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Cucumber grafting onto pumpkin can represent an interesting tool to minimize salinity stress. Physiological and anatomical studies

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ABSTRACT

Cucumber is often affected by salinity due to its highly susceptible to soil salinity even at low electrical conductivity in the saturated soil extract. This study found that cucumber grafting on salt resistance rootstock of pumpkin can represent an interesting tool to alleviate reduce biomass and yield losses caused by salinity stress. Grafted plant grown under salinity often exhibited better growth (plant height, number of leaves, leaf area as well as fresh and dry weight) and yield, higher photosynthesis and greater root-to-shoot ratio, higher accumulation of compatible osmlytes (proline, total free amino acids and total sugars), abscisic acid (ABA) and greater antioxidative enzyme capacity in the leaves and lower accumulations of Na^+ and/or Cl^- in the shoots than in the control (self-grafted plants grown under non-salinized condition). Moreover, grafting nullified the depressing effect of salinity and considerably increased stomatal conductance and leaf water potential in the leaves. The anatomical studies indicated that grafting and/or salinity stress brought about various changes. Salinized cross sections showed compacted cells, as estimated by epidermis diameter and area as well as by cortex diameter in the stem and intermediate tissue in the leaf blade, whereas grafting counteracted these effects. The main vascular bundle (phloem and xylem) dimension and pith area in the stem were increased under salinity and grafting shows an additive effect in this respect. Results suggested that grafting is an integrative reciprocal process and therefore, both scion and rootstock can influence salt tolerance in grafted cucumber plants. It could effectively control plant size through physiological and morphological change mechanisms. Thereby, bringing higher sustainable returns per unit area and greater effectiveness in plant production.

Key words: Cucumber (*Cucumis sativus*), Pumpkin (*Cucurbita pepa*), Grafting, Osmoregulators, Oxidative enzyme, Endogenous phytohormon, Anatomical traits.

Introduction

Cucumber (*Cucumis sativus* L.) is one of the very low calorie vegetables contains many nutrient elements and phenolic compounds. These compounds act as protective scavengers against oxygen drived free radicals and reactive oxygen species (ROS) that play a role in cardiovascular disease prevention and anticancer activity (Yuan, *et al.,* 2008). Cucumber, are glycophytes and highly susceptible to soil salinity even at low electric conductivity in the saturated soil extract (Colla *et al.*, 2010). The deleterious effects of salinity on plant growth are associated with (i) low water potential of the root media which causes a water deficit within the plant; (ii) toxic effects of ions mainly Na^+ and Cl^- (iii) nutritional imbalance caused by reduced nutrients uptake and/or transport to the shoot (Hasegawa *et al.,* 2000). Moreover, aerobic organisms face constant risk from reactive oxygen species (ROS), which are inevitably generated naturally *via* a number of cell metabolic pathways (Munns, 2002). Plant possess antioxidant defense systems, which normally maintain ROS balance within the cell. Most studies have shown that the resistance to the excess production of ROS during salinity stress is usually correlated with a more efficient antioxidant systems (Helaly *et al.*, 2017).

Numerous attempts have been made to improve the salt tolerance of crops by traditional breeding programs. However, commercial success has been very limited due to the complexity of the trait: salt tolerance is complex genetically and physiologically (Flowers, 2004). At present, major

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efforts are being directed towards the genetic transformation of plants in order to raise their tolerance (Borsani *et al.*, 2003) and in spite of the complexity of the trait, the transfer of a single gene or a few genes has led to claims of improvement in salt tolerance, such as occurs with the expression of some genes involved in the control of Na^+ transport (Zhang and Blumwald, 2001). However, the nature of the genetically complex mechanisms of abiotic stress tolerance, and potential detrimental side effects, make this task extremely difficult (Wang *et al.*, 2003; Flowers, 2004). Solving a problem as complex as the profitable use of saline water in irrigated agriculture requires more than one strategy. In addition to tolerant cultivars, several cultural practices, each contributing to a small extent to allow plants to withstand better the deleterious effect of salt, needs to be applied (Cuartero and Fernandez-Muńoz, 1999). Some of the proposed practices, like the application of chemical fertilizers at levels somewhat above the optimum in freshwater irrigation, the application of chemical amendments or leaching salts to deeper soil layers, are hardly compatible with the urgent need to preserve the environment (Cuartero *et al.*, 2006).

One environment-friendly technique for avoiding or reducing losses in production caused by salinity in high-yielding genotypes belonging to Solanaceae and Cucurbitaceae families would be to graft them onto rootstocks capable of ameliorating salt-induced damage to the shoot (Yetisir and Uygur, 2010). This strategy could also enable plant breeder to combine desired shoot characteristic with good root characteristic (Pardo *et al.*, 1998). Proposed explanations for grafting-induced salt tolerance are: (i) higher accumulation of proline and sugar in the leaves (Ruiz *et al.*, 2005); (ii) higher antioxidant capacity in the leaves (López-Gómez *et al.*, 2007); (iii) lower accumulation of Na^+ and/or Cl^- in the leaves (Zhu *et al.*, 2008 a,b).

Grafting is an integrative reciprocal process and, therefore, both scion and rootstock influence salt tolerance of grafted plants (Etehadnia *et al.*, 2008). Romero *et al.*, (1997), observed that root characteristics are of primary importance in determining the salt tolerance of melon plants. In addition, the importance of the root system in the regulation of salt tolerance has also been documented in salt-sensitive and salt-tolerant potato genotypes (Shaterian *et al.*, 2005). In contrast, Chen *et al.*, (2007) concluded that scion genotypes play an important role in the growth of grafted tomato plants, regardless of the salinity in the growing media, whereas rootstock has little influence. Studies on tomato plants suggest that the characteristics of the root stock conferring salt tolerance on the shoot depend also on the salt tolerance of the shoot genotype (Santa-Cruz *et al.*, 2002). Moreover, a recent work on cucumber also suggested that the salt tolerance of grafted cucumber seedlings is related to the shoot genotype (Zhu *et al.*, 2008 a,b). Therefore, more studies are necessary to investigate the primary factor that determine the salt tolerance of grafted plants.

The present investigation aimed to study and characterize the physiological impact of grafting on cucumber growth under saline condition. The anatomical structure of the stem and leaf blade was evaluated in order to elucidate the physiological mechanism of salt stress mitigate by these treatments.

Material and Methods

Two pot experiments were carried out in the Agric. Experimental Station and Laboratories of the Agric. Botany Dept., Faculty of Agric. Mansoura Univ. during the two growing seasons of 2015/2016 and 2016/2017. The Experimenal station is situated at 31°02'40.6"N latitude, 31°22'40.3"E longitude and the altitude is 12 m above the sea level. According to the promising results obtained from a preliminary experiments, two mixture salinity levels (50 and 100 mM) in addition to the control (0 level) were examined. The mixture of salt consist of $MgSO_4$, $CaSO_4$, NaCl, $MgCl_2$ and $CaCO_3$ in ratio of 10:1:78: 2:9 respectively as suggested by Strognov (1964). A cucumber (*Cucumis sativus,* L) cultivar hybrid graft 1010, obtained from Enza Zaden company, Holland was used as a scion. A salt tolerant pumpkin hybride and commercial available (*Cucurbita maxima* x *C. moschata,* obtained from Qingdaod Agric. Acad. of Sci.) was selected as the root stock.

Cucumber and pumpkin seeds were sterilized with 0.5% (w/v) sodium hypochlorite solution for 10 min (s) and then washed thoroughly with deionized water. The washed cucumber seeds were sown in plastic pots, 8 cm in diameter and 10 cm high containing vermiculite on October 10th in both seasons and incubated in the dark at 25℃ for 12 h (s) while the pumpkin seeds were soaked for 24 h (s) (Wang *et al.*, 2012). After that they were sown in plastic trays (41 cm x 41 cm x 5 cm) containing quartz sand and grown at 25-30℃ /15-18℃ (day/night) with about 60-73% relative humidity (RH) in

a grown chamber. The seedling were supplied with half-strength Hoagland's nutrient solution (pH 6.5 ± 0.1, EC 2.0-2.2 dSm^{-1}). Grafting was carried out when the scion's cotyledon fully expanded and rootstock's 2nd true leaf development stage (October 28th) at which time split grafting was performed. The insertion grafting procedure was used as described by Lee (1994).

