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Improving Yield and Quality of Kohlrabi Stems Growing under NaCl Salinity Using Foliar Application of Urea and Seaweed Extract

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Abstract: Under unfavorable growth conditions, the swollen tuber-like stem (marketable part) of kohlrabi plant become woody and tough which reflected on reducing its revenue. Studies on the effect of salinity on growth and quality of kohlrabi stems are extremely limited. Consequently, an outdoor pot experiment was conducted to evaluate the effect of different levels of NaCl salinity (0, 1000, 2000, 3000, 4000, 5000 and 6000 ppm) on the growth attributes of kohlrabi. Results of the preliminary experiment indicated that kohlrabi is a moderate salinity sensitive plant, where growth of the stem significantly reduced after exposing to NaCl at 3000 ppm, whereas the high reduction in growth of both leaves and stems recorded when the applied concentration of NaCl is equal or more than 4000 ppm. NaCl salt as a source of salinity stress was applied at 0 and 4000 ppm in the subsequent main experiment to study the promotive effect of urea and seaweed extract on enhancing the growth and quality of kohlrabi plant under salinity conditions. The concentration of foliar application treatments were four levels for urea (0, 5, 10 and 15 g/l) and two levels of seaweed extract (0 and 0.5 g/l), in addition to their combinations. Application of NaCl at 4000 ppm reduced leaf f.w, leaf area, leaf area index, photosynthetic pigments and total soluble sugars (TSS), which in turn reflected on the reduction of stem f.w, as quantitative trait. In addition to that, salinity has a negative effect on the quality of kohlrabi stems, through increasing firmness value and fibre %. These negative effects of salinity on quantity and quality traits of kohlrabi plant disappeared when urea as individual applications or combined with seaweed extract were applied to the plant as foliar treatments. Moreover, most foliar treatments enhanced the stem f.w, whereas reduced firmness values and fibre %. Application of combined treatment of seaweed extract at 0.5 g/l + urea at 15 g/l maximized the quality and yield of kohlrabi swollen stems under stressed or non-stressed conditions.

Key words: Kohlrabi · *Brassica oleracea* var. *gongylodes* · Urea · Seaweed extract · Yield · Quality · Fibre % · Firmness · Salt stress · Cell concentration index

INTRODUCTION

Kohlrabi (*Brassica oleracea* var. *gongylodes*), from the family Brassicaceae specialized from cabbage and a combination word between khol (cabbage) and rabic (turnip) as German, is widely grown in USA and Northern Europe as a cool season vegetable [1]. The edible part of kohlrabi is the swollen tuber-like stem called knob which located at the base of the stem entirely above the ground which is primarily used as a cooked in soups or raw in salads. It has a highly demand in European countries for its nutritional value and medicinal values due to its rich in vitamins (A, B, C and E), dietary fibre which helpful in controlling body weight, minerals (K, Ca, Mg, Zn) and rich in glucosinolates which determine the characteristic flavour and taste and are known to have anti–carcinogenic activity, in addition to, it has only 27 calories per 100 g, a negligible amount of fat and zero cholesterol [1]. Kohlrabi is untraditional vegetable crop in Egypt, can be promising alternative for vegetable growers due to having a short growing season and its export possibility to European countries.

Since, stem quality of kohlrabi is enhanced by cool, humid weather, which prevents the edible portion from becoming tough and woody [2]. Kohlrabi prefers fertile, well-drained soil rich in organic matter for best growth. Appropriate carefulness allows kohlrabi to accomplish the rapid growth that results in the best quality.

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Salinity affects almost every aspect of the physiology and biochemistry of plants throughout increasing water deficit and ion toxicity which, significantly reduces membrane permeability, plant growth and yield [3]. The osmotic stress is associated with lack of cell wall extension and expansion leading to cessation of the growth. The ionic effect includes interference with nutrient imbalance and lowering the net photosynthetic rates in the affected plants [4]. Salinity reduces the ability of plants to utilize water and causes not only a reduction in growth rate but also changes in plant metabolic processes [5]. Morphology of plant organs subjected to salinity stress have a big change to minimize water loss. Salt stress decreased the stem diameter, epidermis cell size, cortex zone thickness, vascular bundle width, xylem width and phloem width in radish stem [6]. Plant subjected to abiotic stresses increased the percentage of sclerenchyma tissue to minimize the water loss from plant organs and for reinforcing mechanical strength that prevent wilting [7]. When salt concentration increased in irrigated water led to an increase in crude fibre of mandarin fruit, which reducing fruit quality due to thicker deposition in segment and vesicle walls of mandarin fruit [8].

Salinity can reduce nitrogen (N) accumulation in plants by inhibiting its uptake, where NO_3^- competed with Cl^- at the sites of ion transport which led to plant suffering from nitrogen starvation [9]. Foliar application of nitrogen fertilizer could be an efficient alternative route for delivering nitrogen to the plants growing under salinity conditions when nitrogen supply to the roots is impaired. Urea is one of the most widely-used N fertilizers, has a high leaf penetration rate, where it rapidly absorbed and hydrolysed by urease into carbon dioxide and ammonia [10]. It can have accumulated in source tissues such as senescing leaves, which remobilize N to maintain growth in metabolic sinks [10], consequently, foliar application of urea could directly affect N metabolism under saline conditions and therefore amino acids synthesis [11].

Phyto-hormones is another efficient application affected directly the plant response to salinity [11]. Seaweed appears to have some of the qualities of efficient plant growth regulators, which would justify its use as a soil additive. Seaweed liquid fertilizers were found superior than chemical one because of the presence of high levels of organic matter, thus accounting a reduction of 50% cost for chemical fertilizer [12]. Seaweed are known to cause many beneficial effects on plants as they contain growth promoting hormones (IAA, IBA and Cytokinins), trace elements (Fe, Cu, Zn, Co, Mo, Mn and Ni), vitamins and amino acids [13]. Seaweed extract could serve as a bio-stimulant led to an increase in the plant resistance to biotic and abiotic stresses [14].

