. الري بماء ذي توصيل كهربائي 2 أو أكثر تسببت في تخفيض مضطرد بكل المعايير المختبرة (طول النبات، عدد الأفرع، عدد الأوراق، عدد العقد، طول العقدة، عدد الأزهار، عدد الثمار، طول الثمار، قطر الثمار، وزن الثمار). على سبيل المثال زيادة التوصيل الكهربائي لمياه الري إلى 2 (ds m^{-1}) تسببت بانخفاض كل المعايير المدروسة بنسبة (8%، 34%، 12%، 6%، 13%، 18%، 37%، 11%، 6%، 24%) على التوالي. بينما زيادة التوصيل الكهربائي لمياه الري إلى 6 (dc m^{-1}). تسببت في انخفاض كل المعايير المختبرة بنسبة (42%، 67%، 47%، 43%، 22%، 56%، 68%، 42%، 19%، 50%) على التوالي. الأنظمة المسؤولة على محتوى الأوراق و الثمار من حمض الاسكوربيك ومحتوى الأوراق من الكلوروفيل كانت على الأرجح مرتبطة من الناحية الوظيفية بالتغيرات في النبات، على سبيل المثال الري بماء ذي توصيل كهربائي 2 (ds m^{-1}) تسبب في انخفاض محتوى حمض الاسكوربيك بنسبة 12% في الأوراق و 8% في الثمار وانخفاض في محتوى الكلوروفيل في الأوراق بنسبة 21% مقارنة بالشاهد. بينما زيادة التوصيل الكهربائي لماء الري الى 6 (ds m^{-1}) تسببت في انخفاض كمية حمض الاسكوربيك إلى 23% في الأوراق و 26% في الثمار وقللت محتوى الكلوروفيل في الأوراق إلى 24% مقارنة بالشاهد. الري بماء ذي توصيل كهربائي 8 و 10.(ds m^{-1}) أثر بدرجة عالية على كل المعايير المختبرة خاصة عدد الثمار حيث لم نتحصل على ثمار وفي نفس الوقت عدد الأزهار انخفض بدرجة عالية وكانت فقط 4% مقارنة بالشاهد.

الكلمات الدالة: الفلفل (***Capsicum annuum* L. cv., Jalapino**) ، **الاجهاد الملحي، الكلوروفيل، حمض الأسكوربيك.**

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