Mesopotamia Journal of Agriculture

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EFFECT OF SALICYLIC ACID APPLICATION ON GROWTH AND DEVELOPMENT OF GREEN ONIONS GROWN UNDER SALT STRESS

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Article information
Article history:
Received:27/6/2021
Accepted: 12/8/2021
Available: 12/9/2021

Keywords:
Salt stress,
Salicylic acid
green onion.

DOI:
10.33899/magrj.2021.130333.1133

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ABSTRACT

Soil salinity negatively affects crop production and leads to loss of agricultural lands worldwide. In Iraq, poor agricultural practices, such as inadequate drainage facilities and low water quality, are known to be caused soil salinization. Onion (Allium cepa L.) is an economic crop that is consumed heavily as green or dry bulb onion. The cultivated area of green onion was statistically decreased in the Kurdistan region, Iraq, lately. Therefore, this study was conducted in the Directorate of Agriculture, Ankawa, Erbil, to examine whether salicylic acid (SA) mitigate the effect of salt stress and improve the growth and development of green onions. Two different concentrations of NaCl (20 and 40 mM) were injected around the base of each plant and after 24h, SA (0.5 and 1mM) was sprayed to the vegetative parts of each plant, evenly. The results showed that the short-term application of SA was reduced the impact of NaCl. The number of green leaves, leaf area, plant height, root growth, bulb diameter, plant weight and total yield (t. ha-1) was improved by the application of SA. It has been suggested the application of a commercial or SA analogue to the agricultural saline land may alleviate the salt stress and improve the product.

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INTRODUCTION

High salinity stress continues to threat crop production and loss of agricultural land. A case study in Iraq was shown that saline soil increases from north to south and the highest percentage of dissolved salt was found in the irrigated water from Tigris and Euphrates rivers (Jawad, 2020). Salt stress modifies plant physiological and biochemical systems, and results in reduction of plant growth and development (Kamran *et al*., 2020). Onion (*Allium cepa L.*) is an economic crop that is consumed heavily as green or dry bulb onion. The cultivated area of green onion was decreased almost 2% in 2019 in comparison to the previous year in northern Iraq (Anonymous, 2019). Onion species response differently to salt stress according to the recent publication by Plabon (2021). Cell division rate in roots of onion plants was reduced by high salt concentrations, hence the plant growth and productivity was reduced significantly (Singh and Roy, 2016). However, to mitigate the impact of salt stress on plant growth and development, different strategies have been used including intercropping with halophyte species (Lastiri-Hernández, 2021), antioxidant compound (Mohamed and Aly, 2008) and plant growth regulator and widely applied salicylic acid (SA) (Ashraf *et al.*, 2010 and da Silva *et al.*, 2019). SA is known to reduce biotic and abiotic stress in plants by activation of the defense system (Wani *et al.*, 2017). In addition, SA is commonly known as a plant growth regulator, which enhances plant growth and development.

SA application has been used to reduce abiotic stress in plants, however, the plant response to SA application depends on the species, variety and growth stage (Mohammadi *et al.*, 2019; da Silva *et al*., 2019 and Wijoyo *et al*., 2019). In plants, SA is biosynthesized through two different pathways: isochorismate (ICS) and phenylalanine ammonia-lyase (PAL) pathways, and its level varies within different plant organs, it has shown that its level is higher in shoot in compression to the roots in rice (Duan *et al.*, 2014 and Lefevere *et al.*, 2020).The foliar application of 250 ppm of SA at different growing stages, significantly was increased vegetative growth and onions bulbs parameters when compared to the control treatment (Pradhan *et al*., 2016).

It is difficult to maintain the supply vegetables to meet the demands of the market, and is expected to continue increasing over the next decade, due to the conversion of agricultural lands to residential purposes, drought and salinity specifically, in the Kurdistan Region, Iraq. Salt concentration in the soil was found to be increased due to poor management and growth conditions (Singh, 2019). Therefore, this study aimed to examine the impact of SA spraying after 24 h of salt (NaCl) application on the morphology, phenotype and yield of green onion.