Since, any unfavorable change in kohlrabi growth conditions, led to reduction in stem growth rate, which in turn directed the plants to premature aging and forming an unmarketable woody stem. Therefore, the well-drained fertile soil rich in organic matter consider a mandatory demand for kohlrabi production, which could explain the reason behind that until now, there is no previous reports evaluated the growth of kohlrabi plant under salinity stress. So, the objectives of the present study were;

- Evaluating the effect of gradual increase in the concentration of NaCl salt in irrigation water on the leaf and stem growth of kohlrabi plant.
- Defined the first NaCl concentration decreased the growth parameters to a significant level.
- Using foliar application of urea and seaweed extract as plant growth promoters to restore the rapid growth status of kohlrabi plant which could produce non-woody stems (marketable) under selected salinity condition.

MATERIALS AND METHODS

A preliminary pot experiment was conducted in 2012 growing season to evaluate the effect of gradual increase in irrigated water salinity concentration (NaCl at 0, 1000, 2000, 3000, 4000, 5000 and 6000 ppm) on growth of kohlrabi plants. The first NaCl concentration (4000 ppm from Table 2) decreased all studied growth parameters to a significant level had used in the main experiment of this study. The main experiment was pot experiment under outdoor conditions conducted during the two growing seasons of 2013 and 2014 in acid washed sandy soil, at the Experimental farm, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, in order to investigate the effect of foliar application with urea and seaweed extract and their combinations as growth promoters on vegetative yield quality and productivity of kohlrabi plants under NaCl salt stress.

Experimental Design and Treatments: Seeds of Kohlrabi cv. Purple Vienna were sown in the nursery using foam trays on 15th of September in 2013 and 2014 seasons. Kohlrabi seedlings were transplanted into 15-liter plastic pot filled with 14 kg acid washed sandy soil on the first week of November during the two growing seasons of 2013 and 2014. The seedlings were watered with Hoagland solution [15].

Table 1: Chemical and biochemical analyses of seaweed extract, according to UAD® company.

Macro and micro elements	Organic (N)	P_2O_5	K_2O	Ca	S	Mg	Fe	Zn
	2.83%	2.60%	4.47%	0.28%	3.00%	0.65%	0.02%	0.01%
Organic component	Total amino acids	Carbohydrates	Alginic acid	Mannitol	Betaines	IAA	Cytokinins	
	6.11%	35.02%	8.50%	4.23%	0.04%	0.02%	0.02%	

Pots of the experiment were divided into two groups, the first was irrigated with full strength Hoagland nutrient solution to serve as control plants (0 ppm NaCl). The second group was the same plus 4000 ppm NaCl in the nutrient solution. The salinity treatment started at two weeks after transplanting. Treatments were arranged in a complete randomized block design with three replicates.

The concentration of foliar application treatments were four levels for urea (0, 5, 10 and 15 g/l) and two levels of seaweed extract (0 and 0.5 g/l), in addition to a mixture of their combinations. Plants were sprayed four times with 7-day intervals started at one week after transplanting. The structure of seaweed extract used in this study was provided by U.A.D. Co. Egypt (Table 1).

Vegetative Growth Characteristics: The parameters used to explore kohlrabi leaves growth status were leaves no./plant, average leaf f.w, average leaf area and leaf area index (LAI), whereas the parameters used to explore kohlrabi swollen stem growth status and quality were swollen stem diameter, fresh weight, moisture %, firmness and cell concentration index, which recorded at 60 days after transplanting. Leaves area of kohlrabi plant was calculated from analyzing images of total plant leaves by Image-pro plus software (version 6.2, Media Cybernetics Inc., USA). Leaf area index was calculated according to the equation of Hunt [16].

Cell Concentration Index (CCI): Using the optical density (O.D.) or turbidity method recorded by spectrophotometer as an indicator for the organism cell mass status in its growth medium was established as a convenient and rapid method of measuring cell growth rate of an organism. Kubek and Shuler [17] used the same method to measure the growth of plant cell suspension cultures by reading the cell concentration at 525 nm. The authors of the current study, established a new method to explain the relation between plant cell mass, cell volume and cell number in tissues of any plant organ growing under different growth conditions including the optimum conditions (control). The major problem in this method is how to separate plant cells from each other and also maintaining its original size from changing during cell separation. Letham [18] described a new technique for maceration of plant-tissues led to solving this problem by using chelating agent, sodium ethylene-diaminetetra-acetic acid, which had been found to macerate effectively under conditions (pH 7-10, 37-45 °C) without change in cell-volume. Cell concentration index were determined using a modified method depended on combined procedures from the two previously mentioned methods. Cork borer (0.5 cm diameter) was used to take samples with 3 cm length from middle part of kohlrabi stems (swollen stems). A hand microtome was used to make a slices with 2 mm thickness, which used directly to take exactly 1 g fresh weight for all samples. Samples were macerated using 50 ml of 0.1 M Na-EDTA at 75 °C and pH 7 for 6 hours, with occasional shaking, followed by blending for 1-4 min at low speed using a homogenizer with covered blades, followed by recording the turbidity at 525 nm against water. Using the O.D. value of control sample as a reference point (0 %), the increased value in CCI over control will indicating to increasing the cell number with concomitant decrease in cell volume, while decreasing the value of CCI below control indicating to increasing the cell volume rather than cell number.

CCI = ((O.D.sample/O.D.control)-1) X 100

Swollen Stem Firmness and Moisture %: After removing 1 cm of outer peel of kohlrabi swollen stem, the firmness of kohlrabi swollen stem was determined using a drill stand-mounted penetrometer fitted with an 8 mm diameter probe and 7 mm measure distance (Fruit texture analyzer, Guess, South Africa). Values are in kg mm^{-2}. The percentage of kohlrabi swollen stem moisture content was calculated on the basis of fresh weight as described by A.O.A.C. [19].

