Effect of Salinity on Growth and Some Physiological Characteristics of Broccoli Brassica oleracea var. Italica L. plants.

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Abstract

An experiment conducted in the faculty of science and health/ Koya university at winter season 2014-2015 to evaluate the effects of salt stress (tap water as control in addition to 100, 200 and 300 $mM.L^{-1}$ of NaCl) on growth and some physiological characteristics of broccoli *Brassica oleracea var. Italica* L. plants. The results show that increasing salinity concentration in irrigation water caused significant decrease in plants vegetative growth including plant high, leaf number, leaf area, chlorophyll a and b and total chlorophyll compared to control, whereas there were non-significant differences between the treatments in carotenoids content in leaves. Results also show that irrigation with saline water cause a significant decrease in stomata number on leaves abaxial surfaces compared to plants irrigated with tap water. Irrigation with 100 and 300 $mM.L^{-1}$ treatments decreased the stomata width on abaxial leaves surfaces significantly compared to control and 200 $mM.L^{-1}$, whereas in adaxial leaves surfaces the control and 100 $mM.L^{-1}$ salinity record the highest stomata width compared to 200 and 300 $mM.L^{-1}$ treatments.

Key words: *Brassica oleracea var. Italica* L., salinity, plant growth, photosynthesis pigments, stomata.

Introduction

The Broccoli, *(Brassica oleracea var. Italica* L.), a member of the Brassicaceae family, generally it was developed in Italy. Broccoli is considered the most important vegetable crop in the winter season, it is an edible plant whose large flowering head (the closed flowers with peduncle) used as a vegetable crop, it has a high nutritional and good commercial value, it is low in sodium food, fat and calories, high in vitamin C and good source of vitamins A and B_2 and calcium (Brouk, 1975, Matloob, 1979 and Yoldas et al., 2008).

Broccoli is belongs to the moderately tolerant to salinity (Abdul and Mohamed, 1986). Salinity is an environmental stress that limits growth and development of plant. Salt stress is one of the most serious limiting factors for crop growth and production in the arid regions. About 23% of the world's cultivated lands are saline (Khan and Duke, 2001). Soils can be saline due to geo-historical processes or they can be man-made. Soil salinity in agriculture soils refers to the presence of high concentration of soluble salts in the soil moisture of the root zone. These concentrations of soluble salts through their high osmotic pressures affect plant growth by restricting the uptake of water by the roots. Salinity can also affect plant growth because the high concentration of salts in the soil solution interferes with balanced absorption of essential nutritional ions by plants (Tester and Devenport, 2003). General symptoms of damage by salt stress are growth inhibition, accelerated development and senescence and death during prolonged exposure. Growth inhibition is the primary injury that leads to other symptoms although programmed cell death may also occur under severe salinity shock. Salt stress induces the synthesis of abscisic acid which closes stomata when transported to guard cells. As a result of stomatal closure, photosynthesis declines and photoinhibition and oxidative stress occur. An immediate effect of osmotic stress on plant growth is its inhibition of cell expansion either directly or indirectly through abscisic acid. Once sodium gets into the cytoplasm, it inhibits the activities of many enzymes (Zeinolabedin**,** 2012).

The response of plants to excess NaCl is complex and involves changes in their morphology, physiology and metabolism (Hilal et al., 1998). For example Jamil et al. (2005) mentioned that fresh shoot and root weight, leaf area and number of leaves of Brassica species were severely affected at all salinity concentrations (4.7, 9.4 and 14.1 $dS.m^{-1}$). Also Kaouther et al. (2012) reported that watering five cultivars of pepper (*Capsicum frutescens* L.) plants by saline water with NaCl concentrations (0, 2, 4, 6, 8, 10 and 12 $g.L^{-1}$) respectively, they saw that increasing salinity stress, for all cultivars, had a negative impact on roots (length, fresh and dry weights) and leaves (number and area), also, chlorophyll (a and b) amount.

Zahedi and Tohidi Moghadam (2011) reported that antioxidant enzymes activities were increased when plants were exposed to water stress. Under conditions of water stress and other types of environmental stress, reactive oxygen species, such as superoxide anion radicals, hydrogen peroxide and hydroxyl radicals, are generated (Zhu, 2000).