MATERIALS AND METHODS

Experimental site and treatments

A field experiment was carried out at the research center farm of the Directorate of Agriculture, Ankawa, Erbil, Kurdistan Region, Iraq (36.1°N, 44°E and 434 Meters above mean sea level) during the growing season (October–January) (2020–2021). The experimental soil was silty clay in texture, (sand, silt, clay, 90, 569, 341 Kg, respectively), its pH was 8.46; EC 0.72 dS m^{-1}; organic matter 105g. kg^{-1}; available N, P, K 4.6, 3.6 and 1.17 mg. kg^{-1}, respectively. The average temperature and humidity range between (5 -28°C) and (30- 57%) respectively, during the growing season (Anonymous, 2021).

The field was initially ploughed and the organic manure was applied evenly (100 gm.1m^{-2}), and this was followed by rotator for land leveling. A drip irrigation system was installed, the distance between the tape 40 cm and 10 cm between the plants. Onion (cv. Local red) seedlings of seven weeks old were obtained from ARD Kurdistan nursery and transplanted on October 5th during 2020. Two weeks after transplanting, when seedlings were established 5-6 leaves and length between 30-40 cm, irrigation was stopped until the surface of the soil dried. 10 mL of NaCl (0, 20 and 40 mM) was injected once using 10 mL syringes around the base of each plant. 24 h later SA (0, 0.5 and 1mM) was sprayed once evenly to each plant using two-liter sprayer. Irrigation was resume two days after SA treatment. Fertigation was applied according to recommended rate only for the first stage of the growth (Dingre *et al.*, 2016). Weed and pest management was carried out as required.

Experimental design, data collection and analysis

Factorial experiment using randomize complete block design was conducted with three replicates (3×3×3). Ten plants for each experimental unit. A hundred days after transplanting (DAT), four randomly plants were chosen per experimental unit to recorded the data. The number of green leaves was counted from fully developed leaves and the average was computed for each plant. Leaf area (cm^2) was computed using a digital picture captured by a digital camera, then the leaf area was measured using Image J 1.52m software (Córcoles *et al*., 2015). Plant height (cm) was measured from the ground level up to the tip of the longest leaf using measuring tape. Root length (cm) was measured from the stem to the tip of the longest root using measuring tape. Bulb diameters(mm) was measured at the widest circumstance of the bulb using a Vernier caliper. Average bulb weight per plant (gm. $plant^{-1}$) was calculated as the fresh bulb weight after harvesting. The total yield (t. ha^{-1}) was recorded for the total number of plants per experimental unit and then converted into tons per hectare. Data were analyzed using IBM@ SPSS@ statistics, version 25. Duncan multiple range test was applied to compare between the mean treatments at 0.5 level of significance.

RESULTS AND DISCUSSION

Salinity is one of the abiotic stresses that threat the future of agricultural industry (Singh, 2019). Over years of research, different chemical compounds and methods of application have been used to reduce the impact of salt stress on agricultural product (Kamran *et al*., 2020; Silva *et al*., 2019; Singh and Roy, 2016 and Wani *et al*., 2017). Species, varieties, growth stage, and different organs of the plant response differently to salinity tolerance under various growth conditions (Kamran *et al*., 2020). It has been shown that onion is a salt sensitive crop, however, its effect differs at different growth stage (Sta-Baba *et al*., 2010). Multiple phytohormones are involved in abiotic stress resistance in plant, SA stimulate growth and dry matter of onion seedling under salt stress (da Silva *et al*., 2019). In the present study, green onion (cv. Local red) growth and development response positively to different concentration of SA under salt stress.