Stem moisture % = ((Stem f.w-Stem d.w)/Stem f.w) X 100

Biochemical Changes: Photosynthetic pigments of kohlrabi fresh leaves were extracted using 80 % acetone as described by Arnon [20] to determine chlorophyll *a*, chlorophyll *b* and total chlorophylls. Concentrations of chlorophyll *a,* chlorophyll *b*, total chlorophylls and carotenoids were calculated using formulae described by Lichtenthaler and Wellburn [21], after measuring the

absorption of the extracted pigments using a spectrophotometer (Mapada UV 1200) at 470, 647 and 664 nm. The content of photosynthetic pigments was also calculated without further statistical analysis for better understanding to its role in plant physiological status.

Total protein together with free amino acids in both leaves and swollen stems samples, were estimated by digesting the dried leaves and swollen stems samples to amino acids in 6 M HCl, as described by Barbehenn [22]. Total amino acids consisted of total free amino acids plus amino acids from total proteins acid hydrolysis were measured using ninhydrin method. And also, the total free amino acids concentrations in leaves and swollen stems samples were determined according to the method described by Swamy [23]. The developed color with ninhydrin reagent was measured using a spectrophotometer (Mapada UV 1200) at 570 nm. Total protein (TP) in samples was calculated by subtracted the amount of total free amino acids (TFAA) from total amino acids [24]. Proline concentration in the dry samples of both leaf and swollen stem of kohlrabi plant was determined using the acid ninhydrin method described by Bates *et al.* [25].

Total soluble sugars (TSS) were extracted from 0.1 g leaf and swollen stem dry tissues by 80% hot ethanol [19] and determined using anthrone method as described by Sadasivam and Manickam [26]. The total carbohydrates (T.Carbs) were estimated using the same anthrone method, after leaves and swollen stems sample were hydrolyzed in HCl [26]. Crude fibre in kohlrabi swollen stems as percentage of dry matter was determined using digestion method (method 930.10) as described by A.O.A.C. [19].

Statistical Analysis: Data of the two seasons were arranged and statistically analyzed using CoStat software (version 6.4, CoHort Software, USA) according to the method described by Gomez and Gomez [27]. Two-way analysis of variance (ANOVA) was used to test for significant differences among salinity levels at $P < 0.05$, followed by Tukey's HSD test. One-way ANOVA was used to reveal significant differences across foliar application treatments within individual salinity level while a post hoc Tukey's HSD test was used to test for significant differences between individual treatments means. Spearman correlation coefficients between biochemical constituents of kohlrabi leaves and swollen stems and different yield attributes under different NaCl salinity levels were calculated using XLSTAT Addinsoft version 2016 (Addinsoft, NY).

RESULTS AND DISCUSSIONS

First Experiment

Evaluating The Growth Responses of Kohlrabi Plant Under Gradual Increase of Salinity : The growth attributes of kohlrabi plants growing under gradual increase in NaCl salinity from 0 to 6000 ppm were evaluated in preliminary experiment and presented in Table 2. The evaluated parameters (leaves no./plant, average leaf f.w, LAI, swollen stem diameter and fresh weight) significantly decreased when plants grown under NaCl concentration equal or greater than 4000 ppm. Whereas, the first parameter affected by increasing in NaCl concentration was leaves no./plant which start decreasing to a significant level at 2000 ppm NaCl salinity. Increasing the salt concentration to 3000 ppm reduced in addition to leaves no./plant, LAI, swollen stem diameter and f.w, but didn't yet decreased average leaf area to a significant level comparing with 0 ppm NaCl. Whereas, leaves no./plant parameter was the first one affected by salinity increase, the highest affected parameter by gradual increase in salinity was average leaf area which recorded the lowest significant value (e letter) at 6000 ppm NaCl, while its highest value wasn't under 0 ppm NaCl as expected, but under 2000 ppm NaCl. With another expression, increasing salinity level up to 2000 ppm led to a gradual increase in average leaf area and any additional increase in NaCl concentration led to spectacular reduction in leaf area values, which reduced to half its amount when plant growing under 4000 ppm NaCl comparing with plants growing under 3000 ppm NaCl. The second class of the most effected parameters by gradual increase in NaCl concentration was for LAI which started to reduce significantly under the effect of 3000 ppm NaCl and took the same trend of average leaf area under the following higher concentrations of NaCl. Swollen stem f.w reduced to half its value under NaCl at 3000 ppm comparing with its value under 2000 ppm of NaCl.

It was concluded from this experiment that NaCl concentration at 4000 ppm was the first NaCl concentration significantly reduced both of leaf area and swollen stem f.w (the most important parameters affect plant growth) than 0 ppm NaCl. This indicate finally, that kohlrabi is a moderate sensitive plant to salt stress, which lose 50 % of its stem yield under salinity level equal to 3000 ppm NaCl.

Table 2: Effect of gradual increase in NaCl salinity concentration (0: 6000 ppm) on leaves no./plant, average leaf area, leaf area index, swollen stem diameter and swollen stem fresh weight of kohlrabi plant during 2012 season (Preliminary experiment).

	NaCl concentrations (ppm)						
	0	1000	2000	3000	4000	5000	6000
Leaves no./plant	16.8 a	16.7 a	12.7 b	11.8 b	11.7 b	8.3 c	7.3 c
Average leaf area (cm^2)	43.2 bc	45.0 b	55.9 a	40.2 c	21.3 de	24 d	18.1 e
Leaf area index	1.03 a	1.06 a	1.00 a	0.67 b	0.35 c	0.28 c	0.19 d
Swollen stem diameter (cm)	6.47 a	6.43 a	6.19 a	5.62 b	4.31 c	4.09 cd	3.78 d
Swollen stem f.w (g)	167 a	166 a	160 a	83 b	64 c	61 c	56 c

Means followed by different letters are significantly different at $P < 0.05$ level; Tukey's HSD test.