Seedlings of rootstock were picked between the two cotyledons after removing their tops by a razor blade, creating a V-shaped cut. An inverse V-shape cut was made on the stem of the scion, 2 cm below the cotyledons, to fit the cut in the rootstocks were held with a grafting clip. Self-grafted plants were included as control. To maintain a high humidity and facilitate graft formation, seedlings covered with a layer of transparent plastic film and kept in the shade for 6 d (s) under optimum temperature. The compatibility was checked after grafting stage in relative high temperature (25-30℃) ,watching the new growth on the scions. The plastic tunnel film was gradually opened slightly every day to reduce the relative humidity, adaptation and was removed completely 12 d (s) after grafting, preparing the grafting seedlings for transplanting in the plastic house (Oda *et al.,* 1994)

About 30 d(s) after sowing, where the 3rd true leaf was fully expended, seedling of uniform size were transplanted in 35 cm inner diameter plastic pots filled with equal amounts of compost and vermiculite. The chemical analysis of the applied compost was present in Table (1). The transplanting was took place at the rate of 2 seedling/pot and arranged in 6 groups. Each of which was prepared for the specific salinity levels (0, 50 and 100 mM) to evaluate grafted and self-grafted plants (control). Each group was replicated 6 times and each replicate was presented by three pots.

Table 1: Chemical analysis of the compost used.

Weight of m^{-3} (kg)	Moisture Content (%)	pH	EC dSm^{-1}	Total N (%)	O.M (%)	OC (%)	Ashes (%)	C/N (%)	Total P (%)	Total K(%)	Weed seed	Nematoda
6.8	16.6	7.8	4.46	1.03	31	17.5	69	1:17	1.20	1.34	Not found	

Five days after transplanting, seedlings were irrigated with the specific saline water supplemented with half Hoagland's nutrient solution (Hoagland and Arnon, 1950). Irrigation need of the plants were monitored and equal amount of irrigation water (about 65% from WHC) was added. During culturing, plants grown under natural light, ranged between 400-800 µ mol $m^{-2}s^{-1}$ at 25-30℃ /15-18℃ (day night) throughout the experimental period. The plants were thinned to one plant/ plot, trained and pruned by removing the side shoots and flowers up to the 4th internodes . Side shoots were preserved from this point on, but they were pruned, leaving two internodes.

Sampling and biometric observation

A randome samples for biometric observation were taken from each treatment at the active growth period, 90 days from transplanting (85 days from salinity treatment). They were immediately transferred in a cool dry container to the laboratory for measuring the following data:

Growth attributes

Morphological characters:

Main stem height and its branches number as well as number of leaves per plant were estimated. Leaf area of the 4th full expanded leaf from the plant tip was also determined by using disk method (Johanson, 1967) as follows: Samples of ten disks (7.85 cm^2) were taken from the 4th full expanded fresh leaf from plant tip and oven dried to calculate the average dry weight relationship. Leaf area per plant was calculated in square centimeter (Cm^2) using the formula:

$$\text{Leaf area/plant } (cm^2/\text{plant}) = \frac{\textit{Leaf dry weight (g) x Disk area}(cm^2)}{\textit{Disk dry weight (g)}}$$

Dry weight of the shoots as well as the rootstocks were determined. From each treatment, shoots of 3 cucumber plants (the part above the graft union) were separated from the rootstocks (the part below

the graft union), washed using deionizer water and dried carefully with tissue paper. The fresh weight of shoot and root stock were measured, dried at 105℃ for 15 min, in hot air oven, then maintained at 70℃ for two days (48 hours) until reaching a constant weight, and weighted (g/plant). The dried material were ground to a fine powder and kept in tightly closed glass jars for further chemical analysis.

Physiological parameters

Photosynthetic pigments:

Chlorophylls (a and b) as well as carotenoids concentration were analyzed from one gram fresh weight taken from the third and fourth leaf from plant tip. The optical density (O.D.) of the extract was measured at wave lengths 663, 645 and 440.5 nm (Smith and Benitz, 1955) to estimate chlorophyll 'a' and 'b' , and carotenes respectively, using a Spectrophotometer (Spectronic 21D) and a vitreous cell (thickness of photo route 1 cm). Three replicate were used for each treatment, and the amount of pigment concentration (mg/g) in each sample was calculated according to the following equations (Wettstein, 1957) :

1. mg chlorophyll a/g-tissue = 12.7 (O.D.) 663-2.69(O.D.) 645 x *v/w* x 1000
2. mg chlorophyll b/g-tissue = 22.9 (O.D.) 645-4.68(O.D.) 663 x *v/w* x 1000
3. mg total chlorophyll a + b/g-tissue = 20.2 (O.D.) 645-8.02(O.D.) 663 x *v/w* x 1000
4. mg carotenoids/g-tissue = 46.95 (O.D. 440.5- 0.268 x chlorophyll 'a' + 'b')

Where W, the fresh weight by grams for extracted tissue; V, the final size of the extract in 80% acetate; O.D. optical density at specific wave length.

Osmotic potential:

The partial osmotic potential (O.P.) of squeezed sap from the fresh leaf samples collected at 9 am was determined by using the refractometer. Leaves were taken and immediately washed then directly freezed sap cells were extracted in the laboratory with a position press when the frozen tissues had been toward the refractometer. The corresponding osmotic values (bars) due to the soluble sugars were obtained from the tables given by Gosev, (1960) and described (A.O.A.C., 1975).

Stomatal conductance and leaf water potential (deficit)

Stomatal conductance was measured in the 3rd fully expanded leaf from plant tip using the LiCOR- 6200 portable photosynthesis system (Nebraska, USA) with Ca= 0.33 m mol CO_2 mol^{-1} (330 µ1/1), taking care to use leaves of more or less the same age and each observation was replicated twice and clear days between 11.00 and 13.00 solar time.

Leaf water deficit (LWD) was calculated using the following formola:

LWD % = 100-RWC.

RWC (Relative water content) was determined using the following equation:

$$RWC = \frac{Turged\ weight\text{-}Fresh\ weight}{Turged\text{-}Dry\ weight\ at\ 105\ °C} x\ 100$$

as reported by Kalapos (1994).

Endogenous phytohormones:

To ensure uniformity and comparable physiological state in all treatments, the 3rd terminal fully expanded leaf from plant top was collected at approximately mid-day, since there is some evidence that hormonal levels in the leaves showed diural fluctuations . The leaf was excised from intact plants at the inserted base point, then weighted and washed with distilled water prior to the extraction.

Extraction of the endogenous plant hormones was carried out according to Sadeghian, (1971). The methanolic extract was used for cytokinins estimation, (Palmer *et al.*, 1981) . The remaining

aqueous extract was acidified to pH 2.5 and extracted by ethyl acetate, The methanol extract was methylated again and used for determinations of gibberellic acids (GA) and indole acetic (IAA) according to Fales and Jaouni (1973). The quantification of the endogenous phytohormones was carried out by Gas Liquid Chromatography (GLC) ; ATI-Unicum-610 series equipped with flame ionization detector according to the method described by Vogel (1975).
For total soluble phenols determination, the ethanolic extract which prepared for sugar estimation as mentioned below was used. The colorimetric methods of Folin-Denis as described by Swain and Hillis (1959) was employed.

Antioxidative Enzymes Activity:

Leaf tissues were extracted and homogenized in 100 mM chilled sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% polyvinyl pyrrolidone (PVP) (w/v) at 4℃. The extraction ratio was 4 ml buffer for each one gram of plant material (Beauchamp and Fridovich, 1971). The homogenate was centrifuged at 15.000 x g for 15 min at 4℃. Supernatant was used to measure the activities of catalase (Sinha, 1972), peroxidase (Chance and Maehly, 1955) and polyphenol oxidase (Kunwar and Khan, 1982) colorimetrically. The O.D. of catalase (CAT), peroxidase (POX) and polyphenol oxidase (PPO) activities were measured at 570, 430 and 420 nm respectively. The unit per g fresh weight defined as the amount of enzyme that increased 0.01 O.D. per minute of incubation.

Organic compatible compounds (Osmoregulatores):

Sugars, proline and total amino acids were extracted from crude dried material of the 3rd terminal leaf from each treatment by ethanol 70% (Kayani *et al.*, 1990). They were determined colorimetrically in the extraction by the methods described by Naguib, (1964), Bates *et al.*, (1973) and Lee and Takahashi (1966) respectively.

Anatomical investigations:

Specimens of main stem (3rd internode from plant tip) at its medium portion and blade of the leaf developed on the medium portion of the main stem were taken in the second season (2016/2017) at the active growth period of self grafted and grafted cucumber on salt tolerance pumpkin rootstock (90 days). The plant materials were killed and fixed for at least 48 h(s) in FAA (formalin: glacial acetic acid and ethyl alcohol 70%; 2:1:17 ml v/v). They were washed in 50% ethyl alcohol, dehydrated in a normal butyl alcohol series, embedded in paraffin wax of milting point 55-58℃ , transverse sectioned to a thickness of 12-15 microns, double stained with safranin and lightgreen, cleared in xylene and mounted in canada balsam according to Nassar and El-Sahhar, 1998. Slides were examined microscopically and the measurements were taken and average of 10 reading from 3 slids were calculated and evaluated comparatively. Transverse sections were done with rotary microtome model 820.