The objective of this investigation was to evaluate the effects of salt stress on the growth and some physiological characteristics of broccoli plants, grown in Koya city.

Materials and methods

An experiment conducted in Koya city (44°39'E, 36°05' N, and 618m of altitude) in the faculty of science and health on broccoli *(Brassica oleracea var. Italica* L. plants at winter season 2014-2015. The treatments include preparing four irrigation waters with different salinity concentrations (tap water, 100, 200 and 300 $mM.L^{-1}$), by using NaCl salt, each experimental unit consist of five pots.

The seeds were hybrid, named Cham gota and bagged by Barakat company (Syria), planted at October 14, 2014 in boxes then transplanted to plastic pots contain 3Kg of sandy clay soil, and the irrigation with saline water began at December 3, 2014.

The following parameters were studied:

- **Vegetative growth and the heads forming:** vegetative growth includes plant height, leaves number and leaf area which calculated by the method described by Watson and Watson (1953). Head forming calculated by dividing number of headed plants upon the total plants multiply by 100.
- **Percent of shoot and root dry matter**: which were calculated as it described by Al-Sahaf (1989) by divided the wet weight of leaves or root sample by the dry weight of the leaves or root sample multiply by 100.
- **Photosynthesis pigments content:** chlorophyll a, b, total chlorophyll and carotenoids were estimated according to Tang et al. (2004), in botany lab./ Faculty of Science/ Koya University, by putting 0.2 g of leaves into 100 mL mixed extracting solution (acetone : alcohol : distilled water = 4.5 : 4.5 : 1). As the samples were completely whitened at room temperature, the optical density (OD) values at 663 nm, 645 nm and 440 nm of the solution were determined with spectrophotometer (721-2000 SPECTROPHOTOMETER, China). The concentrations of chlorophyll and carotenoid were calculated according to the following formulae.
- Chl a (mg/L) = 9.784 OD663 – 0.990 OD645;
- Chl b (mg/L) = 21.426 OD645 – 4.650 OD663;
- Chl (mg/L) = 5.134 OD663+20.436 OD645;
- Car (mg/L) = 4.695 OD440 – 0.268 (Chl a+Chl b);

In order to convert the values to mg/g, the following formula were used:

Chl (Car)(mg/g) = [Chl (Car) (mg/L)×volume of mixed extracting solution mL)] / [sample mass (g) ×1000].

- **Number, length and width of stomata in adaxial and abaxial leaves surfaces:** measured by the method of lasting impressions as it described in Rai and Mishra (2013).

Statistical analysis

The experiment was conducted in completely randomized design (CRD) with five replications. Variance and comparison between treatments mean were done by Duncan test analyses ($p \leq 0.05$) using SAS statistic program (Reza, 2006).

Results and discussion

- **Effects of salinity on vegetative growth and the heads forming:**

Results in table (1) and figure (1) shows that increasing salinity concentration in irrigation water caused decreasing plant high, leaf number and leaf area significantly as compared to control, which records the highest values (35.3 cm, 12.17 leaves and 406.58 cm^2) for the prior parameters respectively, while the lowest values were record in plants irrigated with 300 $mM.L^{-1}$ salinity water (8.33 cm, 5.00 leaves and 72.02 cm^2) respectively.

Results in table (1) also shown that plants irrigated with 100 mM.L^{-1} don`t show any significant differences compared to control in head forming, while the saline water prevent heading in 200 and 300 mM.L^{-1} salinity respectively.

Table1: Effect of salinity on some vegetative growth and head forming of broccoli.

Salinity concentration	Plant high (cm)	Leaves no.	Leaf area (cm^2)	Head forming (%)
Control (Tap water)	35.3 a *	12.17 a	406.58 a	80.00 a
100 mM.L^{-1}	21.33 b	7.67 b	209.05 ab	60.00 a
200 mM.L^{-1}	9.00 c	5.33 b	83.13 b	0.00 b
300 mM.L^{-1}	8.33 c	5.00 b	72.02 b	0.00 b

* Means followed by the same letters within columns are not significantly different at p ≤ 0.05 according to the Duncan test.