Second Experiment: Enhancing The Quantitative and Qualitative Attributes of Kohlrabi Stems Under 4000 ppm NaCl Salinity

Vegetative Growth Characteristics: Leaf growth parameters of kohlrabi plants grown under the effect of NaCl salinity at 4000 ppm reduced to approximately half its amount for control plants (under NaCl at 0 ppm), especially for average leaf f.w, leaf area and LAI which recorded 2.9 g, 11 cm^2 and 0.17 under NaCl at 4000 ppm comparing with 5.4 g, 23.2 cm^2 and 0.27 for control plants, respectively (Table 3). Leaves no./plant didn't take the same previous trend under salinity condition, where kohlrabi plants grown under salinity condition (4000 ppm) had a higher leaves no./plant comparing with control plants (0 ppm). Correlation between different studied parameters (Table 7), revealed the secret behind this unusual increase in leaves no./plant under salinity stress, where it was strong positive correlated with the concentration of both total free amino acids and total soluble sugars in swollen stems. Since, kohlrabi tuber-like stem is a food storage organ, which in turn considered as source of assimilates to other growing plant organs such as leaves (sink). So, the increase in the concentration of TFAA and proline in swollen stems under NaCl at 4000 ppm led to a consequent increase in total protein in the same organ (Table 5), which could be had a stimulate effect on stem top meristem leading to produce more nodes on the stem with a concomitant formation of new leaves, which finally reflected on increasing leaves no./plant.

Salt stress of NaCl at 4000 ppm led to decrease in the swollen stem f.w and diameter of kohlrabi plants comparing with control plants (under NaCl at 0 ppm), where the fresh weight of the swollen stem was the most stem parameters effected by NaCl salinity which decreased from 127 g in control plants to 77 g in plants subjected to NaCl at 4000 ppm (Table 3). This decrease in stem f.w was strong correlated with the increase in both stem firmness and fibre % (Table 7) which not only led to decrease stem f.w, but also increase the hardness of the tuber as indicated by firmness value. Stem firmness value and cell concentration index (CCI) under the effect of NaCl at 4000 ppm recorded the highest values (10.3 and 4, respectively) among all treatments whether under saline (4000 ppm) or non-saline conditions (Table 3). Strong correlation between stem CCI and stem firmness (Table 7) revealed the reason behind decreasing swollen stem f.w, where increasing in the values of CCI were indicated to increasing the cell number in specific weight, which also indicated to that, cells had a smaller size. Increase cell number led to a consequent increase in supportive cell walls which in turn stand together to be more resisted to the force applied by fruit texture analyzing instrument, leading to an increase in the firmness value and fruit hardening (Table 3).

When kohlrabi plant treated with urea and seaweed extract as foliar applications, it wasn't had the same response trend in vegetative parameters under saline and non-saline stress conditions (Table 3). Whereas, application of urea at 10 or 15 g/l led to a significant increase in leaves no./plant under 0 ppm NaCl, the same urea concentrations decreased the values of leaves no./plant under NaCl at 4000 ppm. Not only leaves no./plant parameter had an opposite response for urea application under saline and non-saline stress conditions, but also parameters of average leaf f.w and leaf area, which reduced when urea foliar applied at 5, 10 and 15 g/l under control condition (0 ppm NaCl), while increased under the effect of NaCl at 4000 ppm for the same urea concentrations (Table 3). The best urea concentration led to significant increase or insignificant decrease in leaf growth parameters under control conditions was urea at 15 g/l. Whereas, under salinity condition (4000 ppm NaCl), the best increase in leaf parameters was recorded with urea at 10 and 15 g/l, respectively.

Table 3: Effect of foliar application of urea and seaweed extract (SWE) and their combinations under different levels of NaCl salinity (0 and 4000 ppm) on leaf and swollen stem (S.Stem) vegetative characteristics of kohlrabi plant during 2013 and 2014 seasons (main of two seasons).

	Leaves no./plant	Average leaf f.w (g)	Average leaf area (cm^2)	Leaf area index	S.Stem diameter (cm)	S.Stem f.w/plant (g)	S.Stem firmness (kg/mm^2)	S.Stem CCI (%)	S.Stem moisture (%)
Foliar treatments (g/l)	---------- NaCl at 0 ppm ----------								
Control	8.3 e	5.4 ab	23.2 a	0.27 ac	5.6 d	127 b	7.1 ab	0 a	84.8 c
Urea 5	9.0 de	3 cd	12.5 bc	0.16 cd	5.9 cd	143 b	5.8 b	-14 ab	88.0 ab
Urea 10	10.0 cd	3.4 bcd	17.9 ab	0.25 bc	6.7 bd	171 b	6.3 b	-25 bc	88.4 ab
Urea 15	11.3 bc	5.1 abc	21.5 a	0.35 ab	7.8 ab	249 ab	6.3 b	-7 a	89.1 ab
SWE 0.5	9.3 de	1.6 d	7.2 c	0.10 d	5.8 d	124 b	6.9 ab	-4 a	86.5 bc
SWE 0.5 + Urea 5	10.3 cd	3.9 abc	19.1 ab	0.28 ac	7.4 ac	224 ab	8.8 a	-35 c	89.7 a
SWE 0.5 + Urea 10	12.3 ab	5.9 a	23.7 a	0.42 a	8.6 a	283 ab	7.4 ab	-7 a	87.0 ac
SWE 0.5 + Urea 15	13.0 a	5.9 a	22.7 a	0.42 a	8.4 a	422 a	6.9 ab	-30 c	87.7 ac
Mean	10.5 B	4.3 A	18.5 A	0.28 A	7.0 A	218 A	7.0 B	-15 A	87.7 A
	---------- NaCl at 4000 ppm ----------								
Control	11.3 ab	2.9 c	11.0 c	0.17 c	5.2 cd	77 c	10.3 a	4 a	86.4 d
Urea 5	12 ab	3.5 c	13.1 c	0.22 bc	6.4 bc	182 bc	8.4 ab	-28 b	89.1 bc
Urea 10	10.3 b	4.2 bc	19.7 bc	0.29 bc	6.9 b	237 b	8.1 b	-18 ab	88.4 c
Urea 15	10.3 b	4.3 bc	18.1 bc	0.26 bc	7.2 b	248 b	6.5 b	-26 b	91.0 a
SWE 0.5	8.0 c	6.3 ab	28.1 ab	0.33 b	5.0 d	68 c	7.8 b	-33 b	87.8 cd
SWE 0.5 + Urea 5	11.7 ab	2.5 c	11.5 c	0.19 bc	6.3 bc	203 b	7.2 b	-39 b	90.1 ab
SWE 0.5 + Urea 10	13.0 a	3.1 c	15.7 c	0.29 bc	6.5 b	226 b	7.5 b	-32 b	88.4 c
SWE 0.5 + Urea 15	12.0 ab	8.1 a	35.3 a	0.58 a	8.7 a	421 a	6.2 b	-30 b	89.2 bc
Mean	11.1 A	4.4A	19.1 A	0.29 A	6.5 B	208 A	7.8 A	-25 B	88.8 A