Yield and its components:

Cucumber plant was harvested every three days intervals, started till the end of the experiments. Average fruit number per plant, fruit weight and yield per plant were estimated. In addition, fruit flavor and taste involved nine untrained panelists were also evaluated. The fruits were picked about 1 h before the beginning of the test and washed with tap water. The panelists were requested to asses fruit flavor and taste based on a scale from 1 to 5 (Saied *et al.*, 2005). Vitamin C, soluble sugars, free amino acids and cellulose content in the fruits were determined by xylene extraction colorimetric test (Nieves *et al.*, 2004) , anthrone colorimetric test (Sivritepe *et al.*, 2005) , ninhydrin test and anthrone test respectively. Titrabale acids were estimated by sodium hydroxide titration (Sivritepe *et al.*, 2005). To calculate water content, around 50 g of each fruit middle was weighted on an electronic scale, then blast dried at 105℃ for 20 min(s), followed by 70℃ for 48 h(s) and the dry weight obtained (Sivritepe *et al.*, 2005).

Statistical analysis:

The experiments in this study were laid out as a factorial randomized block design system, and the analysis of variance (ANOVA) was done as regular two way classification out-lined by Gomez and Gomez (1984). Costal statistics 6.0, release 6.303 software was used. If the F value for entries are found to be significant, the LSD (Least significant difference at 5% ($P \leq 0.01$) of probability) was considered a valid least for the above planned comparisons of means.

Results and discussion

1- Growth characters:

Growth characters of cucumber were affected significantly in a different manner by salinity and/or grafting throughout the study period (Table 2) in both seasons compared with the control. There is general trend for the decrease in all growth attributes studied due to salinity. The decrease was correlated inversely with the increase of salinity levels from 50 to 100 mM. Grafting increased plant height, leaf number, leaf area per plant as well as fresh and dry weight of the shoot and root system of cucumber plants grown under salinized and non-salinized conditions. Grafted plants grown under non-salinized conditions followed by those grown under 50 mM salinity level had higher values compared to the control. Grafted plants under non-salinized conditions produced the long plant having the highest leaf number, highest fresh and dry weights and largest leaf area in both growing seasons. It was followed by plants grown under 50 mM . However, there is less decreases in growth parameters in grafted plants grown under 100 mM salinity level compared with those grown under normal condition. Self grafted plants grown under 100 mM salinity level exhibited the lowest values for all mentioned characters in both seasons followed by plants grown under 50 mM salinity level.

Table 2: Effect of salinity, grafting and their interaction on plant height (cm), number of leaves, leaf area (m^2/plant) as well as dry weight of the shoot and root system (g /plant) of cucumber plants growing in the two growing seasons (2015/2016 and 2016/2017).

Salinity level (S) mM	Grafting (G)	2015/2016					2016/2017				
		Plant height (cm)	No of leaves /plant	Leaf area (m^2)	Dry weight (g/plant)		Plant height (cm)	No of leaves /plant	Leaf area (m^2)	Dry weight (g/plant)	
					Shoot	Root				Shoot	Root
Control (0)	Self grafted plants(control)	329.3	25.6	3.4	42.8	21.7	332.1	26.0	4.2	41.9	22.1
	Grafted plants	336.1	26.3	3.7	44.8	27.9	339.0	27.7	4.4	43.8	28.2
	Mean	332.7	25.5	3.5	43.8	24.8	335.5	26.8	4.3	42.9	25.1
50 mM	Self grafted plants(control)	210.6	21.3	1.5	36.9	20.9	214.5	21.3	2.2	35.2	21.2
	Grafted plants	230.1	22.0	2.8	39.7	25.7	232.8	23.0	3.5	38.2	26.1
	Mean	22o.3	21.8	2.2	38.3	23.3	223.6	22.1	2.8	36,7	23.6
100 mM	Self grafted plants(control)	160.3	17.0	0.9	30.3	20.0	164.1	17.8	1.6	29.1	20.4
	Grafted plants	170.2	17.6	1.7	37.1	25.1	173.6	18.3	2.5	38.2	25.5
	Mean	165.2	17.3	1.3	33.7	22.6	168.8	18.1	2.1	33.6	22.
Mean	Self grafted plants(control)	233.4	21.3	1.9	36.7	20.9	236.9	21.7	2.7	35.4	21.2
	Grafted plants		22.0	2.7	40.5	26.2		23.9	3.5	40.1	26.6
New LSD at 5% for:											
Salinity (S)		10.11	1.31	0.13	3.33	0.70	10.66	1.25	0.11	3.50	0.53
Grafting (G)		2.60	0.07	0.11	1.23	0.50	2.50	0.15	0.05	1.11	0.13
SxG		10.76	1.41	0.15	3.53	0.86	11.37	1.37	0.17	4.00	0.63

Other results that support what has been shown here regarding the effect of salinity are those by Dantus *et al.*, (2005) with their study on cowpea and Memon *et al.*, (2010) on *Brassica camperstris,* L. where they indicated that higher levels of NaCl salinity caused an inhibition in plant growth.

Looking at the statistical analysis, it is appeared there is a general trend for the decrease in all growth characters studied of cucumber plants due to salinity. The decrease was correlated inversely with the increase of the concentration of salinity level from 50 to 100 mM. These results agree with Mathur *et al.*, (2006) who reported that, the stress of the moth bean plant (*Vigna aconitifolia,*L.) with increasing concentration of NaCl led to a decrease in growth as indicating with leaf area. This decrease was inversely by the negative effect of salts on photosynthesis, that leads to the reduction of plant growth, leaf growth and chlorophyll content (Netondo *et al.*, 2004).

Mazhar *et al.*, (2007) attributed the inhibiting effects of salinity on plant growth to the negative effect of it on the rate of photosynthesis, the changes in enzyme activity (that subsequently affects protein synthesis), and also the decrease in the level of carbohydrates and growth hormones, both of which can lead to inhibition of the growth. On *Vicia faba* plants, Abdul Qados (2011) found the decrease of leaf number may be due to the accumulation of sodium chloride in the cell walls and cytoplasm of the older leaves. At the same time, their vacuole sap cannot accumulate more salt and, thereby decrease the concentration of salt inside the cells, which ultimately lead to their quick death and cut down (Munns, 2002).

Other supporting result, include those of Jamil *et al.*, (2006) on sugar cane (*Beta vulgaris,*L.) and Zhao *et al.*, (2007) on oat (*Avena sativa,* L.) and Yilmaz and Kima (2008) with their study on *Fragaria xananssa,* L. Hsiao and Xu (2000) attributed the growth reduction and elongation caused by water stress to turgor reduction at low membrane extensibility (loosening ability) of the cells as a result of dehydration. In line with this, it has been suggested that the cell wall in the basic mechanism for cell expansion as it pushes the cell wall outwards irreversibly, then grown may be survival mechanism for the plants to maintain tissue hydration by reducing the rate of transpiration (Zaharah and Razi, 2009).

The positive effect of grafting on plant growth noticed in the present investigation agree with the result presented by El-Kafafi *et al.*, (2017) in their study on cucumber plant. Munns, (2002) attributed the increases in growth due to grafting to the ability of the plant to increase the size of its sap vacuole, which allows for collection of a lot of water and this in turn dissolves salt ions that have accumulation and leads to subsequent increase in fresh weight.

2. Physiological characters

2.1. Photosynthetic pigments:

Table (3) shows that there is a negative effect of salinity on chl(s), concentration (a,b and total chls.) of the cucumber plants in both seasons. Whenever, the salinity increased, chl(s) decreased and reaching their lower concentration at 100 mM salinity levels for both seasons compared to the control plants. Moreover, salinity was an inhibiting factor for the formation of carotenoids inside the stressed plants where its concentration decreased. The observed differences were statistically significant. Grafting not only countered the depressing effect of salinity on all photosynthetic pigments, but also increased their concentrations in cucumber leaves. Therefore, grafted plants grown under nonsalinized condition and those grown under 50 mM gave the highest values whenever selfgrafted plants grown under 100 mM salinity level produced the lowest values in both seasons. A significant analysis indicated that the observed differences were significant, except for the difference between grafted plants grown under 100 mM salinity level and the control.

The inhibiting effect of salinity on chlorophylls concentration (a,b and total) noticed in the present investigation, agree with what Tort and Turkyilmaz (2004) reported that the exposure barley (*Hordium vulgaris,*L.) to NaCl salinity led to a decrease in chl a, b and total chl(s). Similar results were reported by Taffouo *et al.*, (2010) on *Vigna subterranean,* L.