The number of leaves per plant statistically decreased by increasing the concentration of salt from 20 mM to 40 mM in comparison to the control treatment (Figure 1A). The highest number of leaves per plant was found when the onion plants were spray with 1mM SA (Figure 1B). In the similar way, the interaction effect shows that SA mitigate the effect of NaCl on the number of leaves of green onion plant, the highest value was recorded when 1mM and 0.5mM were applied in the control treatment and 20mM NaCl respectively, and the lowest value was found when plants were treated with 40 mM NaCl in the control treatment (Table 1). The size of leaf area of green onion was reduced significantly with NaCl treatment (Figure 2 A), 40 mM NaCl shows bigger size of leaf area and significantly different when compared with 20 mM NaCl and this is maybe the effect of the interaction in between NaCl and SA (Table 2). The main effect of SA shows the bigger leaf area at 1 mM treatment and both SA treatments (0.5 and 1 mM) increased the leaf area of green onion significantly when compared with the control treatment (Figure 2 B). The interaction effect of NaCl and SA shows that leaf area was bigger when plants were treated with 40mM in comparison with 20mM under the effect of SA, the biggest and smallest leaf area was recorded at 1 mM SA and 40 mM NaCl treatment respectively (Table 2). The plant height, which was measured from the surface of soil to the tallest green leaf, was statistically decreased by increasing the concentration of NaCl treatment (Figure 3A). Contrary, the highest plant was recorded in 1 mM SA treatment in comparison to 0.5 mM SA and control treatment (Figure 3 B). The interaction effect of NaCl and SA treatment shows that 0.5 mM and 1Mm SA significantly decreased the effect of 20 mM and 40 mM NaCl on plant height of green onion when compared to the control treatment (Table 3). These results suggest that SA may moderate the effect of salt concentration and improves the growth of leaves and cell elongation. This is in agreement with the study conducted by Sta-Baba *et al.* (2010) who demonstrated a decrease in the number of onion leaf and plant height with increasing NaCl concentration. SA application was found to have a significant effect on plant height and other growth parameters of onions (Pradhan *et al*., 2016). However, it was reported that SA alleviate germination and growth of onion seeds only under salt stress (da Silva *et al*., 2019). It may suggest that SA reduces the effect of salt stress and interplay with reactive oxygen species (Herrera-Vã¡Squez *at al*., 2015) and modulate the plant growth of onion at the vegetative stage.

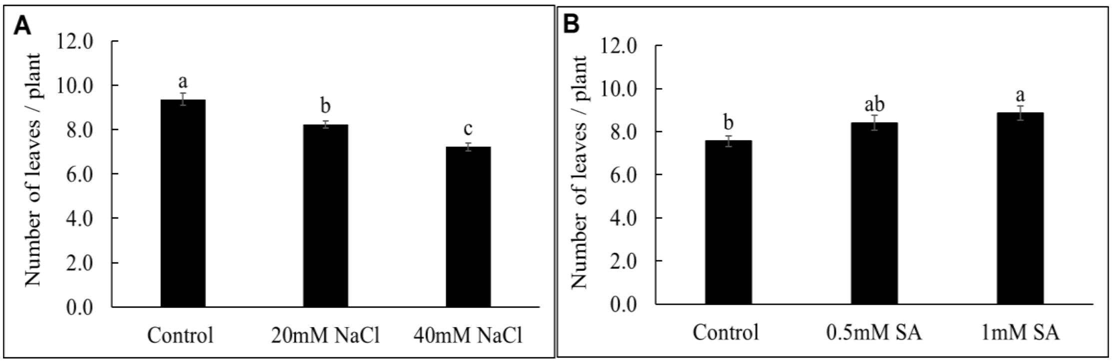

Figure (1): The number of green leaves per plant of green onion under the effect of NaCl and SA. (A) Main effect of NaCl. (B) Main effect of SA on the number of green leaves of green onion at the age of 100 DAT. Values with different letters indicate significant difference at P < 0.05 according to Duncan multiple range test, n = 3, error bars represent ± SE.

Table (1): The interaction effect of NaCl and SA on the number of green leaves per plant of green onion at the age of 100 DAT.