Means followed by different letters are significantly different at $P < 0.05$ level; Tukey's HSD test. CCI = cell concentration index.

All urea individual applications led to an increase in swollen stem diameter and fresh weight under both tested levels of salinity, especially for urea at 15 g/l which recorded the highest levels over the rest of urea concentrations (Table 3). The firmness value which recorded to indicate to the hardness of kohlrabi stems, was decreased to insignificant level for control plants treated with urea (5, 10 and 15 g/l), whereas under salinity condition (4000 ppm NaCl) the effect of urea individual concentrations, especially for 15 g/l significantly reduced the firmness values than its control. The reduction of firmness values under urea applications indicated to that urea had a positive effect on increasing cell size which reflected on decreasing the cell wall network structure in kohlrabi stems which in turn reducing the stem hardness. This suggestion supported by data of swollen stem moisture % and CCI % (Table 3), where application of urea increased the moisture % in kohlrabi stems and decreased value of CCI than control which indicating to an increase in the cell size under the effect of increasing moisture % (Table 3) and decreasing crude fibre % (Table 5). The most impressive observation is that application of urea at 5 and 10 g/l increased the fresh weight of swollen stem under salinity condition (4000 ppm NaCl) more than under 0 ppm NaCl (Table 3). This increase refers to the effect of urea on increasing leaves no./plant, average leaf area and LAI under salinity stress more than its effect under control conditions (Table 3), which allowed plant leaves to capture and utilize more light and synthesis more total carbohydrate in leaves under NaCl at 4000 ppm more than 0 ppm NaCl (Table 5) and also increased the metabolic conversion rate of leaf carbohydrate to leaf amino acids in the presence of urea as a source for N in amino acids synthesis, which translocated to swollen stems where increased significantly leading to increasing total protein of stems (Table 5), which could be, in addition to its effect on enhancing growth, led to decrease the osmotic potential of stem cells in the presence of high NaCl concentration which in turn could be accumulated in the vacuoles as the plant is moderately sensitive to salinity and it could also be sharing with assimilates located in the stem cells in decreasing osmotic potential, which reflected on absorb more water and increase stem moisture %, which in turn reflected on increasing stem f.w under salinity conditions (Table 3).

Under non-stress condition, seaweed extract individual application had a negative effect on leaf and stem studied parameters, leading to decrease stem f.w (Table 3). Whereas, under stress condition (4000 ppm NaCl), although average of leaf f.w, leaf area and LAI significantly increased, the leaves no./plant recorded the lowest value which led also to decrease the fresh weight of swollen stem. Where values of leaf area and leaf f.w were among the highest values under the effect of seaweed extract application and NaCl at 4000 ppm, the reduction in leaves no./plant under NaCl at 4000 ppm wasn't the only explanation for the reduction occurred in stem f.w under treatment of seaweed extract at 0.5 g/l. These observations suggest that application of seaweed extract at 0.5 g/l had a little positive effect on increasing leaves no./plant under non-stress condition through maximizing the concentration of TSS in stem and TFAA in leaves (Table 5), which could serve as raw material to produce new growth including leaves. Whereas, under salt stress (NaCl at 4000 ppm), concentrations of total protein and total carbohydrate in both leaves and stems were increased under seaweed application (Table 5), which suggest that the potential ability of seaweed extract to motivate new growth was repressed under salt stress, which directed the assimilates to synthesis macromolecules (protein and carbohydrate) which led to support leaf growth rather than stem growth. This unsupported action on increasing stem f.w by seaweed extract application could be referred to its biochemical structure, where it has betaine substance (Table 1) which increase in many stress-tolerant plants only under the action of stress to protect macromolecules from damage, so when betaine exogenously applied to some plants support the protection action than enhancing growth action.

The combined treatments between urea (5, 10 and 15 g/l) and seaweed extract at 0.5 g/l under non-stress conditions recorded the highest values in leaves no./plant, LAI, swollen stem diameter and stem fresh weight, where the highest stem f.w values recorded with the treatment of SWE at 0.5 g/l + Urea at 15 g/l and SWE at 0.5 g/l + Urea at 10 g/l, respectively (Table 3). Whereas, under salt stress, the potential ability of seaweed extract on boosting the vegetative growth of kohlrabi plant was released when mixed with urea applications, where recorded the highest value between all foliar application with the treatment of SWE at 0.5 g/l + Urea at 15 g/l which led to boosting the stem f.w to reach 5.5-fold its amount for control plant treated with NaCl at 4000 ppm, whereas the same treatment increased the fresh weight of the stem under NaCl at 0 ppm to 3.3-fold than its control (Table 3). This flawless increase could be referred mainly to the effect of urea which leading to decrease cell wall thickness and increase its extensibility which add more extension to plant cell wall [28], under the addition of seaweed extract due to its IAA content (Table 1), which in turn led to increase cell size 30 % more than control as indicated by CCI value (Table 3).

