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Alleviation of Salt Injury of Cucumber Plant by Grafting onto Salt Tolerance Rootstock

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Abstract: Experiment was conducted in a greenhouse during the two successive seasons of 2008-2009 and 2009-2010 to investigate the effect of grafting technique in improving salt tolerant of grafted cucumber plants (Cucumis sativus L. cv. falcon, Hybrid F1) under saline conditions. Samples were taken at 70 days after planting to determine plant growth, leaf electrolyte leakage percentage (Membrane Permeability) and leaf relative water content (LRWC). As well as, fruits yield and quality, biochemical analyses and antioxidant enzymes activity. Grafting of cucumber on salt tolerance rootstock (Shintosa Supreme pumpkin) significantly increased growth parameters by 44% and 69% for fresh weight, dry weigh by 41% and 69%, plant height 86% and 83% and leaf area by 102% and 57% in both season respectively and enhancement 12% and 13% in leaf relative water content (LRWC) as compared to ungrafted cucumber in both season. Furthermore, These positive effects of grafting cucumber was correlated with significantly increased in chlorophyll, carotenoid, proline and total soluble protein concentrations. Although, grafting reduced membrane permeability and malondialdehyde (MDA) concentration in leaves of cucumber plants under salt stress conditions. As well as, improved fruit number, fruit weight, number of harvest and fruit yield comparing with ungrafted plants. As well as, Grafting increased PAL, POD, CAT, SOD, PPO and APX activities as compared to ungrafted plants under saline conditions. Excluding, grafted plants significantly reduced titratable acidity (TA %), total soluble solids (TSS %) and electrical conductivity (EC) in fruit juice as compared to ungrafted plants.

Key words: Shintosa Supreme pumpkin (*Cucurbita maxima x C. moschata*), cucumber (*Cucumis sativus*), grafting, salt stress

INTRODUCTION

Previous study revealed that cucumber plant is salt-sensitive Jones *et al*, (1989) and Chartzoulakis (1994). Therefore increasing salinity levels up to 80mM NaCl progressively decreased plant height, leaf number and area, fresh and dry weight, fruit growth, fruit numbers of cucumber Abd-Alla *et al* (1993), increasing salt injury index (Zhu *et al*, 2008). Lower osmotic potential and turger Steplien and Klobus (2006), increase in membrane permeability Alpalan and Gunes (2001), decreasing chlorophyll a, b in the leaves Wang *et al* (2006), inhibition of proline dehydrogenase activity in root Duan *et al* (2006), increasing membrane lipid peroxidation of cucumber Zhang and wan (2007), lower antioxidant enzyme activity Wei, *et al* (2004), decreasing K+ concentration in leaves and increasing leaf Na+ and Cl- concentrations Huang *et al* (2009).

Soil salinity has become a serious environmental problem currently, approximately 20% of the world's cultivated land and nearly half of all irrigated lands are affected by salinity (Zhu, 2001). Salinity imposes two constraints on plants: an osmotic effect resulting from the lower soil water potential and an ionic effect resulting from the direct toxicity of saline ions and the ion imbalance in the plants (Munns and Tester 2008). It is well established that the exposure of plants to salt stress can increase the production of reactive oxygen species (ROS), such as superoxide radicals (O2-) and hydrogen peroxide (H2O2) and these ROS are so reactive that they seriously disrupt the normal metabolism of plants through oxidation of membrane lipids, proteins and nucleic acids if the plants do not have sufficient protective mechanisms (Apel and Hirt, 2004). Excessive soil salinity reduces productivity of many agricultural crops, including most vegetables which are particularly sensitive throughout the ontogeny of the plant. Plant sensitivity to salt stress is reflected in loss of turgor, growth reduction and potentially death of the plant (Jones 1989; Cheeseman 1988).Cucumber is highly sensitive to salinity (Zhu *et al*., 2008). Moreover, in protected cultivation, cucumber is the primary vegetable, so the

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secondary salinization of protected soils has badly influenced the growth, development, and yield of cucumber (Wang, 1998).

Great efforts have been made to improve the salt tolerance of many crops by means of traditional breeding programs and, more recently, by genetic transformation (Cuartero *et al*., 2006). However, commercial success has been very limited owing to the complexity of salt tolerance, which is complex genetically and physiologically (Flowers, 2004).