The inverse relationship between salinity and carotene concentration, agree with those registered by Mustard and Renault (2006) when they observed that the stressing of dog wood (*Cornus sericea,* L.) seedling with salinity reduced leaf content of carotenes. However, the favorable effect of grafting on chlorophyll concentration noticed in this study may be refer to that grafting influence absorption and translocation of Mg (Ruiz *et al.*, 1997), also the grafted plants contain more Mg in their leaves than the non-grafted one (Arai *et al.*, 1995) and that may interpret their higher chlorophyll values of mineral concentration obtained from grafting cucumber plants onto pumpkin

may be due to the rootstock many surpass cucumber in size the root system, then a significant amount of xylem sap could be translocate by the rootstock, it is known to contain fairly high concentration of minerals , organic substances and plant biomass as well as higher ability of minerals uptake of grafted plants (Mohsen, *et al.*, 2012).

Table 3: Effect of salinity, grafting and their interaction on chlorophyll a (chl a), chlorophyll b (chl b), total chlorophylls (chls) and carotenoids (cart) concentrations (mg/g F.wt.) of cucumber plants growing in the two growing seasons (2015/2016 and 2016/2017).

Salinity level (S) mM	Grafting (G)	2015/2016				2016/2017			
		Chl a	Chl b	Chls	Cart	Chl a	Chl b	Chls	Cart
Control (0)	Self grafted plants(control)	35.6	10.2	45.8	14.5	36.0	9.7	45.7	15.9
	Grafted plants	13.5	13.5	52.5	16.1	39.6	12.9	52.5	17.3
	Mean	37.3	12.8	49.1	15.3	37.8	11.3	49.1	17.1
50 mM	Self grafted plants(control)	32.9	9.2	42.1	16.2	33.6	9.1	42.7	16.8
	Grafted plants	35.2	11.8	47.0	17.3	35.9	11.6	47.5	17.8
	Mean	34.0	10.5	44.5	16.7	34.7	10.3	45.1	17.3
100 mM	Self grafted plants(control)	27.8	8.2	36.0	16.6	28.2	7.9	36.1	17.4
	Grafted plants	33.5	10.6	44.1	17.7	33.7	10.2	43.9	18.9
	Mean	30.6	9.4	40.1	17.1	30.9	9.1	40.0	18.1
Mean	Self grafted plants(control)	32.1	9.2	41.3	15.8	32.6	8.9	41.5	16.7
	Grafted plants	35.9	12.0	47.9	17.0	36.4	11.6	48.0	18.0
New LSD at 5% for:									
Salinity (S)		1.00	0.43	1.33	0.07	0.89	0.33	1.11	0.07
Grafting (G)		0.73	0.13	0.81	0.03	0.51	0.10	0.81	0,02
SxG		1.11	0.54	1.70	0.14	0.99	0.45	1.34	0.11

2.2. Osmotic potential (O.P.):

Data presented in Table (4) show that, increasing salinity level decreased the osmotic potential of the cucumber plants both grafted and self grafted (control) plants grown in the two growing seasons. This changes is considered one of the defensive mean by which plant tolerate stress as increases its ability to absorb water. The values detected show an inverse relationship between salt stress and osmotic potential for the sap of cucumber leaves, where the reduction of osmotic potential increase with the increase salinity. However, grafted plants showed lower values.

The statistical analysis demonstrated that, the reduction in OP was significant with all treatments in both seasons. These results agree with the result presented by Yagmur *et al.*, (2006) in their study on barley leaves (*Hordium vulgare,* L.) and Gama *et al.*, (2007 and 2009) on beans (*Phaseolus vulgaris,* L.). Similarly, Qin *et al.*, (2009) registered a significant reduction in osmotic potential for leaf sap of seabukthorr (*Hippophae rhamoides,* L.) when treated with NaCl salinity. Abdul Qudos (2011) reported that, the ability of plants to maintain its OP at level below that of OP of the soil surrounding the plant is a means by which it tolerates the harmful effect of the accumulation of salt inside its cells during salt stress.

2.3. Stomatal conductance (g) and leaf water potential (LWD):

All the salinized plant showed a gradual decline in stomatal conductance with all fastest reduction in the self-grafted plants grown under 100 mM salinity level. Stamatal conductance and LWD of grafted plant grown under salinized and non salinized conditions were higher than that of the control in both growing seasons (Table 4)**.**

As for water potential, the same table show that, there are a significant differences between grafted and non-grafted plants grown under different salinity levels for leaf water potential (LWD) through

the two growing seasons. The difference between grafted and non-grafted plants was clearly observed. Thus, grafted plants had consistently higher LWD compared to the corresponding non-grafted, and that grown under the non-salinized condition had higher LWD than that of the salinized one. These results are true during the two growing seasons. The present study showed that LWD of the stressed plants decrease in non-grafted plants than with that of grafted one (Table 4). Also, grafted plants (having high rooting volume) showed highest LWD . This outcome is similar with results reported in mango seedlings (Zaharah and Razi, 2009) which indicate that water stress was identified as the mechanism that decrease LWD.

Table 4: Effect of salinity, grafting and their interaction on osmotic potential (O.P.), stomatal conductance (g_s) and leaf water potential (deficit) (LWD %) of cucumber plants growing in the two growing seasons (2015/2016 and 2016/2017).

Salinity level (S) mM	Grafting (G)	2015/2016			2016/2017		
		O.P. bars	LWD %	g_s	O.P. bars	LWD %	g_s
Control (0)	Self grafted plants(control)	-1.65	42.25	1.00	-1.66	43.00	1.05
	Grafted plants	-1.71	22.01	1.09	-1.69	27.34	1.13
	Mean	-1.68	32.13	1.04	-1.67	35.17	1.09
50 mM	Self grafted plants(control)	-1.74	44.99	0.76	-1.76	43.79	0.75
	Grafted plants	-1.86	29.10	0.81	-2.01	29.30	
	Mean	-1.80	37.04	0.78	-1.88	36.55	0.92
100 mM	Self grafted plants(control)	-2.21	53.67	0.53	-2.41	48.67	0.57
	Grafted plants	-2.54	53.83	0.75	-2.63	32.24	0.75
	Mean	-2.37	53.75	0.64	-2.52	40.45	0.66
Mean	Self grafted plants(control)	-1.87	46.97	0.76	-1.94	45.15	0.79
	Grafted plants	-2.04	34.98	0.88	-2.11	29.63	0.99
New LSD at 5% for:							
Salinity (S)		0.03	1.66	0.01	0.02	1.75	0.01
Grafting (G)		0.04	1.14	0.01	0.03	0.99	0.01
SxG		0.08	1.93	0.01	0.03	1.97	0.02

The relationship between stomatal conductance and LWD indicate that salinity stress and self-grafted cucumber plants had more negative LWD and lower stomatal conductance than non-salinized and grafted plants. On the contrary, Dantus, et al., (2005) reported that the reduction in rooting volume did not reduce stomatal conductance in star fruit plants. The small pots had restricted root system which were then unable to supply sufficient water. The reduction in stomatal conductance under salinized condition might be attributing to the tendency of plants to control water loss through transpiration process.

The interaction treatments (salinity x grafting) indicated that the increment in stomatal conductance was much more pronounced in grafted plants when compared to self-grafted one especially under non-salinized condition. This suggest that a reduction in stomatal conductance is a result of internal water deficit caused by salinity and/or limited root system of the self-grafted cucumber.

Limited nutrients as a result of smallest root volume of self-grafted plant may be introduced, whereas increased root density in grafted plants might cause an estimation of leaf gas exchange. This finding is in agreement with Zaharah and Razi, (2009) who reported that, under well-watered condition, restricted root considerably reduced stomatal conductance and leaf water potential. They added that, more rapid reduction in stomatal conductance and LWD occurred under restricted root and water conditions in mango. However, the hydroactive mechanism or the leaf water status is not the only factor that control, stomatal behavior but also the smaller size of plant cell due to the limited root growth causes lower water translocation from cell to cell. Further investigations are needed to clarify this issue.

2.4. Endogenous phytohormones:

Table (5) shows that under control condition, non-salinized plants and/or grafted cucumber plants resulted in higher level in IAA, GA and CK (promoters) than for self-grafted plants and/or salinized condition. On the other hand, ABA and total phenols level (inhibitors) in the leaves of self-grafted cucumber plants grown under salinity were significantly higher than those in the control plants. Plant grown under the high level of salinity (100 mM) had higher inhibitors and lower level of promoters than the control plants.

Grafting decreased the inhibitors especially that of ABA level even under salinized condition.

Grafted plants under either of non-salinized or salinized conditions had lower ABA and total phenols accumulation in the leaves and higher rate of stomatal conductance than did those with control plants. This outcome is similar with results reported on water-stressed mange plant (Helaly *et al.,* 2017) which indicates that water stress increased ABA levels.