Biochemical Changes: The content of photosynthetic pigments was highly affected by abiotic stress (salinity) and plant nutritional status (Table 4). Subjected plants to NaCl at 4000 ppm significantly reduced all values of chlorophyll fractions in leaves of kohlrabi plants. Correlation analysis in Table 7, revealed that the reduction in total chlorophyll content under salt stress was highly correlated with TSS in stem which reflected on increasing CCI and firmness values, while the highest values of photosynthetic pigments under non-stress condition led to a reduction in CCI values (Table 6). The gradual increase of urea application led to a gradual increase in the chlorophyll content, especially for urea at 15 g/l when plants growing with or free of NaCl stress (Table 4). Under 4000 ppm NaCl, the concentration of chlorophyll fractions didn't take the same trend as done for its content, where urea at 10 g/l decreased the chlorophyll fractions than recorded with 5 g/l of urea. So, the content of chlorophyll is a better indicator for chloroplasts distributions in leaf unit to capture more light. Although, concentrations of chlorophyll fractions were increased under seaweed extract treatment in non- salt stressed kohlrabi plants, its content per leaf unit recorded the lowest values, which strongly suggest that most of assimilates produced by photosynthesis directed to support tuber-like stem growth. The opposite observation for content and concentration of chlorophyll fractions affected by seaweed treatment, recorded under the effect of NaCl at 4000 ppm, which suggest that seaweed extract didn't effect on chlorophyll content directly, but through its direct effect of leaf area and leaf thickness (weight in another word). When urea applied to the plants in the presence of seaweed extract, it led to a gradual increase in total chlorophyll content, which reached to 7.4-fold its amount in plants without foliar treatments under NaCl at 4000 ppm (Table 4).

Table 4: Effect of foliar application of urea and seaweed extract (SWE) and their combinations under different levels of NaCl salinity (0 and 4000 ppm) on photosynthetic pigments concentration and content (per leaf unit) of kohlrabi plant leaves during 2013 and 2014 seasons (main of two seasons).

Foliar treatments (g/l)	Chl *a*	Chl *b*	Chl *a+b*	Carot	Chl *a:b* ratio	Chl *a*	Chl *b*	Chl *a+b*	Carot	Chl *a:b* ratio/leaf
	mg/g f.w					mg/leaf unit				
	NaCl at 0 ppm									
Control	0.47 b	0.26 b	0.73 c	0.26 c	1.81 d	2.51	1.41	3.93	1.38	1.78
Urea 5	0.56 b	0.25 b	0.82 c	0.27 bc	2.24 ac	1.70	0.76	2.47	0.82	2.23
Urea 10	1.00 a	0.40 a	1.40 a	0.46 a	2.51 a	3.44	1.37	4.80	1.57	2.51
Urea 15	0.82 a	0.36 ab	1.18 ab	0.37 ab	2.31 ab	4.19	1.82	6.01	1.90	2.30
SWE 0.5	0.57 b	0.33 ab	0.90 bc	0.27 c	1.74 d	0.93	0.53	1.46	0.43	1.75
SWE 0.5 + Urea 5	0.60 b	0.33 ab	0.93 bc	0.30 bc	1.81 d	2.31	1.28	3.59	1.15	1.81
SWE 0.5 + Urea 10	0.57 b	0.28 b	0.85 c	0.27 bc	2.06 bd	3.38	1.66	5.04	1.61	2.04
SWE 0.5 + Urea 15	0.61 b	0.32 ab	0.93 bc	0.30 bc	1.93 cd	3.59	1.88	5.48	1.78	1.91
Mean	0.65 A	0.32 A	0.97 A	0.31 A	2.05 A	2.76	1.34	4.10	1.33	2.04
	NaCl at 4000 ppm									
Control	0.30 d	0.18 c	0.47 d	0.12 e	1.67 c	0.86	0.51	1.37	0.34	1.67
Urea 5	0.56 c	0.27 b	0.84 bc	0.23 bd	2.04 ab	1.97	0.97	2.94	0.79	2.04
Urea 10	0.51 c	0.27 b	0.78 c	0.20 d	1.92 ac	2.16	1.13	3.30	0.84	1.91
Urea 15	0.61 bc	0.29 b	0.90 bc	0.24 bd	2.08 ab	2.58	1.25	3.83	1.01	2.07
SWE 0.5	0.54 c	0.30 b	0.84 bc	0.22 cd	1.83 bc	3.43	1.89	5.32	1.38	1.81
SWE 0.5 + Urea 5	0.85 a	0.42 a	1.27 a	0.33 a	2.04 ab	2.13	1.05	3.19	0.82	2.03
SWE 0.5 + Urea 10	0.72 ab	0.33 b	1.05 b	0.30 ab	2.19 a	2.25	1.02	3.27	0.93	2.19
SWE 0.5 + Urea 15	0.84 a	0.42 a	1.26 a	0.29 ac	2.01 ab	6.78	3.36	10.14	2.38	2.01
Mean	0.62 A	0.31 A	0.93 A	0.24 B	1.97 A	2.77	1.40	4.17	1.06	1.97

Means followed by different letters are significantly different at $P < 0.05$ level; Tukey's HSD test. Chl = chlorophyll; Carot = carotenoids.

Plants grown under stress or non-stress conditions exhibited that the concentrations of TFAA in leaves and stem approximated to each other's (Table 5), whereas TSS concentration in stems was 3-fold its concentration in leaf, which suggest that the driven force for plant stem growth is the concentration of TSS especially under salt stress where it had a strong positive correlation with leaves no./plant, total chlorophyll and total protein (Table 7). Whereas, increase TSS concentration in leaves under non-stress conditions led to an increase in leaf f.w and leaf area which was high correlated with increasing stem firmness value (Table 6). Proline concentrations in leaf and stem of kohlrabi plant under the effect of 0 ppm NaCl were approximated to each other's (Table 5), whereas under salt stress (4000 ppm), proline concentration decreased in leaves and boosted in swollen stem to reach 6.4-fold its amount in leaf. This observation suggest that kohlrabi stems is more tolerate to salinity stress than its leaves. All foliar treatments decreased proline concentration in kohlrabi stem than its control for plant grown under NaCl at 4000 ppm, especially for seaweed extract at 0.5 g/l, urea at 15 and 10 g/l, respectively (Table 5), which suggest the abilities of these treatments to restore the plant homeostasis found under non-stress conditions, even under stress conditions. All foliar treatments increased the total protein concentration in leaves and stems of kohlrabi plant under salt stress than non-salt stress, which leading to increase the nutritional value of the leaves and swollen stems. Protein concentrations were more correlated with urea concentrations than seaweed extract. All combined treatments under stress conditions recorded the highest values for most of biochemical parameters, except for crude fibre % which decreased significantly than its control, which in turn indicated to those treatments had a promotive effect on growth and quality of kohlrabi stems.