The above mentioned deleterious effects of NaCl salt stress on salt sensitive cucumber led to decrease plant growth and productivity to alleviate the salt injury of cucumber by grafting salt-sensitive cucumber onto appropriate rootstock could be improved plant growth and productivity.

Shintoza Supreme Punpkin was found to be relative salt tolerant comparing with gourd black seed, bottle gourd and pumpkin (Amal El-shraiy *etal* 2011). Therefore Shintoza Supreme Punpkin was selected as appropriate rootstock in the oresent study under greenhouse experiment.

The present study was undertaken to investigate the effect of grafting using salt tolerant rootstock on cucumber plants under saline condition.

MATERIALS AND METHODS

Plant Materials and Cultivation:

Salt sensitive Cucumber (Cucumis sativus, L. cv. falcon, Hybrid F1) from Sakata seed, Europe was used in this study as a scion, and Shintosa Supreme pumpkin (Cucurbita maxima x C. moschata), was used as rootstock. Seeds of rootstock 'Shintosa Supreme pumpkin' were sown on October 18th, 2008 in 84 seedling plug trays filled with 2:1 (v:v) mixture of peat and vermiculite. Three days later, seeds of cucumber scion were sown. When seedlings of the rootstock had developed two cotyledon leaves (after 14 day from sowing), the cucumber seedlings with one true leaf were grafted onto the rootstock, using the procedure 'Root removed single cotyledon splice grafting - RRSG' described by Seong *et al*., 2003. The seedlings were incubated for 3-5 days on 25-30°C air temperature, relative humidity of 80-90% and 30-50% shading in the plastic tunnel in greenhouse. Ten days after grafting (5 days after incubation), plants were transferred to production greenhouse. Ungrafted cucumber plants were used as control and were sown at the same time with cucumber plants used as scions. The electrical conductivity (EC) of the soil and ground water used for irrigation were 2.1 dS m-1and 3.9 dS m-1, respectively. The pH of the soil and ground water were maintained close to 7.8 and 8, respectively. Fertilization was performed as recommended by Ministry of Agriculture. Experiments were arranged in a randomized block design with three replicates each replicate was divided into 2 plots (1 m×45 m /plot) with 100 plants per plot.

Samples were taken at 70 days after planting for growth measurements, determination of growth parameters, fruit quality and yield, leaf relative water content, electrolyte leakage, chemical analysis and antioxidant enzymes activity.

Growth Parameters:

Three plants from each replicate were harvested for growth measurement; plant height, shoot fresh and dry weight, and leaf area as previously.

Fruits Yield and Quality Measurements:

Fruits were harvested during the period of 21- 70 days after sowing and total numbers of harvesting were calculated. At each harvest, the total number of fruits per plant (N0 Plant-1) was recorded separately. Mean fruit weight (g fruit-1) and total fruit yield per plant (g Plant-1) were calculated.

In each treatment, 15 representatives marketable fruits were sampled (5 fruits/replicate) for determination of total soluble solid (TSS), titratable acidity, and ascorbic acid (Vit. C) concentrations.

Determination of Leaf Relative Water Content (LRWC):

Samples were taken from two plants per replicate (the sixth leaf from the top). Individual leaves first detached from the stem and then weighed to determine fresh weight (FWt.). In order to determine turgid weight (TWt.), leaves were floated in distilled water inside a closed Petri dish. Leaf samples were weighed periodically, after gently wiping the water from the leaf surface with the tissue paper until a steady state achieved. At the end of imbibitions period, leaf samples were placed in a pre-heated oven at 80○C for 48 h, in order to determine dry weight (DWt.). Values of FWt., TWt., and DWt. were used to calculate LRWC using the equation below (Kaya *et al*., 2003):

LRWC (%) = [(FWt. – DWt.) / (TWt. – DWt.)] × 100

Determination of Membrane Permeability:

Electrolyte leakage was measured in two plants per replicate in the young fully expanded leaves as mentioned previously in the preliminary experiment.