SA \ NaCl	Control	20 mM NaCl	40 mM NaCl
Control	8.333 ± 0.08 d	7.717 ± 0.12 e	6.617 ± 0.07 g
0.5mM SA	9.667 ± 0.08 b	8.300 ± 0.12 d	7.250 ± 0.14 f
1mM SA	10.073 ± 0.21 a	8.683 ± 0.04 c	7.817 ± 0.04 e

Values with different letters indicate significant difference at P < 0.05 according to Duncan multiple range test, n = 3, ± standard error (SE).

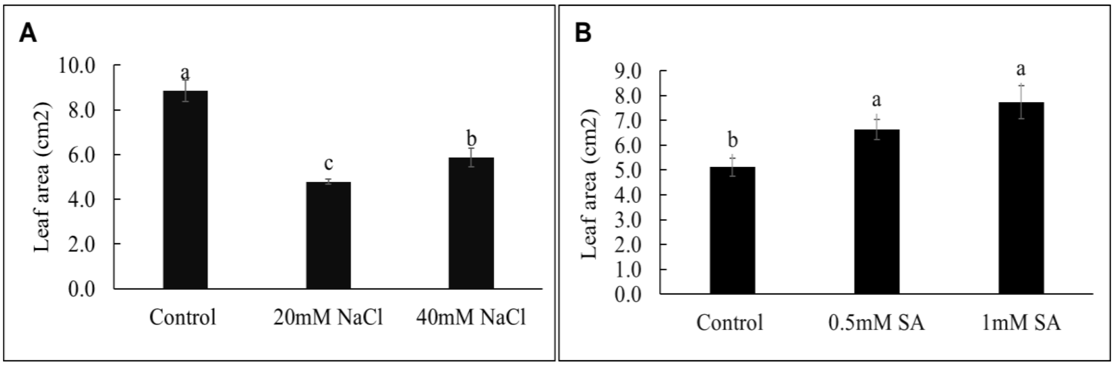

Figure (2): Leaf area of green onion under the effect of NaCl and SA. (A) Main effect of NaCl. (B) Main effect of SA on leaf area of green onion at the age of 100 DAT. Values with different letters indicate significant difference at $P < 0.05$ according to Duncan multiple range test, n = 3, error bars represent ± SE.

Table (2): The interaction effect of NaCl and SA on leaf area (cm^2) of green onion at the age of 100 DAT.

SA \ NaCl	Control	20 mM NaCl	40 mM NaCl
Control	6.786 ± 0.38 c	4.862 ± 0.64 d	3.721 ± 0.37 e
0.5mM SA	8.412 ± 0.17 b	4.640 ± 0.39 d	6.849 ± 0.35 c
1mM SA	11.341 ± 0.23 a	4.866 ± 0.44 d	7.028 ± 0.25 c

Values with different letters indicate significant difference at $P < 0.05$ according to Duncan multiple range test, n = 3, ± standard error (SE).

