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Salinity tolerance of grafted watermelon

Bóhm Viktória

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PhD School

name: PhD School of Horticultural Science

field: Crop Sciences and Horticulture

head: Dr. Zámbooriné Németh Éva
professor, DSc, habil dr., doctor of MTA
SZENT ISTVÁN UNIVERSITY, Faculty of Horticulture,
Department of Medicinal and Aromatic Plants

Supervisors: Dr. Kappel Noémi
senior lecturer, PhD
SZENT ISTVÁN UNIVERSITY, Faculty of Horticulture,
Department of Vegetable Growing

Dr. Gáspár László
senior lecturer, PhD
SZENT ISTVÁN UNIVERSITY, Faculty of Horticulture,
Department of Plant Biology and Plant Biochemistry

.....
Head of PhD School

.....
Supervisor

.....
Supervisors

1. Introduction

Salinity is one of the major abiotic stresses that could reduce plant growth. It can be a serious problem in arid and semi-arid regions where the area is prone to salinity due to irrigation. Moreover in many irrigated areas of the arid and semi-arid regions, farmers are forced to use saline water to irrigate their crops due to an inadequate supply of fresh water. Salinity can cause complex and several physiological, morphological and metabolic changes in plants through physical and chemical stresses (BOROCHOV-NEORI and BOROCHOV, 1991). The deleterious effects of salinity on plant growth are associated with low water potential of the root medium which causes a water deficit within the plant; toxic effects of ions mainly Na^+ , Cl^- , and SO_4^{2-} and nutritional imbalance caused by reduced nutrient (e.g., K^+ , Ca^{2+} , Mg^{2+}) uptake and/or transport to the shoot (MARSCHNER, 1995; HASEGAWA et al., 2000). The weight of the shoot, root and leaf area can be reduced. The main reason of the leaf area reduction is due to the less water uptake of the plants because of the osmotic changes that results in developing smaller leaf cells (KAYA et al., 2002). Grafted plants develop numerous physiological and biochemical mechanisms to cope with salt stress including: salt exclusion in the shoot and retention of salt ions in the root, better maintenance of potassium homeostasis, compartmentation of salt ions in the vacuole, accumulation of compatible solutes and osmolytes in the cytosol, activation of an antioxidant defense system, and induction of hormones mediated changes in plant growth (COLLA et al. 2010). Rootstocks can decrease the accumulation of Cl^- and Na^+ in *Cucumis melo* scion leaves (ROMERO et al. 1997). Apart from the level of Na^+ in the shoot, another component of plant salinity tolerance is the capability of the tissue to tolerate Na^+ (MUNNS and TESTER, 2008). In contrast to the exclusion of saline ions in the shoot, the root of rootstocks generally includes more Na^+ and Cl^- compared with self-rooted plants, as has been reported for eggplants (WEI et al., 2007), watermelons (GORETA et al., 2008), and cucumbers (ZHU et al., 2008).

Using rootstocks capable of ameliorating salt-induced damage to the shoot can be a solution to avoid yield loss in salt sensitive genotypes belonging to *Cucurbitaceae* family (YETISIR and UYGUR, 2009). Recently, grafting onto a salt-tolerant rootstock was shown to be an efficient and environmental friendly technique for eliminating or reducing losses in production caused by salinity in high-yielding genotypes belonging to the *Solanaceae* and *Cucurbitaceae* families (COLLA et al., 2010).

2. Objectives

The aim of this study was to compare the salinity tolerance of two commonly used rootstock for watermelon (interspecific squash hybrid and *Lagenaria*). Both nongrafted and self-grafted plants are rarely used simultaneously for control in grafting experiments. In this study the effect of

grafting itself on salinity tolerance were also studied, since both ungrafted and self-grafted watermelon were used for control.

3. Material and methods

3.1 Location of the experiments

In order to evaluate the salinity tolerance of grafted watermelon four experiments were conducted:

- An openfield experiment conducted in containers in Soroksár at the Research and Experimental Farm of the Vegetable and Mushroom Growing Department of Corvinus University of Budapest in 2012 (Soroksár experiment)
- 2 experiments in controlled environment (autumn 2012 and spring 2014) conducted in Conviron growing chamber at Corvinus University of Budapest, Faculty of Horticultural Science, Department of Plant Physiology and Plant Biochemistry (fitotron experiment).
- A greenhouse experiment conducted in the 50 m² greenhouse belongs to the Vegetable and Mushroom Growing Department in the Arboretum of Corvinus University of Budapest in 2013 (greenhouse experiment).

3.2 The scion and rootstock

Citrullus lanatus (Thunb.) Matsum & Nakai 'Esmeralda' F1 was used as scion and two commonly used rootstock for watermelon were chosen: *Lagenaria siceraria* 'DG-01' F1 (**L**) and *Cucurbita maxima* x *Cucurbita moschata* 'Shintosa F-90' F1 interspecific squash hibrid (**INT**).