Figure1. Growth and head forming of broccoli plants irrigated with different saline water.

- **Effects of salinity on shoot and root dry matter percentage:**

The results in table 2 shows increasing the salinity of irrigation water to 300 $mM.L^{-1}$ increased the shoot dry mater percentage significantly to 20.97%, while there were non-significant differences between other treatments, whereas both of control and irrigation with 300 $mM.L^{-1}$ increased the root dry mater percentage significantly to 20.35 and 18.27% compared to 100 and 200 $mM.L^{-1}$.

Table 2: Effects of salinity on shoots and root dry matter percentage of broccoli.

Salinity concentration	Shoot dry matter (%)	Root dry matter (%)
Control (Tap water)	15.62 b*	20.35 a
100 $mM.L^{-1}$	13.51 b	15.26 bc
200 $mM.L^{-1}$	14.16 b	13.58 c
300 $mM.L^{-1}$	20.97 a	18.27 ab

* Means followed by the same letters within columns are not significantly different at $p \leq 0.05$ according to the Duncan test.

The decrease in plant growth with increasing the salinity concentration may due to salinity water have high osmotic pressures which affects plant growth by restricting the uptake of water by the roots and interferes with balanced absorption of essential nutritional ions by plants (Tester and Devenport, 2003). An immediate effect of osmotic stress on plant growth is its inhibition of cell expansion either directly or indirectly through abscisic acid (Zeinolabedin, 2012).

Nutrient disturbances under salinity reduce plant growth by affecting the availability, transport, and partitioning of nutrients. However, salinity can differentially affect the mineral nutrition of plants. Salinity may cause nutrient deficiencies or imbalances, due to the competition of Na^+ and Cl^- with nutrients such as K^+, Ca^{2+}, and NO_3^-. Under saline conditions, a reduced plant growth due to specific ion toxicities (e.g. Na^+ and Cl^-) and ionic imbalances acting on biophysical and/or metabolic components of plant growth occurs (Grattan and Grieves, 1999). Leaf area decrease has been considered as the major cause of growth reduction, due to the decline in the photosynthetic area (Rawson and Munns, 1984).

Salt stress induces the synthesis of abscisic acid which closes stomata when transported to guard cells, and as a result of stomatal closure, photosynthesis declines and photoinhibition and oxidative stress occur (Zeinolabedin, 2012), also the photosynthetic products and dry matter accumulation will decreased. But as we show the percentage of dry matter was high, because the accumulation for new growth is districted, whereas the percentage of the dry matter was increase for same weight of plant especially for 300 $mM.L^{-1}$.

Salinity reduces the ability of plants to take up water, and this quickly causes reductions in growth rate, along with a suite of metabolic changes identical to those caused by water stress. The initial reduction in shoot growth is probably due to hormonal signals generated by the roots. If excessive amounts of salt enter the plant, salt will eventually rise to toxic levels in the older transpiring leaves, causing premature senescence, and reduce the photosynthetic leaf area of the plant to a level that cannot sustain growth (Munns, 2002).

A similar decrease in the growth of broccoli plants under saline conditions was observed by L´ opez-Berenguer et al. (2006 and 2008) also by Verma (2008) who cleared that excess of salt in water not only lowers the absorption of water but also decreases the productivity of plant and overall growth of plants. The results also agree with Al- Seedi and Gatteh (2010) whom found that the increase in salinity concentration from 0 to 200 $mM.L^{-1}$ caused a decrease in mung bean plant stem lengths , fresh and dry matter weights as a result of the increase of salinity and with Chokri et al. (2012) were the shoot and root dry weights and leaf area of two broccoli cultivars were decreased by increasing salinity from 0 to 90 $mM.L^{-1}$.

- **Effects of salinity on some photosynthetic pigments:**

Results in table 3 shows that the irrigation with saline water decreased chlorophyll content of leaves significantly as compared to control, were irrigation with 100 and 200 $mM.L^{-1}$ decreased chlorophyll b and total chlorophyll significantly compared to control, and the highest values records in plants irrigated with tap water (1.50, 1.84 and 3.33 mg.100g fresh $weight^{-1}$ for each of chlorophyll a, b and total chlorophyll respectively. There were non-significant differences between the treatments in there effects in carotenoids content in leaves.