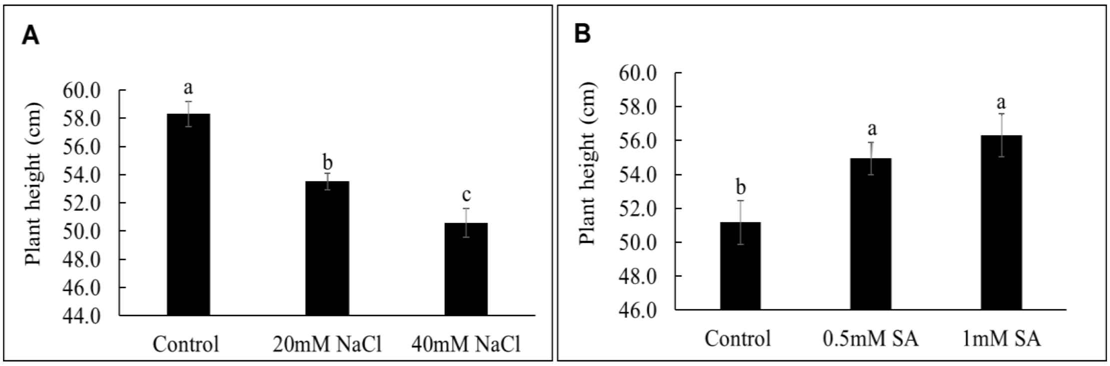

Figure (3): Plant height of green onion under the effect of NaCl and SA. (A)Main effect of NaCl. (B) Main effect of SA on plant height (cm) of green onion at the age of 100 DAT. Values with different letters indicate significant difference at $P < 0.05$ according to Duncan multiple range test, n = 3, error bars represent ± SE.

Table (3): The interaction effect of NaCl and SA on plant height (cm) of green onion at the age of 100 DAT.

SA \ NaCl	Control	20 mM NaCl	40 mM NaCl
Control	55.333 ± 0.51 c	51.500 ± 0.50 e	46.667 ± 0.94 f
0.5mM SA	58.500 ± 0.52 b	53.917 ± 0.51 cd	52.417 ± 0.36 de
1mM SA	61.083 ± 0.65 a	55.167 ± 0.22 c	52.667 ± 0.58 de

Values with different letters indicate significant difference at $P < 0.05$ according to Duncan multiple range test, n = 3, ± standard error (SE).

Root growth, bulb size and yield of green onion were also affected by the short period of time of NaCl and SA treatment. Root length, bulb diameter, total weight per plant and yield ($t.h^{-1}$) were statistically and significantly decreased at 20 and 40 mM NaCl treatment when compared to the control treatment of green onion (Figure 4A, 5A, 6A and 7A). Oppositely, SA application significantly increased the values of the same parameters of green onion (Figure 4B, 5B, 6B and 7B). Onion root, bulb diameter and total weight response positively and similarly to 0.5 and 1mM SA treatment under different salt concentration 20 mM and 40 mM (Table 4, 5 and 6). In agreement with previous studies, it was reported that SA had a greater effect on root growth and root biomass of sorghum and maize under slat stress (El-Katony *et al*., 2019 and Rajabi *et al*., 2019). It was also reported that onion bulb diameter statistically reduced and increased under the effect of salt stress and SA, respectively (Pradhan *et al*., 2016 and Sta-Baba *et al*., 2010). Green onion yield ($t.\ ha^{-1}$) responses positively to the effect of short-term SA treatment under NaCl stress, the lowest yield was recorded when plants were treated with 40 mM NaCl (Table 7). Statistically, no significant difference was found in the yield of plants treated with SA 0.5 and 1 mM under the effect of 20 and 40 mM NaCl, respectively (Table 7). Many studies examined the response of plant to SA under salt stress (Pradhan *et al*., 2016; Singh and Roy, 2016 and Wani *et al*., 2017). Different plant species at different growth stages influenced differently to abiotic and biotic stress under the effect of SA application (Dingre *et al*., 2016; El-Katony *et al*., 2019; Mohammadi *et al*., 2019 and Singh and Roy, 2016). In addition to the defense mechanism against pathogen, SA play a big role to enhance antioxidant system in the plant, and also regulate cell growth and enlargement (Bhasker *et al*.,2020; Li *et al*., 2014 and Vanacker *et al*., 2001). It may suggest that the short- term effect of NaCl in the field was alleviated by SA application in the early stage of growth and development of green onion (cv. Local red).