Biochemical Analyses:

5.1. Determination of Chlorophylls and Carotenoids:

Leaf discs (0.1g) were taken from the interveinal areas of the fully opened sixth leaf from the top of three plants per replicate. The total chlorophyll and carotenoid contents were determined by UV–vis spectrophotometery (CT 200 spectrophotometer). The absorbance of the solution was measured at 470, 647 and 664 nm. Formulae and extinction coefficients used for the determination of chlorophyllous pigments (total chlorophyll and carotenoids) were described by Lichtenhaler and Wellburn (1983). Carotenoids were determined spectrophotometrically at 470 nm (CT 200 spectrophotometer) using the method of Shlyk (1971).

5.2. Determination of Proline:

Proline concentration was determined according to the method of Troll and Lindsley (1955) modified by Petters *et al*. (1997). Fresh samples (0.5 g) were ground and homogenized with one volume of 100 mM sodium phosphate buffer (pH 6.0). The samples were centrifuged for 10 min at 16.000 x g. The reaction proceeded for 1 h in boiling water bath and the developed dye was extracted with 1 ml of toluene and measured by the spectrophotometer at 515 nm by using UV–vis spectrophotometer (CT 200 spectrophotometer).

5.3. Determination of Malondialdehyde:

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) concentration by thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). One gram of fresh seedling tissue was homogenized in 5 ml of 0.1% (w/v) trichloroacetic acid (TCA). The contents were centrifuged at 10000 x g for 15 min and the absorbance of suspension was measured at 532 nm in spectrophotometer (CT 200 spectrophotometer). The concentration of MDA was calculated using an extinction coefficient of 155 mM-1cm-1and was expressed as µmol g-1 FW.

Determination of Antioxidant Enzymes Activity:

6.1. Preparation of Enzymes Extract:

Leaf tissues were 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 ºC. The extraction ratio was 4 ml buffer for each one gram of plant material. The homogenate was centrifuged at 15.000 x g for 15 min at 4 ºC. Supernatant was used to measure the activities of phenylalanine ammonia-lyase (PAL), peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) and polyphenol oxidase (PPO). Protein concentration was determined according to the method of Bradford (1976). All enzymes activity was calculated per milligram of protein per minute. The proteins concentration were calculated by using the standard curve of bovine serum albumin (BSA).

6.2. Determination of Phenylalanine Ammonia-lyase Activity:

The activity of phenylalanine ammonia-lyase (PAL; E.C 4.3.1.5) was determined by He *et al*., (2001). The PAL assay reaction mixture consisted of 100 µL crude enzyme extract and 900 µL of 6 µmol L-phenylalanine in 500 mM Tris-HCl buffer (pH 8.5). The mixture was incubated at 37 °C for 1 h. The absorbance was measured by spectrophotometer (CT 200 spectrophotometer) at 290 nm.

6.3. Determination of Peroxidase Activity:

The activity of peroxidase (POD; EC1.11.1.7) was assayed by the method of Hammerschmidt *et al.* (1982). The reaction mixture (2.9 ml) consisted of 0.25 % (v/v) guaiacol in 10 mM sodium phosphate buffer (pH 6 containing 10 mM hydrogen peroxide H2O2). Volume of 100 µl of the crude enzyme extract was added to initiate the reaction which was measured spectrophotometrically (CT 200 spectrophotometer) at 470 nm per min. One international (IU) of enzyme activity was expressed as Δ OD= 0.01. POX activity expressed as unit .min-1.mg-1 protein.

6.4. Determination of Catalase Activity:

The activity of catalase (CAT; EC 1.11.1.6) was determined according to Aebi (1984). Enzyme extract (100 µl) was added to 2.9 ml of a reaction mixture containing 20 mM H2O2 and 50 mM sodium phosphate buffer (pH 7.0). The activity of CAT was measured by monitoring the reduction in the absorbance at 240 nm as a result of H2O2 consumption.

6.5. Determination of Superoxide Dismutase Activity:

The activity of superoxide dismutase (SOD; EC 1.15.1.1) was assayed by the method of Beauchamp and Fridovich (1971) by measuring its ability of enzyme to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). A reaction mixture (3 ml) containing 40 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 2 µM riboflavin, 0.1 mM EDTA and 100 µl of the crude enzyme extract was shaken and placed 30 cm below light source consisting of 15 W fluorescent lamp. The absorbance was recorded at 560 nm.