Table 3: Effect of salinity on some photosynthetic pigments of broccoli leaves.

Salinity concentration	Chlorophyll a	Chlorophyll b	Total Chlorophyll	carotenoids
	Mg.100g fresh $weight^{-1}$			
Control (Tap water)	1.50 a	1.84 a	3.33 a	0.53 a
100 $mM.L^{-1}$	0.93 bc	1.36 b	2.28 b	0.43 a
200 $mM.L^{-1}$	0.66 c	1.32 b	1.98 b	0.35 a
300 $mM.L^{-1}$	1.31 b	1.67 ab	2.98 ab	0.49 a

* Means followed by the same letters within columns are not significantly different at $p \leq 0.05$ according to the Duncan test.

One of the stress symptoms in salt treated plants was a decrease in chlorophyll content. Changes in leaf chlorophyll content with stresses may involve a severe chlorophyll photo-oxidation mediated by oxy-radicals (Wise and Naylor, 1987). Stress leads to an increase in free radicals in chloroplasts and destruction of chlorophyll molecules by reactive oxygen species, this result in reduction of photosynthesis and growth. Singlet oxygen atoms and O_2^- radicals predominantly attack double bond-containing compounds (unsaturated fatty acids and chlorophyll), thus damaging the chloroplast membrane system and photosynthetic reaction center (Zhang et al, 2003), which, in turn, may result in the release of chlorophyll from the thylakoid membranes, whereas, Rahimi and Biglarifard (2011) and Suriyan and Chalermpol

(2009) reported that salt stress has significant effect on PSII photochemical activity in photosynthesis process in strawberry and maize.

Parida and Das (2005) suggested that decrease in chlorophyll content in response to salt stress is disordering synthesizing chlorophyll and appearing chlorosis in plant, or by increasing the activity of the chlorophyll degrading enzyme according to Rao and Rao (1981), or the inhibitory effect of the accumulated ions of various salts on the biosynthesis of the different chlorophyll fractions according to Ali et al. (2004).

Also increased NaCl concentration has been reported to induce increases in Na and Cl as well as decreases in N, P, Ca, K and Mg level in peppermint and lemon verbena (Tabatabaie and Nazari, 2007). As a result the chlorophyll synthesis will affect.

Different responses were records in different plants as a result to salt stress, it has been reported that chlorophyll content decreases in salt susceptible plants such as pea (Hamada & El-Enany, 1994), but increases in salt tolerant plants such as mustard (Singh et al., 1990). This difference response shown in table 3 in increasing in photosynthesis pigments in 300 $mM.L^{-1}$ salinity compared to other saline waters, this increase also may due to decreasing in leaf area (table 1) which may result in accumulation the cell components including chloroplasts per area (Ah-Shahwani, 2006). These results agree with Muhammad et al. (2007) whom found that leaf chlorophyll content increased significantly with increasing salt concentration both in the seedlings of sugar beet (*Beta vulgaris* L.) and cabbage (*Brassica oleracea capitata* L.) grown in sand culture at salinities of 0 (control), 50, 100 and 150 $mM.L^{-1}$ NaCl.

- Effects of salinity on number, length and width of stomata in adaxial and abaxial leaves surfaces:

Results in table 4 shows that irrigation with saline water decreased the stomata number on abaxial surfaces of leaves significantly compared to stomata number in plants irrigated with tap water (570 stomata.$(mm^2)^{-1}$), whereas treatments don`t show any significant differences in stomata number on adaxial surfaces.

As shown in the same table, there were non-significant differences between abaxial stomata length, except the significant increase in stomata length in plants irrigated with 200 $mM.L^{-1}$ salinity compared to plants irrigated with 300 $mM.L^{-1}$, while there were non- significant differences between stomata length in adaxial leaves surfaces.

Irrigation with 100 and 300 $mM.L^{-1}$ saline water decreased significantly the stomata width on abaxial leaves surfaces to 8.33 micron compare to control and 200 $mM.L^{-1}$, were records 11.67 and 11.25 micron respectively. In adaxial surfaces the control and 100 $mM.L^{-1}$ record the highest stomata width (13.33 and 11.67 micron) respectively, compared to 200 and 300 $mM.L^{-1}$ treatments (Figure 2 and 3).