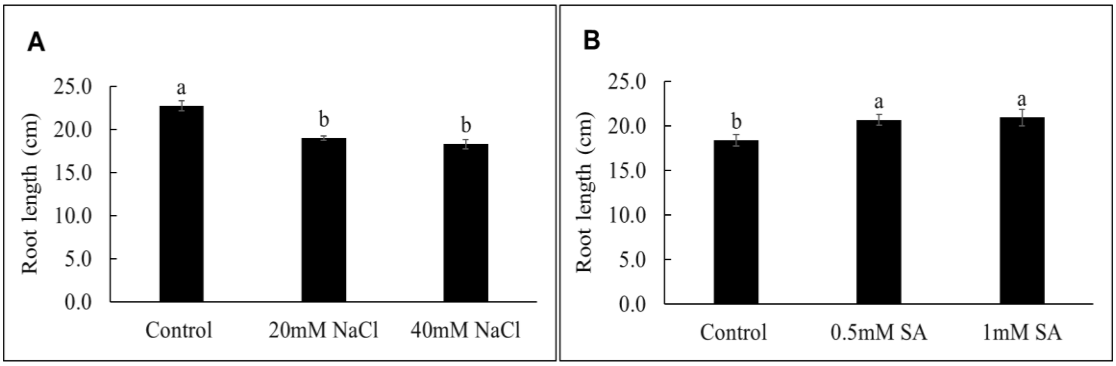

Figure (4): Root length of green onion under the effect of NaCl and SA. (A)Main effect of NaCl. (B) Main effect of SA on root length (cm) of green onion at the age of 100 DAT. Values with different letters indicate significant difference at $P < 0.05$ according to Duncan multiple range test, n = 3, error bars represent ± SE.

Table (4): The interaction effect of NaCl and SA on root length (cm) of green onion at the age of 100 DAT.

SA \ NaCl	Control	20 mM NaCl	40 mM NaCl
Control	20.667 ± 0.36 c	18.083 ± 0.22 e	16.300 ± 0.29 f
0.5mM SA	22.900 ± 0.37 b	19.250 ± 0.14 d	19.750 ± 0.38 d
1mM SA	24.458 ± 0.44 a	19.500 ± 0.14 d	18.783 ± 0.24 de

Values with different letters indicate significant difference at P < 0.05 according to Duncan multiple range test, n = 3, ± standard error (SE).

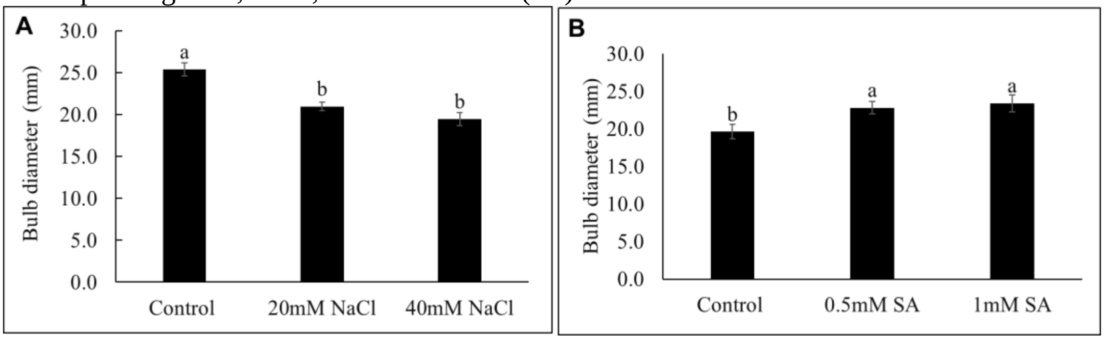

Figure (5): Bulb diameter of green onion under the effect of NaCl and SA. (A)Main effect of NaCl. (B) Main effect of SA on bulb diameter (cm) of green onion at the age of 100 DAT. Values with different letters indicate significant difference at P < 0.05 according to Duncan multiple range test, n = 3, error bars represent ± SE.

Table (5): The interaction effect of NaCl and SA bulb diameter (cm) of green onion at the age of 100 DAT.