6.6. Determination of Polyphenol Oxidase Activity:

The activity of polyphenol oxidase (PPO; EC 1.14.18.1) was determined by the method of Oktay *et al.* (1995). The reaction mixture contained 600 µl catechol (0.1 M) and 100 µl enzyme extract was completed to 3 ml with 0.1 M phosphate buffer pH 7. The absorbance was recorded at 420 nm by spectrophotometer (CT 200 spectrophotometer).

6.7. Determination of Ascorbate Peroxidase:

Ascorbate peroxidase (APX; EC 1.11.1.11) was assayed by recording the decrease in absorbance at 290 nm due to a decrease in ascorbic acid content (Nakano and Asada, 1981). Reaction mixture (3 ml) contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM EDTA, and 1.5 mM H2O2 and 0.1 ml enzyme extract. The reaction was started with the addition of H2O2 (ε = 2.8 mM cm-1). Absorbance was measured by spectrophotometer (CT 200 spectrophotometer) at 290 nm.

Statistical Analysis:

Growth Parameters (plant height, leaf area, stem and root fresh and dry weights , fruit quality and yield, membrane permeability (%),leaf relative water content and the average of two season (2008 and 2009) for antioxidant enzymes activity in ungrating and grafting cucumber were determined by analysis of variance using SAS software (SAS Institute, Cary NC). Differences between means treatment were separated by the least significant difference (L.S.D.) test at a 0.05 probability level.

Results:

Growth Parameters:

Grafting technique significantly increased cucumber growth comparing with ungrafted plants under salt stress conditions, data in table (1) reveal that grafting of cucumber on salt tolerance rootstock (Shintosa Supreme pumpkin) gave more better growth comparing with ungrafted plants in the two successive seasons (2008/2009, 2009/2010), as indicated by fresh and dry weights, plant height and leaf area under salt conditions. Grafted plants had higher fresh weight by 44% and 69%, dry weigh by 41% and 69%, plant height 86% and 83% and leaf area by 102% and 57% in both season respectively.

Table 1: Effect of grafting cucumber on salt tolerance rootstock (Shintosa Supreme pumpkin) (GC) and ungrafting cucumber on the growth under salinity stress after 70 days from planting during the two seasons.

Treatments	Season 2008-2009 Shoot				Season 2009-2010 Shoot			
	Fwt. (g)	Dwt. (g)	Height (cm)	Leaf area (cm^2)	Fwt. (g)	Dwt. (g)	Height (cm)	Leaf area (cm^2)
Cucum.	89	32.72	79	98.62	158.4	43.91	124	180.25
GC	128	46.22	147	217.49	268.46	74.33	227	282.73
MSD	1.9567	1.3188	2.094	1.3187	1.332	1.309	0.9255	3.3433

Fruit Quality and Yield:

Data presented in table (2) showed that mean fruit weight, fruit number, fruit yield and number of harvest significantly increased in grafted plants comparing to ungrafted plants almost by 16.6 % and 18.2 % for mean fruit weight and 32.6 % and 25.8 % for fruit yield in two successive seasons under saline conditions. Data

presented in Table (3) showed that grafted plants significantly reduced titratable acidity (TA %), total soluble solids (TSS %) and electrical conductivity (EC) in fruit juice, at 70 days after planting in both seasons. There were no significant differences in TA between grafted plants and ungrafted plants under saline conditions in the first season; however, these differences were significant in the second season. Data indicated that, grafted plants significant increased TSS and Vit C as compared with ungrafted plants in both seasons. On the other hand, grafted plants significant decreased EC in comparison with ungrafted plants under saline condition in both season.

Table 2: Effect of grafting cucumber on salt tolerance rootstock (Shintosa Supreme pumpkin) (GC) and ungrafting cucumber on fruit yield under salinity stress after 70 days from planting during the two seasons.

Treatments	Season 2008-2009				Season 2009-2010			
	Mean fruit weight (g fruit^{-1})	Fruit number (N^0 Plant^{-1})	fruit yield (g Plant^{-1})	Number of harvesting per plant	Mean fruit weight (g fruit^{-1})	Fruit number (N^0 Plant^{-1})	fruit yield (g Plant^{-1})	Number of harvesting per plant
Cucum.	90.21	29	2616	10	86.42	31	2679	10
GC	105.15	33	3470	10	102.12	33	3370	10
MSD	1.1564	1.851	2.267		0.8211	1.3088	1.851	

Table 3: Effect of grafting cucumber on salt tolerance rootstock (Shintosa Supreme pumpkin) (GC) and ungrafting cucumber on fruit quality under salinity stress after 70 days from planting during the two seasons.