Since urea is a soluble N source, it is commonly utilized as a foliar N source. The nitrogen in urea is not directly available to the plant until it is hydrolyzed into ammonia by urease enzyme in the cytosol. Urea is metabolized quickly and does not accumulate in the cytosol. Very little is known about the importance of the form of N transported and stored. However, urea is constantly produced in metabolism and can be used as a source of nitrogen. The rapid distribution of urea after foliar application and the increase in N from urea in xylem sap indicated that urea has the ability to transport directly. Urea could be sequestered into the vacuole for

Table 5: Effect of foliar application of urea and seaweed extract (SWE) and their combinations under different levels of NaCl salinity (0 and 4000 ppm) on concentration of total free amino acids (TFAA), proline, total protein (TP), total soluble sugars (TSS) and total carbohydrates (T.Carbs) in leaf and swollen stem (S.Stem) of kohlrabi plant, in addition to crude fibre percentage (C. fibre) in swollen stem during 2013 and 2014 seasons (main of two seasons).

	mg/g d.w										(%)
	TFAA		Proline		TP		TSS		T.Carbs		C. fibre
	Leaf	S.Stem	Leaf	S.Stem	Leaf	S.Stem	Leaf	S.Stem	Leaf	S.Stem	S.Stem
Foliar treatments (g/l)	NaCl at 0 ppm										
Control	21.9 c	20.5 b	8.1 c	5.0 c	99 a	156 cd	44.5 c	150 bc	672 a	639 ab	19.3 a
Urea 5	38.9 ab	13.8 c	10.4 ab	4.4 c	129 a	170 bd	32.5 d	159 ac	560 a	661 ab	15.1 a
Urea 10	50.9 a	14.1 bc	11.1 ab	7.9 bc	103 a	183 ac	23.2 e	130 c	540 a	602 ab	19.1 a
Urea 15	29.7 bc	29.8 a	11.5 a	15.7 a	103 a	203 ab	42.8 c	135 bc	694 a	599 ab	19.0 a
SWE 0.5	48.8 a	14.7 bc	10 abc	6.1 c	91 a	138 d	23.9 e	205 a	622 a	740 a	18.6 a
SWE 0.5 + Urea 5	32.7 bc	27.5 a	9.2 bc	6.3 c	126 a	169 bd	50.6 b	166 ac	675 a	685 ab	15.0 a
SWE 0.5 + Urea 10	29.4 bc	17.7 bc	9.2 bc	5.2 c	102 a	189 ac	58.9 a	185 ab	714 a	677 ab	18.7 a
SWE 0.5 + Urea 15	38.0 ab	16 bc	9.6 abc	13.3 ab	129 a	215 a	42.1 c	119 c	701 a	594 b	18.0 a
Mean	36.3 A	19.3 B	9.9 A	8.0 B	110 B	178 B	39.8 A	156 A	647 A	650 A	17.8 A
	NaCl at 4000 ppm										
Control	27.7 b	30.2 ab	3.9 b	25.0 a	91 b	153 e	32.2 ab	104 c	604 ab	637 ab	20.1 a
Urea 5	22.2 bc	39.8 a	5.1 b	20.4 ab	143 ab	219 bc	24.9 b	122 bc	671 ab	643 ab	18.0 ac
Urea 10	9.1 c	21.8 bc	4.6 b	9.3 cd	150 ab	294 a	36.3 ab	120 bc	723 a	559 b	15.4 d
Urea 15	25.7 b	21.8 bc	5.1 b	8.2 cd	187 a	242 b	36.8 ab	155 ab	605 ab	597 ab	15.4 d
SWE 0.5	27.9 b	10.6 c	6.3 b	6.9 d	140 ab	158 e	24.2 b	88 c	691 a	694 a	16.8 cd
SWE 0.5 + Urea 5	49.2 a	27.0 ab	12.7 a	15.2 bc	181 ab	185 de	21.6 b	194 a	508 b	634 ab	17.5 bd
SWE 0.5 + Urea 10	14.0 bc	30.9 ab	11.1 a	15.4 bc	123 ab	195 cd	47.0 a	191 a	760 a	689 a	19.7 ab
SWE 0.5 + Urea 15	22.3 bc	30.3 ab	12.8 a	15.2 bc	176 ab	226 bc	35.2 ab	121 bc	612 ab	610 ab	15.4 d
Mean	24.8 B	26.7 A	7.7 B	14.5 A	149 A	209 A	32.3 B	137 B	647 A	633 B	17.3 A

Means followed by different letters are significantly different at $P < 0.05$ level; Tukey's HSD test.

Table 6: Coefficients of correlation between biochemical constituents of kohlrabi leaves and swollen stems and different vegetative yield attributes for the combined two seasons under 0 ppm NaCl salinity.