SA \ NaCl	Control	20 mM NaCl	40 mM NaCl
Control	22.897 ± 0.88 c	19.143 ± 0.42 d	16.779 ± 0.61 e
0.5mM SA	25.507 ± 0.57 b	22.199 ± 0.44 c	20.752 ± 1.38 cd
1mM SA	27.783 ± 0.21 a	21.573 ± 0.15 c	20.865 ± 0.48 cd

Values with different letters indicate significant difference at P < 0.05 according to Duncan multiple range test, n = 3, ± standard error (SE).

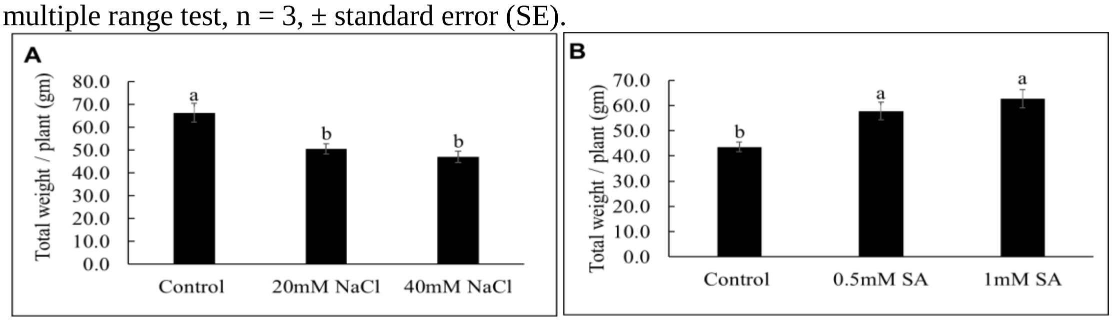

Figure (6): Total weight per plant (gm) of green onion under the effect of NaCl and SA. (A)Main effect of NaCl. (B) Main effect of SA on the total weight (gm /plant) (root, bulb and green leaves) of green onion at the age of 100 DAT. Values with different letters indicate significant difference at P < 0.05 according to Duncan multiple range test, n = 3, error bars represent ± SE.

Table (6): The interaction effect of NaCl and SA on total weight per plant (gm) of green onion at the age of 100 DAT.

SA \ NaCl	Control	20 mM NaCl	40 mM NaCl
Control	50.583 ± 0.93 e	42.250 ± 0.87 f	37.700 ± 0.98 g
0.5mM SA	71.917 ± 0.93 b	51.917 ± 0.98 de	49.667 ±1.45 e
1mM SA	76.667 ± 0.73 a	57.333 ± 1.36 c	54.017 ± 1.41 d

Values with different letters indicate significant difference at P < 0.05 according to Duncan multiple range test, n = 3, ± standard error (SE).

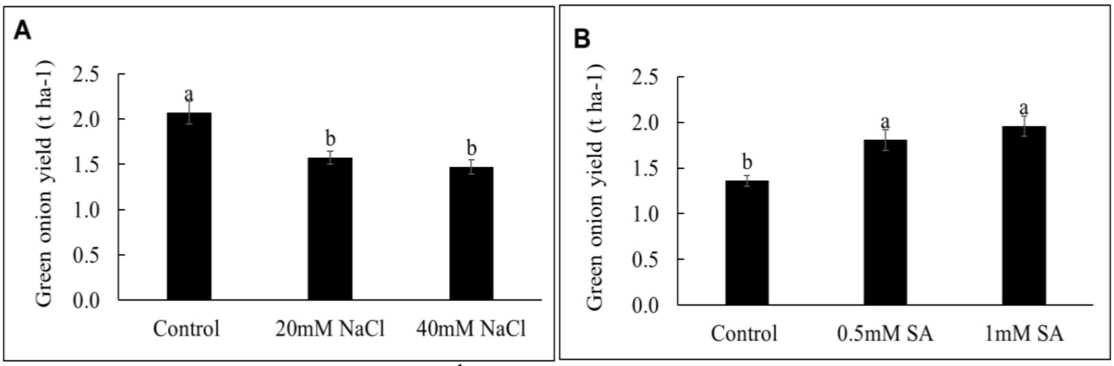

Figure (7): Green onion yield (t ha^{-1}) under the effect of NaCl and SA. (A)Main effect of NaCl. (B) Main effect of SA on green onion yield (tha-1) at the age of 100 DAT. Values with different letters indicate significant difference at P < 0.05 according to Duncan multiple range test, n = 3, error bars represent ± SE.