Table 5: Effect of salinity, grafting and their interaction on indol acetic acid (IAA), gibbrelins (GA), cytokinin (CK), abscic acid (ABA) and total phenols (Tph) concentrations (mg/100 g F.Wt.) of cucumber plants growing in the two growing seasons (2015/2016 and 2016/2017).

Salinity level (S) mM	Grafting (G)	2015/2016					2016/2017				
		IAA	GA	CK	ABA	Tph	IAA	GA	CK	ABA	Tph
Control (0)	Self grafted plants(control)	26.8	34.0	21.3	0.63	150.5	19.3	30.9	21.3	0.62	152.8
	Grafted plants	27.8	35.8	21.6	0.65	160.3	24.4	33.4	21.7	0.66	162,6
	Mean	27.3	35.3	21.4	0.64	155.4	21.8	32.1	21.5	0.64	157.7
50 mM	Self grafted plants(control)	27.9	32.2	19.2	0.69	155.1	23.8	32.1	19.2	0.71	157.4
	Grafted plants	24.7	33.1	20.5	0.70	161.7	35.3	33.4	20.6	0.74	164.0
	Mean	26.3	34.6	19.8	0.69	158.4	24.6	32.7	19.9	0.72	160.7
100 mM	Self grafted plants(control)	18.9	30.0	18.1	0.74	158.4	27.3	34.3	18.2	0.78	160.7
	Grafted plants	24.1	33.7	20.0	0.85	163.3	28.6	36.1	20.1	0.87	165.6
	Mean	21.5	31.8	19.0	0.79	160.8	28.0	35.3	19.1	0.82	163.1
Mean	Self grafted plants(control)	22.9	32.0	19.5	0.69	154.7	23.4	32.4	19.6	0.70	157.0
	Grafted plants	25.5	33.9	20.7	0.71	161.8	26.2	34.3	20.8	0.76	164.1
New LSD at 5% for:											
Salinity (S)		0.33	0.12	0.03	0.11	2.11	0.41	0,07	0.03	0.13	2.33
Grafting (G)		0.35	0.10	0.01	0.03	1.07	0,30	0.06	0.03	0.09	167
SxG		0.51	0.17	0.05	0.27	2.33	0.70	0.11	0.07	0.17	3.17

Similarly, like many other plant species, there must be a chemical signal responsible for inhibition of growth. It also, suggested that when adequate water is available to part of a restricted root system, there may be no change in the ABA concentration in the leaves since a substantial uptake of water will dilute any increased ABA production by the roots (. Zaharah and Razi, (2009) indicated that, high ABA accumulation were closely correlated with the higher rate of stomata aperture in plant under water stress condition.

Data in the present investigation supported this conclusion, since the reduction in stomatal conductance with salinity in self-grafted and grafted cucumber plants could be attributed to the increased ABA and phenolic compound accumulation (Table 5). In agreement with these findings, Helaly *et al.,* (2017) reported that under water stress condition sufficient ABA synthesis occurred to tigger the processes that cause stomatal conductance to decline. These results indicate at negative correction between stomatal conductance and ABA accumulation because of the nonhydroulic root-to shoot communication *via* ABA. This is a good evidence that, ABA is originates from the roots in a drying soil (water stress) and is transported to the shoot part to inhibit leaf growth and stomatal conductance.

2.5. Antioxidative enzyme activities

CAT, POX and PPO activities in cucumber leaves as affected by salinity, grafting and their interactions is shown in Table (6). Activities of all these enzymes significantly increased due to salinity with maximum values achieved for self-grafted plants grown under the high level (100 mM). Grafted plants showed a significant decrease in the activity of CAT, POX and PPO in cucumber leaves. However, the values of activities were still significantly affected by salinity level and grafting. Salinized self-grafted plants exhibited the highest activities of CAT, POX and PPO in both seasons followed by salinized grafted plants.

The increase in antioxidative enzyme activities due to stress condition noticed in this study indicating that one of the plant adaptive mechanism is to respond under different abiotic stress (Mazorra *et al.*, 2002).The increase in POX activity under stress was similar to those reported in mango with Zaharah and Razi, (2009) who suggested that the availability of water is the key factor in determining the role of peroxidase activity, which in turn control leaf expansion during drought stress (Netodo, *et al.*, 2004).

Table 6: Effect of salinity, grafting and their interaction on the activities (unit g F. Wt.)of catalase (CAT), peroxidase (POX) and polyphenol oxidase (PPO) of cucumber plants growing in the two growing seasons (2015/2016 and 2016/2017).

Salinity level (S) mM	Grafting (G)	2015/2016			2016/2017		
		CAT	POX	PPO	CAT	POX	PPO
Control (0)	Self grafted plants(control)	11.01	2.11	2.51	10.70	3.01	2.81
	Grafted plants	12.00	2.33	2.86	12.00	3.51	3.20
	Mean	11.50	2.22	2.68	11.35	3.25	3.00
50 mM	Self grafted plants(control)	13.11	2.27	2.77	11.56	3.97	3.18
	Grafted plants	15.33	2.56	3.06	12.91	4.11	3.42
	Mean	14.22	2.41	2.91	12.23	4.04	3.30
100 mM	Self grafted plants(control)	17.00	2.31	3.11	13.11	4.25	3.46
	Grafted plants	17.76	2.96	3.25	15.65	5.00	3.52
	Mean	17.38	2.63	3.18	14.38	4.62	3.49
Mean	Self grafted plants(control)	13.71	1.47	2.80	11.79	3.74	3.15
	Grafted plants	15.03	2.62	3.06	13.52	4.21	3.38
New LSD at 5% for:							
Salinity (S)		1.01	0.03	0.07	0.93	0.01	0.09
Grafting (G)		0.63	0.01	0.03	0.34	0.01	0.17
SxG		1.17	0.05	0.16	1.11	0.01	0.17

2.6. Compatible organic solutes:

Free proline, total sugars and total soluble amino acids as an osmoregulatory solutes were all significantly affected by salinity, grafting combination and their interactions (Table 7). Generally, salinity irrespective to grafting increased all these organic solutes and the respective increases were concentration dependent. Grafted plants accumulated more proline and total sugars compared with self-grafted plants under normal and/or salinized conditions. These results are true during the two growing seasons indicating that grafted plants has a greater ability of tolerate salinity stress compared to self-grafted plants. Bartels and Sunkar (2005) found a strong correlation between osmotic stress tolerance and sugar accumulation. The accumulation of compatible solutes as organic solutes in stressed plants especially proline and sugars may lead to osmoprotection of tolerant cv(s) of wheat plant as reported by Misra and Saxena (2009) and upregulation of specific enzymes of proline metabolism (Misra and Gupta, 2006). Moreover, the accumulation of proline and sugars in the present investigation in self-grafted and grafted plants grown in salinized condition indicate that the increase

in these compatible solutes seems to be related to salinity stress rather than grafting combination and their interactions. In addition, the present study indicated an opposite relationships between proline level and LWD in grafted and self-grafted cucumber plants grown under salinized and non-salinized condition. With an increase in leaf proline, more negative, water potential was detected.

Table 7: Effect of salinity, grafting and their interaction on free proline, total sugars (T.S) and soluble amino acids(AA) concentrations (mg/g D. Wt.) of cucumber plants growing in the two growing seasons (2015/2016 and 2016/2017).

Salinity level (S) mM	Grafting (G)	2015/2016			2016/2017		
		Proline	T.S	AA	Proline	T.S	AA
Control (0)	Self grafted plants(control)	15.50	16.50	10.83	12.10	16.57	11.10
	Grafted plants	11.60	18.60	11.43	10.10	18.76	11.70
	Mean	13.55	17.55	11.13	11.10	17.66	11.40
50 mM	Self grafted plants(control)	15.00	19.60	11.50	13.70	18.63	11.76
	Grafted plants	17.70	20.50	11.96	15.90	20.56	11.90
	Mean	16.35	20.05	11.73	14.80	19.60	11.83
100 mM	Self grafted plants(control)	15.60	21.56	12.83	14.70	19.40	12.83
	Grafted plants	18.10	22.50	13.56	17.50	22.36	13.76
	Mean	16.85	22.03	13.19	16.10	20.88	13.30
Mean	Self grafted plants(control)	13.30	19.22	11.05	12.63	18.20	11.36
	Grafted plants	17.10	30.53	12.12	15.17	20.56	12.25
New LSD at 5% for:							
Salinity (S)		0.93	1.11	0.54	0.86	0.96	0.63
Grafting (G)		0.66	0.93	0.31	0.53	0.75	0.34
SxG		1.33	1.51	0.67	1.11	1.11	0.79

Plant growth developed is generally through to be an energy-requiring processes therefore the increase in proline accumulation in response to salinity and self-grafted plants are most likely acting as a way to save energy by inhibiting roots growth in pot experiment and/or in self-grafted cucumber plants. Proline play an important role in many biochemical processes, including osmoregulation, protecting enzyme denaturation, acting as a source of carbon and nitrogen, stabilizing the machinery of protein synthesis, regulating the cytosolic acidity and scavenging hydroxyl radiacals (Jain *et al.*, 2001). Venkamp (1989) added that an increase in proline levels could be related to a decrease in electron transport in plants under stress conditions. Wu, (2013) found that proline accumulation causes an increased synthesis rate, or a lowered turnover rate of intracellular proline. As a result, there would be an accumulation of $NADH^+$ and H^+. In addition, the increase in $NADH^+$ might even affect phosphorylation substrate level while inhibiting important metabolic reactions that need $NADH^+$ (Venekamp *et al.,* 1989). Misra and Saxena, (2009) suggested that proline may be acting as a regulator of osmotic pressure, protecting membrane integrity and stabilizing enzymes.