Table 4: Effects of salinity on some stomata characteristics for broccoli leaves.

Salinity concentration	Stomata Number.$(mm^2)^{-1}$		Stomata Length (micron)		Stomata Width (micron)	
	Abaxial surface	Adaxial surface	Abaxial surface	Adaxial surface	Abaxial surface	Adaxial surface
Control (Tap water)	570.00 a*	296.30 a	18.33 ab	20.00 a	11.67 a	13.33 a
100 $mM.L^{-1}$	346.67 b	366.70 a	16.67 ab	19.16 a	8.33 b	11.67 a
200 $mM.L^{-1}$	301.00 b	243.30 a	20.9 a	17.50 a	11.25 a	8.33 b
300 $mM.L^{-1}$	310.00 b	223.30 a	14.19 b	15.00 a	8.33 b	8.33 b

* Means followed by the same letters within columns are not significantly different at $p \leq 0.05$ according to the Duncan test.

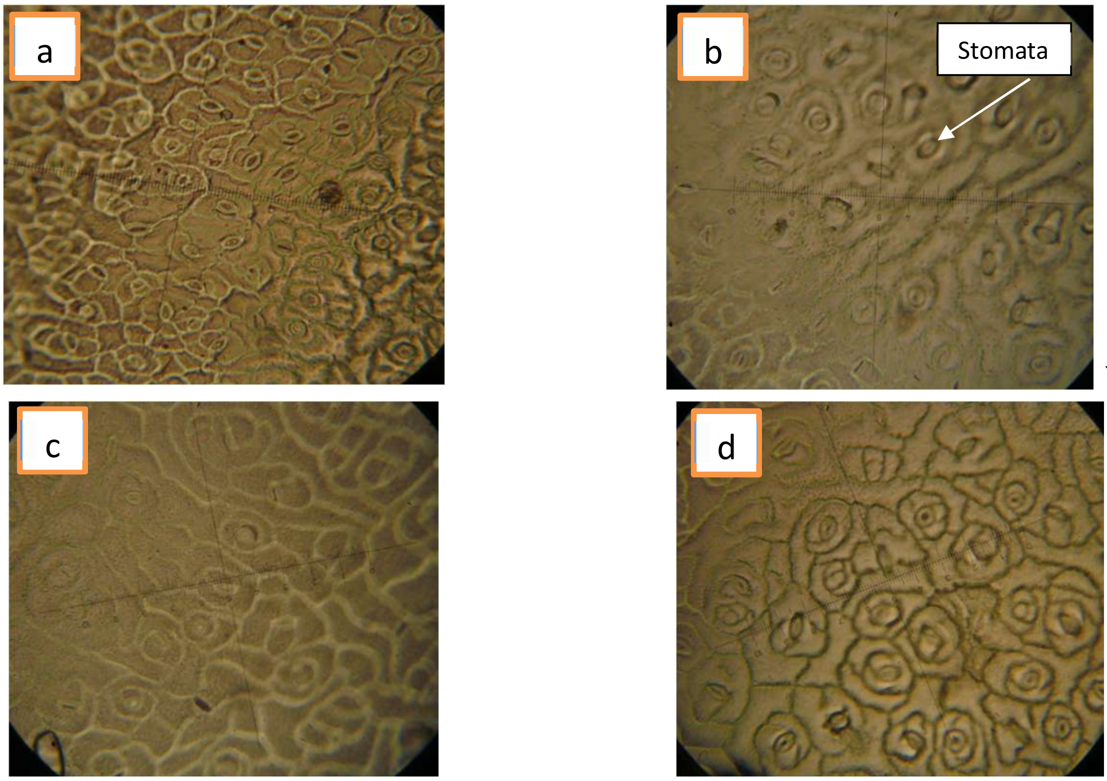

Fig.2. Detail of 400X microscopic observation of abaxial surface stomata in a broccoli leaves irrigated with (a) tap water (b) 100 $mM.L^{-1}$ salinity water (c) 200 $mM.L^{-1}$ salinity water (d) 300 $mM.L^{-1}$ salinity water.