Table (7): The interaction effect of NaCl and SA on green onion yield (t ha^{-1}) at the age of 100 DAT.

SA \ NaCl	Control	20 mM NaCl	40 mM NaCl
Control	1.581 ± 0.03 e	1.3203 ± 0.03 f	1.1781 ± 0.03 g
0.5mM SA	2.247 ± 0.03 b	1.622 ± 0.03 de	1.552 ± 0.04 e
1mM SA	2.396 ± 0.02 a	1.792 ± 0.04 c	1.688 ± 0.04 d

Values with different letters indicate significant difference at P < 0.05 according to Duncan multiple range test, n = 3, ± standard error (SE).

In conclusion, onion is one of the most important commercial vegetable crops grown in Iraq. Green onion is heavily consumed as salad or cocked. The yield and planting area of green onion and other economic crops in Iraq, specifically, is reduced because of different reasons including poor irrigation practice, drainage infrastructure and water quality, which cause soil salinization (Qureshi and Al-Falahi, 2015). Salt stress adversely affects plant growth and development at different stages, generally. Various studies were conducted over decades to mitigate the negative response of plant to salt stress and other causes of slat accumulation on the soil surface, including, drought, water quality etc. Attempts were made using various chemical compounds and / or plant regulator to alleviate the adverse effect of salt stress on plant. SA partially neglected the effect of slat stress at the early stage of green onion growth and development. It is suggested firstly, improve the agricultural practices including facilitate drainage and use clean water for crop irrigation. Secondly, introduce commercial version of SA to agricultural market. However, further studies are required to examine the effect of SA at different stages of onion growth and development (germination, vegetative and seed production) under abiotic stress.

Acknowledgment

The research was financially supported by Qudra 2, Hungarian Interchurch Aid (HIA), Erbil, Iraq.

تأثير اضافة حامض الساليسليك على نمو وتطور البصل الاخضر تحت تأثير الشد الملحي

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الخلاصة

البصل من المحاصيل الاقتصادية التي تستهلك بكثرة كبصل اخضر وبصل الجاف. في العراق ضعف العمليات الزراعية مثل قلة البزل ورداءة نوعية المياه المروية، تعتبر من اهم العوامل التي تسبب ملوحة التربة. ان ملوحة التربة تؤثر سلبيا على انتاج المحاصيل وتؤدي الى فقدان الأراضي الزراعية. ان الاراضي المزروعة بالبصل الاخضر إحصائيا قلت معنويا في اقليم كوردستان العراق. أجربت هذه الدراسة في مديرية الزراعة، عنكاوة، اربيل لتخفف تأثير الجهد الملحي على نمو وتطور البصل الاخضر باستخدام حامض الساليسليك. تبين من النتائج ان اضافة حامض السالسليك لفترة قصيرة وبتركيز 0.5 و 1 ملي مولار قلل من تأثير NaCl 20 & 40 ملي مولار وازداد وتحسن عدد الاوراق الخضراء والمساحة الورقية وطول النبات ونمو الجذور وقطر البصلة والوزن الكلي لنبات البصل والحاصل الكلي (طن / هكتار). ان من أهم المقترحات المبنية على هذه النتائج، اضافة النوع التجاري المماثل والمشابه لحامض الساليسليك للأراضي الزراعية التي تعاني من الملوحة الذي ربما يقلل الملوحة ويحسن المحصول.

الكلمات المفتاحية: الشد الملحي، حامض الساليسليك، محصول البصل الاخضر

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