3.3 Fertilizers and substrates

Yara Fericare and Yara Liva Calcinit fertilizers with macro and microelements were used (15:30:15 in all experiment, and 14:11:25 in the experiment in Soroksár during the salinity treatments). In case of the experiment in Soroksár the containers were filled with peat, in the experiment in growing chamber and greenhouse perlite were used as a substrate.

3.4 Sowing and grafting

In case of the Soroksár experiment the rootstock and non-grafted plant were sown to plastic pots (9x9x10 cm) filled with peat while in the fitotron experiments the non-grafted watermelon and the rootstocks were sown into plastic pots (9x9x10 cm) filled with 600 ml perlite precisely. The scion used for grafting and all plants in the greenhouse experiments were sown in trays filled with peat. Because of differences in growth vigor, the interspecific rootstock and the non-grafted watermelon were sown 7 days later than the *Lagenaria* and watermelon rootstock. The watermelons used as scion were sown in trays filled with peat. Seedlings were grafted by hand, applying the one-

cotyledon grafting method on. After 1 week in grafting chamber the plants were grown in controlled environment (100-250/0 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 16 h/8 h, 25 °C/20 °C, 70%/70 % relative humidity). The seedlings were transplanted to the containers filled with peat 3 weeks after grafting. The seedlings were irrigated with Yara Ferticare (NPK 15:30:15 + microelements) in 0,2 m/m% concentration before grafting and for one week after grafting. The concentration was increased to 0,4 m/m% from the second week after grafting and was supplemented with Yara Liva Calcinit in 0,2 m/m% concentration on the 3rd week from grafting.

3.5 Experiments

3.5.1 Soroksár experiment

An openfield experiment was carried out in Soroksár at the Research and Experimental Farm of the Vegetable and Mushroom Growing Department of Corvinus University of Budapest in 2012. Plants were planted out to 12 l container filled with peat on 23rd May. The peat did not contain any nutrients. The fertigation solutions were prepared by the use of YaraLiva water-soluble fertilizer containing macro- and micro-nutrients in 0.2% (m/m) concentration completed with Yara Liva Calcinit in 0.2% (m/m). The salinity treatment started one month after transplanting. The saline solution used for the treatments were prepared by adding NaCl to the basic fertilizer solution in 100 mmol and 150 mmol concentration. The plants were irrigated with 2l/container/day saline solution for 15 days while the control plants were irrigated with the same amount of the basic fertilizer solution.

3.5.2 Fitotron experiments

Two experiment in autumn 2012 and spring 2014 were conducted in Conviron growing chamber at Corvinus University of Budapest, Faculty of Horticultural Science, Department of Plant Physiology and Plant Biochemistry. Before the salinity treatments have started every pot had been placed into a nylon bag and tied at the plant base. The weight of the plants were measured after irrigation and before irrigation in 2 days interval. The transpiration of the plants were calculated from the weight difference. The irrigation were planned based on the average transpiration of the control plant group from each grafting combination. The experiment consisted of 3 treatments (control (0), 2,85 (I.) and 4,28 (II.) mM/l substrate NaCl/irrigation) initialized on 28.11.2012. and 07.05.2014. Every plant (each pots) were irrigated with the same amount of fertilizer solution and NaCl solution (0, 1ml, 1,5 ml of 100g/l NaCl solution) completed with deionized water based on the average transpiration rate of the control plants. So 0, 2,85 and 4,28 mM/l substrate NaCl dosage were added to the plants with each irrigation. The treatments were done in 2 days interval for 23

days. The treatments were arranged in a randomized complete-block design with 4 replicates per treatment.

3.5.3 Greenhouse experiment

The greenhouse experiment was conducted in the 50 m² greenhouse belongs to the Vegetable and Mushroom Growing Department in the Arboretum of Corvinus University of Budapest. The seedlings were planted out to 3 l containers filled with perlite on 26 March. The salinity treatments were began 3 weeks after transplanting (15th April). The method of the treatments were similar to the method in the experiments in the chamber with one difference only. To ensure the same salinity concentration in the substrate in the bigger container the NaCl solution added/each irrigation were raised to 5 and 7,5 ml. The composition of the fertilizer solution, the calculation and frequency on the irrigation (2 days interval), the length of the experiment (23 days) were similar to the experiments in the chamber.