Treatments	Season 2008-2009				Season 2009-2010			
	TA %	Vit.C mg 100g^{-1}	TSS %	EC	TA %	Vit.C mg 100g^{-1}	TSS %	EC
Cucum.	0.40	3.56	2.2	2.03	0.44	3.53	2.4	2.23
GC	0.31	3.61	1.8	1.96	0.36	3.69	2.1	1.95
MSD	0.054	0.0171	0.148	0.014	0.012	0.0193	0.129	0.013

Titratable acidity (TA), L-ascorbic acid (Vit.C), Electric conductivity (EC), Total soluble solid (TSS)

Membrane Permeability and Malondialdehyde (MDA):

At 70 days after planting, the plasma membrane permeability (MP) significantly decreased by 20% and 22% in grafted plants compared with ungrafted plants under saline conditions in both seasons (Table 4). From table (4) it is clear that, under salt stress, MDA concentrations in cucumber leaves was 13.13 and 12.44 µmol g-1 FWt. After grafting, the accumulation of MDA was significantly reduced to 9.81 and 8.79 µmol g-1 FWt. at 70 days after planting in both seasons respectively.

Table 4: Effect of grafting cucumber on salt tolerance rootstock (Shintosa Supreme pumpkin) (GC) and ungrafting cucumber on malondialdehyde (MDA) and membrane permeability (MP) under salinity stress after 70 days from planting during the two seasons.

Treatments	Season 2008-2009		Season 2009-2010	
	MDA (µmol g^{-1} FWt).	MP (%)	MDA (µmol g^{-1} FWt).	MP (%)
Cucum.	13.13	41.67	12.48	42.51
GC	9.81	33.33	8.97	33.01
MSD	2.865	2..223	2.608	3.33

Leaf Relative Water Content:

Data in Table (5) illustrated that leaf relative water content (LRWC) was significantly affected by grafting under salt condition, however, for grafted plants the enhancement in LRWC was 12% and 13% comparing with ungrafted plants at first date in both seasons respectively.

Table 5: Effect of grafting cucumber on salt tolerance rootstock (Shintosa Supreme pumpkin) (GC) and ungrafting cucumber on leaf relative water content under salinity stress after 70 days from planting during the two seasons.

Treatments	Season 2008-2009 LRWC (%)	Season 2009-2010 LRWC (%)
Cucum.	69.86	70.11
GC	78.50	79.36
MSD	2.3651	1.891

Biochemical Constituents in Leaves:

Chlorophyll and Carotenoids Content:

Data presented in Table (6) showed that, after 70 days from planting, grafted cucumber plants had higher

leaf chlorophyll content than ungrafted plants under salt conditions in two successive seasons, but this difference was not significant. In comparison to ungrafted plants, carotenoids significantly increased in grafted plants, this increment was not significant in the second season.

Total Soluble Protein and Proline Content:

Data presented in Table (6) showed that grafted plants recorded higher total soluble protein values than ungrafted plants at 70 days after planting in both seasons under saline conditions, however, the differences between grafted and ungrafted plants in total soluble protein concentrations was significant in the second season. Furthermore, Proline concentrations were significantly increased in grafted plants more than in ungrafted plants at 70 days after planting in both seasons under salt stress conditions.

Table 6: Effect of grafting cucumber on salt tolerance rootstock (Shintosa Supreme pumpkin) (GC) and ungrafting cucumber on chlorophylls (Chl.), carotenoids (Carot.), proline (pro.) and total soluble protein (TSP) concentrations under salinity stress after 70 days from planting during the two seasons.