The low level of proline accumulation in self-grafted cucumber plants and grown under non-salinized condition may be due to its oxidation and conversion to glutamic acid and other compounds (Delauney and Vrena, 1993). In addition, it was observed that the increase in proline accumulation in stressed and/or grafted cucumber plants were closely related to the decrease in leaf water potential (Table 4). At low LWP, the accumulation of such compatible osmolytes increased and improved the capacity of the cell to maintain its turgor. These non-participatory levels, however, are essential for the physiological processes such as photosynthesis, enzyme activity and cell expansion (Zahrah and Razi, 2009). There could also be a casual link between ABA and proline accumulation in stressed plants (Savoure *et al.*, 1997). Therefore, the accumulation of proline promoters recovery and contributes to stress tolerance. Alquariany (2007) reported that over accumulation of proline in stressed plants may be due to the strategies adapted by the plants to cope up with the stress condition, Xia *et al.*, (2014) added that the accumulation of proline may be associated with the increase in

synthesis of Δ pyrroline-5 carboxylaate synthetase (p5cs) and p5cs m-RNA and pyrroline-5 carboxylaate reductase (p5cp, Misra and Gupta, 2006) as well as δ-glutamyl kinase activity (Misra and Sexene, 2009) . Alternatively, low or reduced activity of the proline degrading enzyme, proline oxidase, localized in inner mitochondrial membrane (Misra and Sexene, 2009) and cytoplasmic proline dehydrogenase (Al-Zubaydi *et al.*, 2012) may account for increase in free proline content.

As for total free amino acids (TAA) , results in (Table 7) indicate also a positive effect for salinity using various concentrations on total free amino acids of cucumber plant shoots. There are a general increase in total free amino acids that corresponded with the increase in salinity level. However, there are a general reverse in total amino acids in grafted plants. Statistical analysis did not show a significant differences regarding salinized grafted plants and self-grafted ones. These results, in general, agree with what Kapoor and Srivastave, (2010) had presented. They observed an increase in protein content when increasing salt concentration. Contrary results were reported by Chen *et al.*, (2007) who indicating a negative effect of salt stress on protein content in *Vigna unguiculate* L. plants.

El-Beltagi *et al.*, (2013) attributed the accumulation of total free amino acids, under salinization to a consequence of either synthesis or degradation of proteins and/or to an activated synthesis of adaptive protein. The relation between grafted and self-grafted plants in response to salinity seems to be associated with the ability of plant tissues to survive the stress conditions. The possibility that plants can form specific protein types for adaptation to high stress condition had been reported (Al-Mulla *et al.*, 2013) . Also, the hydrophilic protein may protect the plasma colloids from coagulation caused concentrated electrolytes, as a consequence of cell degradation (Sperling *et al.*, 2014). Hormonal modification of protein metabolism due to stress has also been reported (Dawood *et al.*, 2012).

Concerning, sugar accumulation, Sperling *et al.*, (2014) reported that sucrose and other sugars have dual roles in plant metabolisms both for sucrose metabolism and as osmoprotectants during dehydration stress. The data in the present investigation showed a correlation between the accumulation of total sugars and proline (Table 7) in response to salinity. This relation may be due either to increased proline synthesis and/or reduced degradation of proline. Amirjani (2010) found that mRNA transcript encoding p5cp was increased in phloem tissue in response to water deprivation. The dramatic increase in transcription of the gene may be related to the finding of Verslues and Sharma (2010) who found for potato, that when sucrose phloem loading was blocked, proline accumulations at a high level. Ma *et al.*, (2004) attributed the accumulation of proline and sugars as an organic osmolytes (Kholva *et al.*, 2009) under stress to the presence of glycine. Betain (GB) in stressed plants as an osmoprotectant. All these molecules act as osmolytes and play a role in osmotic adjustment in non-halophytes (Tajdoost *et al.*, 2007).

3. Anatomical observation:

The anatomical observation of cucumber were focused on the medium portion of the 3rd internode from cucumber plants tip of the main stem and leaf blade developed on the same internode as affected by grafting and salinity stress. The microscopical measurements and counts of certain anatomical characters in transverse section are presented in Tables (8 and 9 and illustrated in Figures (1 and 2).

3.1. Main stem structure:

Table (8) and Fig (1) show that salinity at 50 and 100 mM decreased stem diameter. The reduction in thinning was linked with a remarkable decrease in diameter of cortex, as well as xylem and pith diameter. In addition, the reduction in number of cortex diameter could be due to a reduction in number of cortical layers. The same data indicate that salinity decreased diameter of vascular bundle which due to the reduction in xylem dimensions which attributed to the less production of the vascular elements accompanied with small size of the vessels. Similar results were reported by Nofal *et al.*, (2015) on *Calendula* sp.

Regarding the effect of grafting, data in Table (8) and Fig (1) show that, grafting on pumpkin rootstock caused an increase in stem diameter of cucumber more than the control. This increase could

Table 8: Measurements in microns (µ) of certain anatomical characters in transverse section through the medium portion of the 3rd internode on the main stem of self-grafted (control) cucumber plants (90 days old) and that grafted onto pumpkin plant rootstock grown under different levels of salinity during the second season (2016/2017).

Treatments	Anatomical characters									
	Stem diameter	Epiderm diameter	Cortex diameter	Vascular bundle (Average diameter)	Phloem diameter	Xylem diameter	Xylem vessel	Pith diameter	No of cortex lavers	No of xylem vessel
Control	3924	17	270	186	63	117	12	3461	8	9
S 50 mM	3830	16	256	152	60	87	12	3411	8	9
S 100 mM	3111	11	110	97	40	51	11	2876	6	7
Grafting (G)	4183	17	504	490	101	380	13	3660	11	11
S 100 x G	3566	15	300	256	86	167	12	2980	8	8
New LSD at 5%	7.11	1.10	2.33	4.33	2.61	3.31	0.07	19.21	0.03	0.02