Table 1: Summary of the experiments

Date of experiment	Reference in text	locatio	medium	sowing	grafting	planting	first salt treatment	end of the experiment	duration of salt treatment	number of salt treatment
2012 spring-summer	soroksár experiment	Soroksár	peat	2012.04.20 (<i>Lagenaria</i> +watermelon rootstock and scion) 2012.04.27. (interspecific+non-grafted wm.)	2012.05.04	2012.05.23	2012.06.27	2012.07.11	15 days	11
2012 autumn	fitotron experiment (2012)	fitotron	perlite	2012.10.15. (<i>Lagenaria</i> + watermelon rootstock and scion) 2012.10.22. (interspecific+non-grafted wm.)	2012.10.29	_	2012.11.28	2012.12.19	22 days	11
2013 spring	greenhouse experiment	greenhouse	perlite	2013.02.15 (<i>Lagenaria</i> + watermelon rootstock and scion) 2013.02.22. (interspecific+non-grafted wm.)	2013.03.01	2013.03.26	2013.04.15	2013.05.07	23 days	11
2014 spring	fitotron experiment (2014)	fitotron	perlite	2014.04.03. (<i>Lagenaria</i> + <i>Lagenaria</i> + watermelon rootstock and scion) 2014. 04.10. (interspecific+non-grafted wm.)	2014.04.18	_	2014.05.07	2014.05.29	23 days	11

3.6 Measurements

Morphological measurements and laboratory analysis were carried out during the salinity treatments and after the experiments. The photosynthesis activity were measured by LCi SD Portable Photosynthesis and transpiration rate analyzer on the 13th day (Soroksár experiment) and on the 8th and 18th day from the initialization of the salinity treatment (greenhouse and growing chamber experiment). Parallel with the second photosynthesis activity measurements leaf samples for water potential, and epidermis samples for stomatal density measurement were collected. The leaves were frozen and the water potential was measured later with WP4 Water Potential Meter (Decagon Devices, USA) according to operator's manual sample preparation of plant samples. The stomata number were calculated for an area of 0,038 mm² under an Olympus CX41 microscope and the density of 1 mm² were calculated from the data. In case of the greenhouse and growing chamber experiments the transpiration were calculated from the weight of the plants before and after irrigation measured in 2 days interval during the treatments. The transpiration were calculated to g water/24 hours.

At the end of the treatments the plants were harvested and the fresh weight (FW) of the different plant parts were measured (shoot, leaf, stem, root). The leaves were scanned and the leaf area was calculated by photoshop software afterwards. The parts of the plants were placed into a forced air oven at 60 °C for 3 days to determine their dry weights (DW). In case of the experiment 2013 and 2014 leaves from different development stage were collected from 4 plants per grafting and treatment combination during harvest and the polyphenol content and antioxidant capacity (FRAP= Ferric Reducing Ability of Plasma) were measured. The polyphenol content were determined by UV-vis spectrophotometric procedure with Folin Ciocalteu reagent at $\lambda = 760$ nm (SINGLETON and ROSSI,1965). The antioxidant capacity were determined by the the method of BENZIE and STRAIN (1965) by spectrophotometry at $\lambda=593$ nm. Na⁺, Mg²⁺, K⁺, Ca²⁺ content of leaves and roots were determined from the dried homogeneous sample of 4 plants by ICP-OES (IRIS Thermo Jarrel ASH, Corp., Franklin, MA, USA) spectrometer. The Cl⁻ content of the leaves were determined by titration with silver nitrate based on the Mohr method.

3.7 Statistical analysis

All of the data (except Cl⁻, Na⁺, Mg²⁺, K⁺, Ca²⁺ content, and the change of transpiration) obtained from the measurements were evaluated statistically by IBM SPSS (SPSS Inc. 2004) software. To compare data multifactor ANOVA with Tukey post-hoc tests were used. In some cases when variances were unequal Games-Howell tests were used instead of Tukey. To indicate how or to

what extent variables are associated with each other correlation analysis were done. The statistical analysis were performed at 95% significance level.

4. Results and discussion

4.1 Biomass

The shoot and root weight of the salinity treated plants were dropped in all grafting combination comparing to control. Although the reduction was not alike, it was more manifested in case of ungrafted plants. Among the parts of the shoot the reduction of leaves weight were the most observable. Plants grafted on interspecific rootstock showed the best salinity tolerance among all graft combinations, since the lower salinity dosis scarcely reduced the leaves weight comparing to control (except in the open field experiment (68%)). Moreover, in the fitotron experiments the leaves weight of salinity treated interspecific grafted plants were increased in salt treatment II. compared to salt treatment I. (Figure 1.).

The roots DW of INT and L in experiment 2012 and roots of L in experiment 2014 were significantly bigger compared to NG and SG plants. Significant difference caused by the salt treatments could only be detected in case of NG in 2014, where the roots DW in II. salt treatments dropped significantly compared to control. These findings are in parallel with the study of COLLA et al. (2006) where the root weights of the watermelon grafted onto *Lagenaria* and interspecific rootstock were plural compared to non-grafted ones but did not decreased significantly by salinity. In our experiment even a slight increase in roots dry weight were observed in case of interspecific-grafted (2012, 2014) and *Lagenaria*-grafted (2014) plants in II. salt treatment compared to control, while non-grafted and self-grafted dropped. However the I. treatment did not affected the root dry weight of self-grafted plants but the root dry weight of non-grafted plants dropped gradually due to salinity.