Treatments	Season 2008-2009				Season 2009-2010			
	Chl. (mg g^{-1} Fwt.)	Carot. (mg g^{-1} Fwt.)	TSP (mg g^{-1} Fwt.)	Pro. (µg g^{-1} FWt.)	Chl. (mg g^{-1} FWt.)	Carot. (mg g^{-1} Fwt.)	TSP (mg g^{-1} Fwt.)	Pro. (µg g^{-1} Fwt.)
Cucum.	1.67	0.15	5.23	26.18	1.82	0.24	6.80	28.79
GC	2.24	0.31	6.76	33.78	2.30	0.34	7.84	37.15
MSD	0.54	0.081	1.42	0.423	0.498	0.17	1.261	0.409

Antioxidant Enzymes Activity:

Grafting of cucumber on salt tolerance rootstock (Shintosa Supreme pumpkin) significantly increased PAL, POD, CAT, SOD, PPO and APX activities comparison to ungrafted plants under saline conditions at 70 days after planting in both season (Figure 1).

Discussion:

Experiments was conducted in greenhouse to compare grafted cucumber on relative salt tolerant rootstock (Shintosa Supreme pumpkin) and own-rooted cucumber under 3800 ppm of salinity (soil + irrigation water). Recently, many studies have shown that salt tolerance of vegetable plant can be improved by grafting onto appropriate rootstock such as melon, tomato and cucumber (Zhu, et al , 2008). Pervious study revealed that cucumber plant is salt-sensitive (Jones, et al, 1989 and Chartzoalakis 1994). There is a great need to better understand the mechanism of salt tolerance in grafted plant.

Proposed explanation for grafting included salt tolerance are:

- Higher accumulation of proline and sugar in the leaves (Xu, *et al*, 2006)
- Higher antioxidant capacity in leaves (López-Gómez, *et al* 2007)
- Lower accumulation of Na+ and/or Cl- in the leaves (Goreta *et al* 2008 and Zhu, *et al* 2008)

However, water status in plant under salt stress is the most limiting factor allow to resume growth (Yeo *et al*, 1985) to maintain relative water content under existing NaCl stress, plant need to uptake same in gram solutes such as Na+, Cl- and K+ and synthesize some compatible solutes (proline, sugar and glycin betaine (Munns and Tester 2008). Therefore, higher concentration of Na+ and Cl- are often detrimental to salt-sensitive plants. At 70 days after planting of grafting cucumber plants showed improving vegetative growth comparing with ungrafted plants under salt conditions. Grafted plants showed better growth and development. Thus, grafting increased plant height, number of internodes per plant and root dry weight. Also grafting produced 43% more shoot dry matter than non-grafted plants (Canizares, *et al*., 2000; Zeng *et al*., 2004). The present results showed more stimulation on growth parameters reaching 43% and 72% at shoot dry weight. As well as, grafted plants enhancement in leaf relative water content (LRWC) comparing with ungrafted plants. Also, membrane permeability (MP) decreased in grafted plants compared with ungrafted plants under saline conditions. There are numerous reports in the literature showing that the cell membrane permeability in leaves were dramatically lower in grafted seedlings than own-rooted seedlings Chen and Wang (2008) and Zhu, *et al*., (2008b) investigated the effects of grafting with different rootstocks on cucumber growth and physiological trait, using 'Chaofeng 8848' and 'Heizinangua' as rootstocks, and 'Jinchun No.2' as scion under NaCl stress. The ungrafted cucumber plants were used as the controls. The shoot water content in the leaves of grafted plants was higher, than those of ungrafted plants at the same NaCl stress. These results were confirmed by