Variables	(01)	(02)	(03)	(04)	(05)	(06)	(07)	(08)	(09)	(10)	(11)	(12)
01-Leaves no./plant	1.00											
02-Average leaf f.w	0.62*	1.00										
03-Average leaf area	0.43	0.93*	1.00									
04-S.Stem f.w/plant	0.93*	0.76*	0.60*	1.00								
05-Firmness (S.Stem)	0.19	0.36	0.48	0.12	1.00							
06-Swollen stem CCI	-0.49	-0.10	0.13	-0.54*	0.05	1.00						
07-Chl a+b	0.48	-0.10	-0.24	0.36	-0.19	-0.55*	1.00					
08-TFAA (leaf)	-0.10	-0.67*	-0.83*	-0.26	-0.57*	-0.37	0.45	1.00				
09-TFAA (S.Stem)	0.31	0.48	0.55*	0.33	0.60*	0.08	0.17	-0.74*	1.00			
10-TP (leaf)	0.55*	0.24	-0.02	0.64*	-0.24	-0.75*	0.19	0.14	-0.07	1.00		
11-TP (S.Stem)	-0.17	-0.29	-0.21	0.05	-0.55*	-0.34	0.26	0.19	-0.19	0.29	1.00	
12-TSS (leaf)	0.31	0.62*	0.76*	0.43	0.76*	0.04	-0.31	-0.88*	0.71*	0.07	-0.14	1.00
13-TSS (S.Stem)	-0.26	-0.43	-0.21	-0.43	0.50	0.36	-0.40	-0.10	0.00	-0.33	-0.24	0.29
14-Fibre (S.Stem)	-0.24	0.19	0.36	-0.19	-0.12	0.63*	0.00	-0.26	0.14	-0.74*	-0.05	-0.10

Values with asterisk (*) are different from 0 with a significance level alpha=0.05. CCI = cell concentration index, Chl = chlorophyll, TFAA = total free amino acids, TP = total protein, TSS = total soluble sugars, S.Stem = swollen stem.

transient storage or to avoid uncontrolled urea breakdown by urease [10]. Foliar application of urea on potato increased urease activity which reflected on increasing urea metabolism (ammonium accumulation) and noted that the rapid export of N from urea-treated leaves to the tubers within 48 h, followed by a more gradual redistribution during the subsequent days [29]. In an attempt to combat slow growth of kohlrabi plants under salinity, foliar application with urea was used and showed a 21% increase in chlorophyll content together with a reduction in the chlorophyll *a:b* ratio [30]. However, increase in chlorophyll content per leaf area or weight of

Table 7: Coefficients of correlation between biochemical constituents of kohlrabi leaves and swollen stems and different vegetative yield attributes for the combined two seasons under 4000 ppm NaCl salinity.

Variables	(01)	(02)	(03)	(04)	(05)	(06)	(07)	(08)	(09)	(10)	(11)	(12)
01-Leaves no./plant	1.00											
02-Average leaf f.w	-0.30	1.00										
03-Average leaf area	-0.24	0.93*	1.00									
04-S.Stem f.w/plant	0.27	0.38	0.45	1.00								
05-Firmness (S.Stem)	-0.14	-0.43	-0.52*	-0.71*	1.00							
06-Swollen stem CCI	-0.29	-0.12	-0.29	-0.10	0.64*	1.00						
07-Chl a+b	0.40	0.05	0.21	0.43	-0.86*	-0.88*	1.00					
08-TFAA (leaf)	-0.35	-0.19	-0.26	-0.45	-0.14	-0.40	0.29	1.00				
09-TFAA (S.Stem)	0.93*	-0.29	-0.36	0.12	0.07	-0.07	0.14	-0.33	1.00			
10-TP (leaf)	-0.10	0.14	0.21	0.57*	-0.69*	-0.48	0.62*	0.21	-0.21	1.00		
11-TP (S.Stem)	-0.37	0.76*	0.83*	0.21	-0.10	-0.05	-0.14	-0.43	-0.40	0.14	1.00	
12-TSS (leaf)	0.23	0.21	0.24	0.62*	-0.21	0.43	-0.07	-0.71*	0.17	-0.17	0.07	1.00
13-TSS (S.Stem)	0.58*	-0.45	-0.33	0.40	-0.48	-0.40	0.69*	-0.02	0.43	0.52*	-0.45	0.17
14-Fibre (S.Stem)	0.31	-0.79*	-0.81*	-0.69*	0.71*	0.29	-0.36	0.07	0.40	-0.69*	-0.64*	-0.14

Values with asterisk (*) are different from 0 with a significance level alpha=0.05. CCI = cell concentration index, Chl = chlorophyll, TFAA = total free amino acids, TP = total protein, TSS = total soluble sugars, S.Stem = swollen stem.

the leaves did not yield a larger net photosynthesis. This may be a result of saturating chlorophyll concentration exceeding that required for maximum photosynthesis [30]. Foliar spray of urea to the salinized broccoli plants appeared to partially counteract the deleterious effects of salinity and partially minimized the salt-induced nutrient deficiency and increased photosynthesis, photosynthesis-related parameters, yield and yield components. Additionally, foliar application of urea also appeared to counteract the stress-induced damage, maintaining growth and photosynthesis at moderate stress [11]. Foliar application of urea at 10000 ppm increased the yield of tomato plant 2-fold its amount under control treatment [31] and led to greater increase in the size of broccoli heads and leaf chlorophyll content [32]. In this regard, Gooding and Davies [33] reported that foliar urea sprays can be of greater benefit than soil applications, where foliar application of urea have increased wheat grain yield and quality.

Salt stress decreased the radish stem diameter, epidermis cell size, cortex zone thickness, vascular bundle width, cambium thickness, xylem width, trachea diameter and phloem width in the seedlings non-pretreated with the growth regulators. On the other hand, many of the growth regulator pretreatments more or less stimulated the stem diameter, epidermis cell width, cortex zone thickness, vascular bundle width, xylem width, trachea diameter and phloem width in comparison with the control seedlings grown on saline medium [6]. Since, seaweed extracts have plant growth hormones, it acts as biostimulants, improving plant growth, yield, flower set and fruit production, increasing resistance to biotic and abiotic stresses. This stimulant effects often have been attributed to the presence of plant growth hormones and related low molecular weight compounds present in the extracts [14].

CONCLUSION

Results of this study concluded that, kohlrabi plant is a moderate sensitive plant to salinity, where the stem fresh weight start to reduce when grown and salt stress equal to 3000 ppm and above. The best treatment led to eliminate the negative effects of salinity on qualitative and quantitative traits of kohlrabi stems is the combined foliar treatment between seaweed extract at 0.5 g/l and urea at 15 g/l.

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