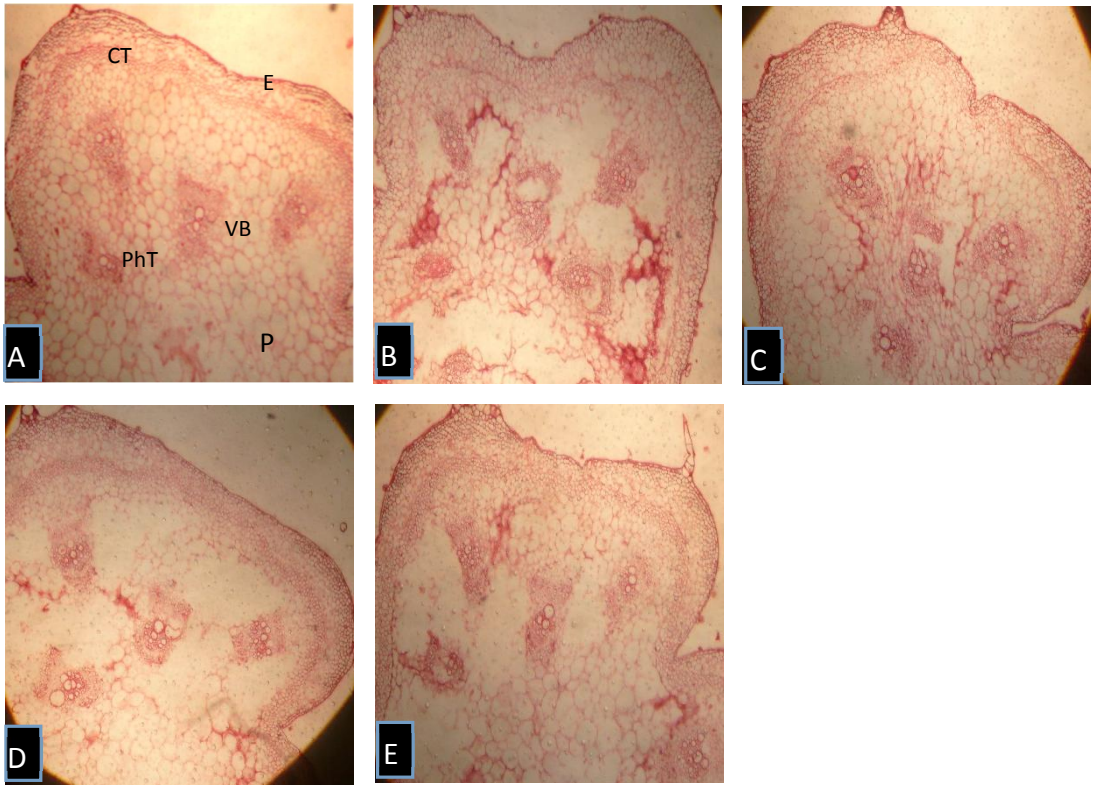

Fig 1: Transverse section through medium portion of the 3rd internode from plant tip on the main stem of cucumber plants (90 days old) grown under different salinity levels and grafting ; A- Control (self grafted plants grown under non-salinized condition. B- Self grafted plants grown under 50 mM of salinity. C- Self grafted plants grown under 100 mM of salinity. D- Grafted plants grown under 0 level of salinity. E- Grafted plants grown under 100 mM of salinity.

be due to the increase in the different tissues which comprise the stem including epidermis, cortex, phloem, xylem and pith. Similarly, the increment in cortex diameter could be due to the increase in cell diameter and number of parenchymatous cell layers. Moreover, the increase in vascular bundle dimensions is mainly due to the increase in vessel diameter and average number of vessel rows compared with the control. Pith diameter was also increased due to grafting which could be due to the enlargement in average pith paranchymatous cell and their diameters and numbers. No available data on the effect of grafting on the anatomical structure of other plant species were detected. However, the increase phloem, epidermis diameter and area, and cortex diameter thus increasing the efficiency of the grafted plants to absorb essential nutrition and water inclosing plant growth. The increased

xylem diameter (thickness) in stressed condition could be interpreted as plant adaptation to unfavorable conditions. Similar results were reported by Zahrah and Razi (2009) who indicated that when water availability is low the cortex thickness decreases and collapses the outer cortex layer to the branches under both restricted and control root growth.

3.2. Leaf blade structure :

Table (9) and Fig (2) show that, salinity decreased diameter of the midvein and the decrease was a concentration dependent. Similarly, salinity decreased diameter of lamina in the blade of the leaf developed on the medium portion of the main stem and the decrease was accompanied with a reduction in palisade and spongy tissues diameter. Also, comparing to the corresponding control, the midvein bundle dimensions (length and width) were decreased due to salinity and the decrease was accompanied with a decrease in the average number of xylem rows per midvein bundle and vessel diameter relative to the control. Similar results were reported by Dawood *et al.*, (2014) on *Vicia faba.*

Table 9: Measurements in microns (μ) of certain anatomical characters in transverse section through the blade of the 3rd leaf from the plant tip developed on the main stem of self-grafted (control) cucumber plants (90 days old) and that grafted onto pumpkin plant rootstock grown under different levels of salinity during the second season (2016/2017).

Treatments	Anatomical characters						
	Mid vein diameter	Lamina diameter	Palisade diameter	Spongy diameter	Main vascular bundle (Length x width)	No of xylem vessels/ mid vein bundle	Vessel diameter
Control	479	275.1	30.5	180.0	184.0 x179.5	8	15.7
S 50 mM	470	259.7	83.5	171.1	198.0 x190.0	7	15.9
S 100 mM	420	237.3	71.6	147.5	169.2 x160.0	5	10.7
Grafting (G)	627	364.7	121.0	235.5	211.5 x200.0	7	18.1
S 100 x G	580	347.3	115.4	230.8	206.5 x200.0	6	13.7
New LSD at 5%	2.97	4.63	2.78	3.67	2.11 x0.31	0.31	1.33

Comparing self-grafted cucumber plants (control) with those grafting on pumpkin rootstock, the microphotographs and the tabulated data show that grafting increased diameter of both midvein and lamina of cucumber leaf blades more than the control. The increase in lamina diameter was accompanied with an increase in diameter of palisade and spongy tissues compared to the self-grafted plants. Likewise, the main vascular bundle of the midvein was increased in size (length and width) as a result of grafting which mainly due to the increase in its dimensions. However, the average number of xylem rows per midvein bundle was increased due to production of more vessels which amount to more total active conductivity area to cope with vigorous growth resulting from grafting. No available data were detected in this respect. However, it could be concluded that although the processes that regulate water and nutrients uptake are complex, it is clear that roots and shoot structures plays an important role in maintaining favorable plant water relations (Stendle and Peterson, 1998). In line with this Dawood *et al.*, (2014) has suggested that the vessel density provides an estimate of the mean diameter of its components in the xylem, a factor strongly related to water conductivity. Accordingly, the changes in cell structure in the stem and leaves of cucumber plants. In the present investigation might have improved plant tolerance to salinity stress and/or self grafted grown in pots (restricted roots) situation and induced suitable morphological and physiological responses to adapt to the adverse condition. Zaharah and Razi (2009) found that a striking secondary branch feature in the mango plants grown under restricted rooting volume and water stress conditions was a significant reduction in the size of epidermal cells. The smallest size of straight (not sinuous) walls of the epidermal cells may contribute to the greater resistance against collapse than in the large cells. Furthermore, when water availability is low, the cortex thickness decreases and collapses the outer cortex layer in the branches under both restricted and control growth.

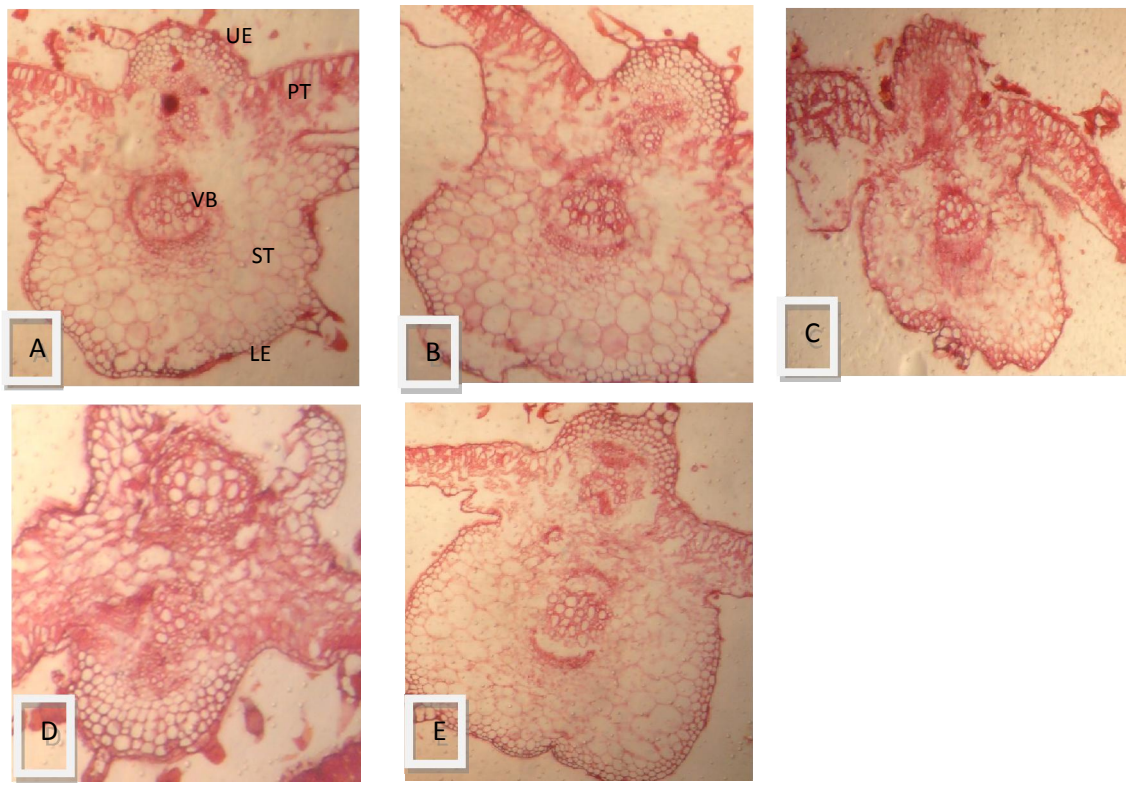

Fig 2: Transverse section through the blade of the 3rd leaf from the plant tip developed on the main stem of selfgrafted (control)_of cucumber plants (90 days old) grown under different salinity levels and grafting ; A- Control (self grafted plants grown under non-salinized condition. B- Self grafted plants grown under 50 mM of salinity. C- Self grafted plants grown under 100 mM of salinity. D- Grafted plants grown under 0 level of salinity. E- Grafted plants grown under 100 mM of salinity.