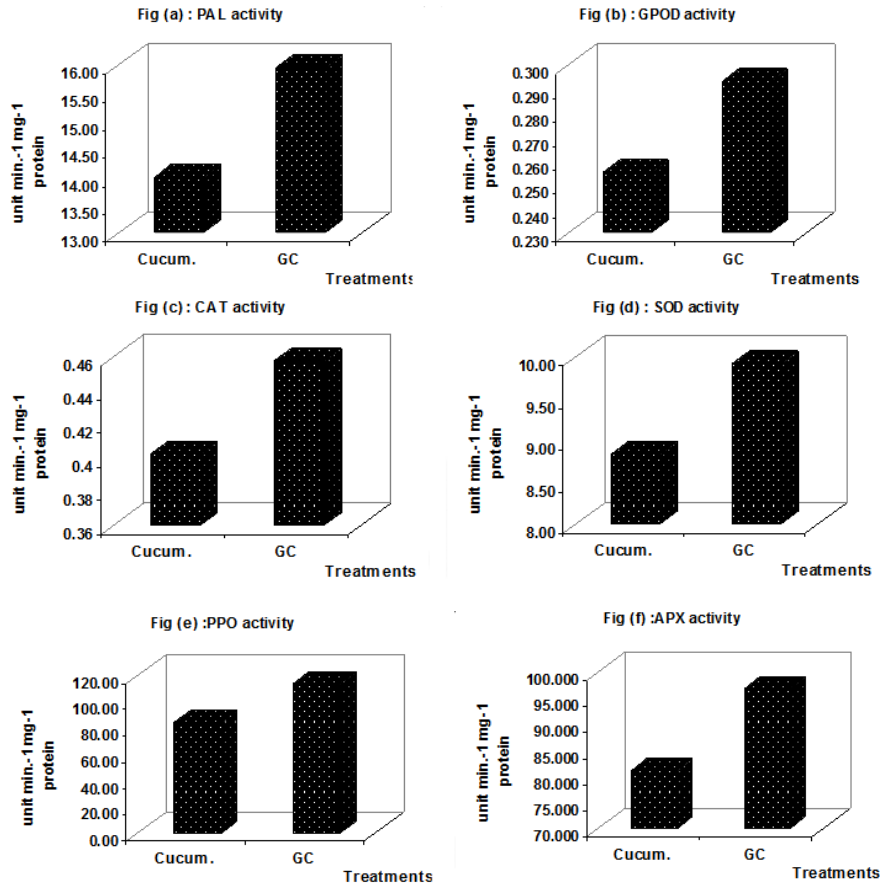

Fig 1: Effect of grafting cucumber on salt tolerance rootstock (Shintosa Supreme pumpkin) (GC) and ungrafting cucumber on Antioxidant enzymes activity **[(a)**Phenylalanine ammonialase (PAL), (b)Guaiacol peroxidase (GPOD), (c)Catalase (CAT), (d)Superoxide dismutasa (SOD), (e)Polyphenol oxidase (PPO), (f)Ascorbate peroxidase (APX). under salinity stress after 70 days from planting during the two seasons.

the biochemical constituents which showed an increase in chlorophyll and carotenoids concentrations, total soluble protein, proline, malondialdehyde and antioxidant enzymes activity (PAL, POD, CAT, SOD, PPO and APX) in grafted plants. An interpretation to this improvement of grafting cucumber plants was mentioned by Yang, *et al*., (2006) and Hang, *et al*., (2009) grafted a salt-sensitive cucumber cultivar Jinchun No. 2 (Cucumis sativus L.) on to the commercial salt-tolerant bottle gourd cultivar Chaofeng 8848 (Lagenaria siceraria Standl.), to determine if salt tolerance of grafted cucumber can be associated with the accumulation of organic solutes and inorganic solutes in the leaves and roots. Compared with the ungrafted plants, salinity increased the malondialdehyde contents (MDA) to a lesser extent in the grafted plants, especially in the roots. At the end of the experiment, salinity increased MDA contents by 72.4 and 51.3% in the leaves and roots of grafted plants, respectively, while the respective values were 90.1 and 93.4% in the ungrafted plants. Also, Zhu, et al., (2008b) investigated the effects of grafting with different rootstocks on cucumber physiological trait, using 'Chaofeng 8848' and 'Heizinangua' as rootstocks, and 'Jinchun No.2' as scion under NaCl stress. The ungrafted cucumber plants were used as the controls. The H2O2 contents, and O2- production rate were lower, whereas SOD, POD, CAT and APX activities in the leaves of grafted plants were higher, than those of ungrafted plants at the same NaCl stress. The antioxidant system was the important reason for the higher salt tolerance of grafted 'Chaofeng 8848' plants than that of grafted 'Heizinangua' plants.

Grafting of cucumber on salt tolerance rootstock (Shintosa Supreme pumpkin) significantly increased growth and fruits yield, leaf relative water content (LRWC) and antioxidant enzymes activity. Furthermore, grafting significantly increased chlorophyll, carotenoid, proline and total soluble protein concentrations

comparing with ungrafted plants. Grafting reduced membrane permeability and malondialdehyde (MDA) concentration in leaves of cucumber plants under salt stress conditions.