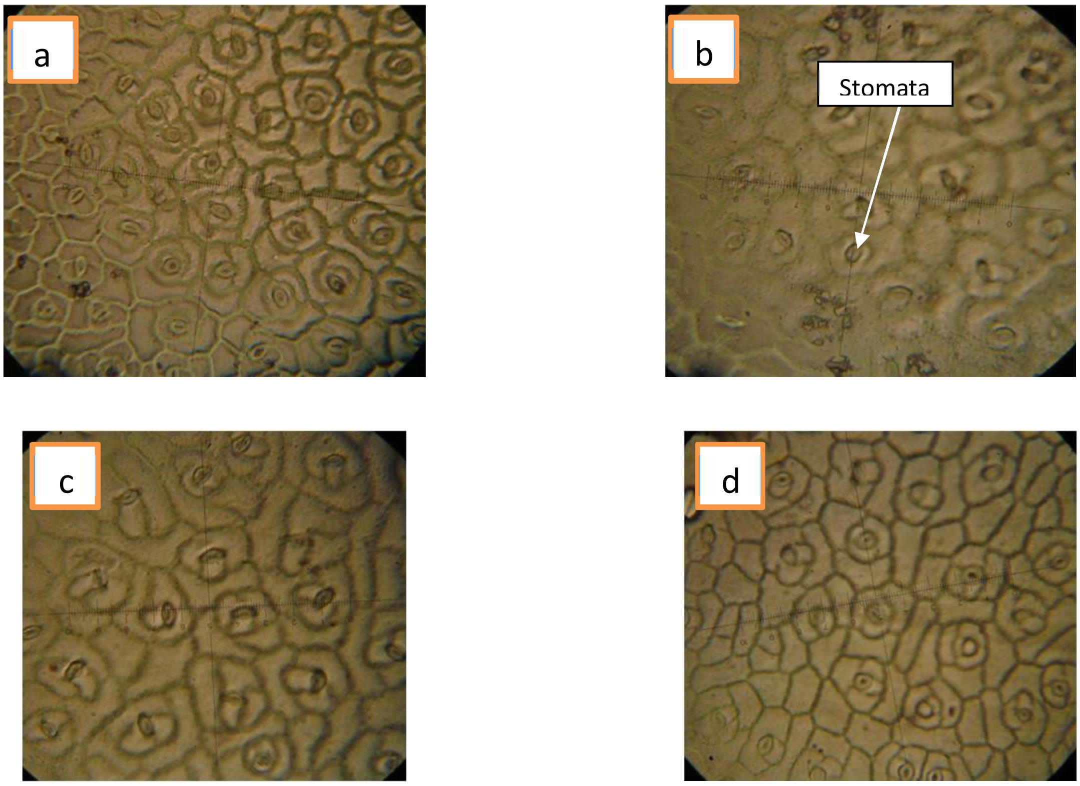

Fig.3. Detail of 400X microscopic observation of adaxial surface stomata in a broccoli leaves irrigated with (a) tap water (b) 100 $mM.L^{-1}$ salinity water (c) 200 $mM.L^{-1}$ salinity water (d) 300 $mM.L^{-1}$ salinity water.

The decrease in stomata density with saline water irrigation agree with Romero-Aranda et al. (2001) who clear that the presence of salt reduces the stomatal density in tomato leaves. As many other plants broccoli decrease the stomata length and width in most saline treatments were these plants have developed different mechanisms to reduce the effect of salt stress, including stomatal closure (Mansour et al., 1994 and Iyengar and Reddy, 1996). Also Perera, et al. (1994), record that there was a suppression of stomatal opening by increasing NaCl concentrations in *Aster tripolium* L. plants

From the results of this work we conclude that irrigation with saline water reduced the growth of broccoli plants and decrease head forming percentage. Also it decreased the photosynthesis pigments and effects on stomata densities and dimensions. More studies by using other materials that decreasing the inhibitor effects of salinity is recommended, in addition to using alternative irrigation with non-saline water in order to decreasing the effects of salts stress.