4. Yield and its components:

Tables (10 and 11) show that total cucumber yield in term of total fruits number/plant as well as fruit fresh weight were decreased significantly with salinity level increase. This inhibition was true in the two growing seasons and may be due to ion toxicity, photosynthesis decrease , accumulation of toxic substances, water and nutrient deficiencies , blocked protein synthesis and reduction on biomass (Trajkova *et al.*, 2006).

Grafting counteracted the depressing effects of salinity on cucumber yield , since grafted plants showed higher total yield than those of self-grafted one (Table 10). These results indicating the superiority of pumpkin salt-tolerant rootstock under salinized condition on product yield, compared with the control. The promoting effect of grafting on yield and its components of cucumber noticed in this study are in agreement with Edelstein (2004) who reported that grafted plants may have higher yields improved tolerance to environmental stress such as soil salinity. Colla *et al.*, (2010) found that grafting can represent an interesting tool to avoid or reduce yield losses caused by salinity stress in high yield genotypes belonging to solanaceae and cucurbitaceae families.

The interaction treatments showed that grafted plants grown under 50 mM salinity gave best results in the fruit yield and its components. This may be due to the higher nutritional capacity of the rootstock. There elevates can encourage vegetative growth, total Chls and photosynthetic rate which enhance flowering and fruiting leading to an increase in early fruit maturity. The important of nutrition balance were previously reported (Hatung, 2004) on growth, flowering and fruiting.

As for marketable yield, data in Table (11) show that salinity increased abnormal fruit rate, whereas grafting decreased it in both growing seasons. Fruit quality as indicated by average water content (reflects fruit brittleness) was decreased due to salinity irrespective to grafting. However, grafted plants showed higher values than the control. Similarly, the highest average fruit weight, vitamine C , total sugars (TS), total amino acids (TAA) and cellulose percentages were obtained from

grafted plants compared with the control overall salinity levels. The fruits were good flavor and taste as well as panelists under non-salinized condition whereas, salinized condition recorded less fruit quality. Flavor and tastes are the major factors that affect the fruit nutritional value. Zhou *et al.*, (2010) reported that free amino acids are one of the important members in nitrogen metabolism and the basis for the construction of organs shape. They added that cellulose content reflects the toughness of the cucumber, where increasing concentrations of cellulose correspond with worsening taste. However, Yuan *et al.*, (2009) stated that although salinity reduced fruit yield of cucumber owing to decrease both fruit weight and number, it improved fruit quality by increasing fruit dry matter, soluble sugars and titratable acidy content but had no significant effect on vitamin C content.

It could be concluded that grafting cucumber on salt-resistance rootstock of pumpkin improved it to tolerate salinity stress. The malfunctioning of enzymes, osmotic imbalance, membrane disorganization, reduction in evaporation and the production of ROS caused by salinity may be counteracted by grafting. Grafted plants had higher accumulation of osmoregulators in term of proline , sugars , free AA. Stem and leaf blade structures especially vessel density plays an important role in maintaining favourable plant water relations improving plant tolerance to salinity.

Table 10: Effect of salinity, grafting and their interaction on number of fruits/plant, average fruit weight (g) and average plant yield (g) of cucumber plants growing in the two growing seasons (2015/2016 and 2016/2017).

Salinity level (S) mM	Grafting (G)	2015/2016			2016/2017		
		Number of fruit/plant	Average fruit weight	Average plant yield	Number of fruit/plant	Average fruit weight	Average plant yield
Control (0)	Self grafted plants(control)	35.5	62.6	2306.3	38.7	66.2	2335.2
	Grafted plants	40.3	68.0	2740.4	43.4	71.5	2769.3
	Mean	37.9	65.3	2523.3	41.0	68.8	2552.2
50 mM	Self grafted plants(control)	31.1	50.0	1554.0	34.2	53.5	1582.9
	Grafted plants	37.6	50.1	2259.8	40.8	53.7	2288.7
	Mean	34.3	50.0	1906.9	37.5	53.6	1935.8
100 mM	Self grafted plants(control)	23.5	36.3	853.1	26.6	39.8	833.8
	Grafted plants	31.2	40.0	1252.0	34.4	43.5	1232.7
	Mean	27.3	38.1	1052.5	30.5	41.6	1033.2
Mean	Self grafted plants(control)	30.0	49.6	1571.1	33.2	53.2	1583.0
	Grafted plants	36.4	52.7	2084.1	39.5	56.2	2096.9
New LSD at 5% for:							
Salinity (S)		1.13	2.71	22.31	1.70	2.22	20.42
Grafting (G)		2.11	1.33	21.01	1.12	1.17	20.13
SxG		1.50	2.96	27.80	2.31	1.34	24.50

Table 11: Effect of salinity, grafting and their interaction on abnormal fruit, water content, total sugars, cellulose, vitamin C, total amino acid, fruit flavor score and fruit tasta score of cucumber plants growing in the two growing seasons (2015/2016 and 2016/2017).

Salinity level (S) mM	Grafting (G)	Season, 2015/2016							
		Abnormal fruit %	water content %	Total sugars mg/g	Cellulose mg/g	Vitamin C mg/g	Total amino acids mg/g	Fruit flavor score	Fruit taste score
Control (0)	Self grafted plants(control)	10.3	94.2	2.75	1.79	116.11	0.50	9.51	9.40
	Grafted plants	8.6	95.6	8.61	2.88	90.76	0.43	8.25	8.33
	Mean	9.4	94.9	5.68	2.33	103.43	0.46	8.88	8.86
50 mM	Self grafted plants(control)	47.4	92.3	1.79	2.88	59.36	0.30	9.27	8.00
	Grafted plants	21.0	94.0	2.00	3.00	65.66	0.35	8.39	7.85
	Mean	34.2	93.2	1.89	2.94	62.51	0.33	8.80	7.92
100 mM	Self grafted plants(control)	71.5	91.0	1.56	3.11	47.21	0.25	9.21	7.01
	Grafted plants	26.2	92.4	2.27	3.33	62.33	0.30	8.41	6.97
	Mean	48.8	91.7	1.92	3.22	54.77	0.27	8.81	6.99
Mean	Self grafted plants(control)	43.1	92.5	2.03	2.59	74.23	0.35	9.33	8.14
	Grafted plants	18.6	94.0	4.29	3.07	72.92	0.35	8.35	7.72
New LSD at 5% for:									
Salinity (S)		2.76	2.95	0.21	0.07	1.63	0.02	0.19	0.15
Grafting (G)		1.33	2.11	0.07	0.05	1.55	0.01	0.16	0.11
SxG		4.07	3.97	0.63	0.11	2.31	0.02	1.03	0.64
Season, 2016/2017									
Salinity level (S) mM	Grafting (G)	Abnormal fruit %	water content %	Total sugars mg/g	Cellulose mg/g	Vitamin C mg/g	Total amino acids mg/g	Fruit flavor score	Fruit taste score
Control (0)	Self grafted plants(control)	11.7	92.0	3.1	2.02	120.21	0.50	9.94	9.11
	Grafted plants	10.0	93.4	9.0	3.11	94.85	0.41	8.68	8.04
	Mean	10.85	92.7	6.1	2.56	107.53	0.45	9.31	8.57
50 mM	Self grafted plants(control)	48.7	90.1	2.2	3.09	58.31	0.34	9.70	7.71
	Grafted plants	22.4	91.9	2.4	3.23	64.62	0.36	8.82	7.56
	Mean	35.55	91.0	2.3	3.16	61.47	0.35	9.26	7.63
100 mM	Self grafted plants(control)	72.9	88.9	1.9	3.31	47.72	0.24	9.64	6.72
	Grafted plants	27.6	90.3	2.6	3.57	62.83	0.31	8.84	6.68
	Mean	50.25	89.6	2.2	3.44	55.27	0.27	9.24	6.70
Mean	Self grafted plants(control)	44.4	90.1	2.4	2.81	75.41	0.36	9.76	7.85
	Grafted plants	20.0	91.7	4.6	3.30	74.10	0.36	8.78	7.43
New LSD at 5% for:									
Salinity (S)		4.66	4.51	0.16	0.13	3.03	0.02	0.13	0.98
Grafting (G)		3.07	2.70	0.09	0.05	2.10	0.01	0.11	0.70
SxG		6.11	5.07	0.63	0.53	4.67	0.07	0.47	1.75

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