An improvement in yield parameters, and biochemical contents of grafted cucumber plants compared to ungrafted plants under salt conditions were found to be agree with Zhong and Bie (2007) they grafted cucumber cultivars 'Jinyu No.1' and 'Jinchun No.4' (scion) on to rootstock fig-leaf gourd (C. ficifolia), and fruit development and quality were investigated. The results showed that grafting significantly increased the growth of cucumber fruits. Eight days after pollination, fruit fresh weight of 'Jinyu No.1' and 'Jinchun No.4' grafted plants increased by 38.4% and 36.1% compared with non-grafted cucumber plants, respectively, whereas fruit length increased by 23.7% and 30.2%, respectively. With the development of cucumber fruit, fruit ascorbic acid concentration gradually decreased, and there was no significant difference in fruit quality between grafted and non-grafted plants. Also, Zhang HongMei *et al*. (2008) mentioned that the ascorbic acid concentration in fruits of all the grafting combinations was lower than that of self-root cucumber. An interpretation to this improvement of yield and quality confirmed by Yuan, *et al*., (2009b) carried out a greenhouse experiment tusing cucumber plants (Cucumis sativus L. cv. Jinchun No. 2), either self-grafted or grafted onto the commercial salt tolerant rootstock Figleaf Gourd (Cucurbita ficifolia Bouche) and Chaofeng Kangshengwang (Lagenaria siceraria Standl) to investigate the feasibility of using salt tolerant rootstock to increase fruit yield and quality of cucumber under 60 mM NaCl stress. Plants grafted onto Figleaf Gourd and Chaofeng Kangshengwang had a higher fruit number and marketable fruit yield compared to the selfgrafted plants at all salt levels. The total fruit yield of plants grafted onto Figleaf Gourd increased by 15%, 28%, and 73% under 0, 30, and 60 mM NaCl stress, respectively, whereas the respective values were 14%, 33%, and 83% in the plants grafted onto Chaofeng Kangshengwang, with respect to the self-grafted plants. Salinity had significant effect on fruit dry matter, soluble sugar, and titratable acidity contents, but had no significant effect on vitamin C content. Rootstock had no significant effect on fruit dry matter and soluble sugar contents, but had significant effect on titratable acidity and vitamin C contents. Meanwhile, fruit dry matter and soluble sugar contents were significantly affected by salinity × rootstock interaction. However, a higher content of soluble sugar in the plants grafted onto Figleaf Gourd was observed under salt stress. In addition, an overall increase of titratable acidity and vitamin C contents of fruits was obtained by grafting onto Figleaf Gourd and Chaofeng Kangshengwang, whether saline-challenged or not. Overall, it is suggested that the use of salt tolerant rootstock could provide a useful tool to improve fruit yield of cucumber under NaCl stress.

Conclusion:

The results obtained from the previous study could be summarized as follow:

- Grafting of cucumber on salt tolerance rootstock (Shintosa Supreme pumpkin) significantly gave better growth, as indicated by fresh and dry weights, plant height and leaf area comparing with ungrafted plants in the both seasons under salt stress conditions.
- Leaf relative water content (LRWC) was improved in grafted plants comparing with ungrafted plants under salt condition.
- Plasma membrane permeability (MP) and malondialdehyde (MDA) concentration significantly decreased in grafted plants compared with ungrafted plants under saline conditions. Grafted cucumber plants had higher chlorophyll, carotenoid, total soluble protein and proline concentrations than ungrafted plants under salt conditions.
- Grafting of cucumber on salt tolerance rootstock (Shintosa Supreme pumpkin) significantly increased PAL, POD, CAT, SOD, PPO and APX activities comparison to ungrafted plants under saline conditions.
- Grafted cucumber plants significantly increased the features of fruits quality, as indicated by reduced titratable acidity (TA %), total soluble solids (TSS %), electrical conductivity (EC) and increased Vit.C concentrations in fruit juice comparing with ungrafted plants under salt stress conditions.

It was concluded in this study that, salt-tolerant rootstock (Shintosa Supreme pumpkin) has a primary effect in determining the salt tolerance of grafted cucumber seedlings under salt stress conditions compared with ungrafted plants.

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