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تأثير الملوحة في النمو وبعض الصفات الفسلجية لنبات البروكولـــــي
Broccoli *Brassica oleracea var. Italica* L.

المستخلص

نفذت التجربة في فاكلتي العلوم والصحة/ جامعة كوية خلال الموسم الشتوي 2014-2015 بهدف تقييم تأثير الأجهاد الملحي (ماء الحنفية كمعاملة كقارنة و 100 و 200 و 300 مليمول/لتر) في النمو وبعض الصفات الفسلجية لنبات البروكولـــــي ,.*Brassica oleracea var. Italica* L. أظهرت النتائج ان زيادة تركيز الملوحة أدت الى انخفاض معنوي في صفات النمو الخضري من ارتفاع النبات و عدد الأوراق والمساحة الورقية، فضلا عن محتوى الأوراق من كلوروفيل أ و ب والكلوروفيل الكلي بالقياس الى معاملة المقارنة، في حين لم تكن هناك فروق معنوية بين المعاملات المختلفة في تأثيرها على محتوى الأوراق من الكاروتينات. كما أظهرت النتائج أن الري بالمياه المالحة سبب انخفاض معنوي في عدد ثغور السطح السفلي من الأوراق قياساً بمعاملة المقارنة والمروية بماء الحنفية. أدى الري بالمياه المالحة الى انخفاض معنوي في عرض ثغور السطح السفلي مقارنة بمعاملتي الري بـ 100 و 300 مليمول/لتر على التوالي، بينما أعطت معاملتي المقارنة والري بماء ملوحته 100 مليمول/لتر الى إعطاء أعلى عرض للثغور على السطح العلوي للأوراق قياسا بمعاملتي 200 و 300 مليمول/لتر.

كلمات مفتاحية: *Brassica oleracea var. Italica* L، ملوحة، نمو النبات، صبغات التمثيل الضوئي، ثغور.

کاریگەری سوێری لە سەر گەشەو هەندێ خەسلەتی فەسلەجی ڕووەکی برۆکۆلی
Broccoli *Brassica oleracea var. Italica* L.

بوختە

ئەم توێژینەوە جێبەجێ کرا لە فاکەڵتی زانست وتەندروستی/زانکۆی کۆیە لو وەرزی زستانی 2014-2015 بە مەبەستی هەڵسەنگاندنی کاریگەری سوێری ئاوی ئاودان (ئاوی بۆری وەکو کۆنترۆل، 100 و 200 و 300 مليمول. لەتر$^{-1}$) لەسەر گەشەو هەندێ لە خەسلەتە فەسلەجیەکانی ڕووەکی برۆکۆلی *Brassica oleracea var. Italica* L. ئەنجامەکان دەریان خست کەوا زیادبوونی خەستی سوێری، کەمبوونێکی بەرچاوی لێ کەوتوەوە لە خەسلەتەکانی بەرزی ڕووەك و ژمارەی گەڵا و ڕووبەری گەڵاکان و هەروەها ڕێژەی کلوروفیل أ و ب و گشتی لە گەڵاکان بەبەراورد بە مامەڵەی کۆنترۆڵ. لەهەمان کاتدا جیاوازێکی بەرچاو لە نێوان مامەڵەکان نەبوو دەربارەی کاروتینات. ئەنجامەکان دەریانخست کەوا ئاو دان بە ئاوی سوێر ژمارەی دەمیلەکانی ڕووی خوارەوەی گەڵاکانیان بەبەرچاوی کەمکرتەوە، بە بەراوەرد بە مامەڵەی کۆنترۆل (ئاوی بۆری). هەروەها ئاودان بە ئاوی سوێر کەمبوونێکی بەرچاوی هەبوو لە پانی دەمیلەکانی ڕووی خوارەوەی گەڵاکان بە بەراوەرد بە مامەڵەی کۆنترۆل (ئاوی بۆری) و 100 مليمۆل.لەتر$^{-1}$، کەوا زیاترین پانی دەمیلەیان هەبوو لە ڕووی سەرەوەی گەڵاکان، بە بەراوەرد بە مامەڵەی 200 و 300 مليمۆل.لەتر$^{-1}$.