Table 2: Signatures of the treatments on the graphs

Grafting combinations	Meaning	
	NG	non-grafted watermelon 'Esmeralda' variety
SG	self-grafted watermelon	
L	watermelon grafted onto <i>Lagenaria</i> rootstock	
INT	watermelon grafted onto Interspecific rootstock	
Salinity treatments	greenhouse and fitotron experiments	Soroksár experiment
0	control	control
I	2,85 mmol NaCl/l media/treatment	100 mmol NaCl/l nutrient solution
II	4,28 mmol NaCl/l media/treatment	150 mmol NaCl/l nutrient solution

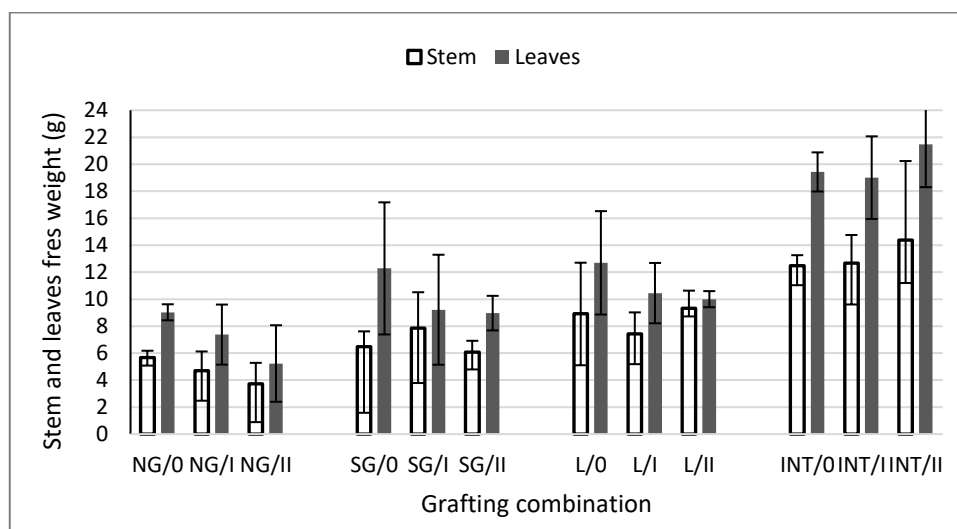


Figure 1.: The effect of salinity stress on stem and leaves fresh weight in the fitotron experiment (2012)

4.2 Leaf area

The changes in leaf area are parallel to the leaf fresh weight and dry weight, affected significantly by the grafting combination ($p < 0,001$) and also by the salinity stress ($p < 0,05$), although not in case of all grafting combination. Similar to the shoot weights, the interspecific grafted plants produced the highest results and the less reduction (in salinity treatments) in leaf area compared to the other grafted combination. In case of the fitotron experiments even a slight increase were observed due to salt treatment. Comparing the effect of salinity stress in the grafting combination, treatment II. caused significant decrease in the leaf area of self-grafted plants in 2012 and non-grafted plants in 2014. The leaf area of interspecific-grafted plants did not decreased similarly to the other grafting combinations nor yet increased compared to control under salinity stress (both treatment in 2012 and treatment I. in 2014). In contrast to our findings COLLA et al. (2006) observed the same decrease in leaf area by salinity stress in grafted watermelon onto interspecific and *Lagenaria* rootstock and non-grafted plants.

4.3 Stomata number

The salinity treatments did not caused significant difference in stomata number, only in case of non-grafted plant in case of Soroksár experiment, where the II. treatment significantly increased the stomata number of leaves. In the fitotron and greenhouse experiment the salinity treatment did not, but the grafting combination statistically affected the stomata number. The stomata number of interspecific-grafted plants were higher than the other grafting combination, on the other hand their reaction to the salinity treatment were different. The leaves stomata number of all the other

grafted combination increased parallel by salinity dosage. The interspecific-grafted plants' leaves stomata number dropped by the lower salinity dosage, then it was augmented a bit by the higher salinity dosis, but still remained lower than measured in the control treatments. This tendency was observed in case of the self-grafted plants as well. Regarding other studies the decrease in stomata number of leaves in response to salinity stress could indicate salinity tolerance (Alnayef, 2012., Kadam és Pravin 2010).

4.4 Transpiration

The salt treatments decreased the transpiration of the plants but there were differences in the level of decrease by grafting combinations (Figure 2.). The transpiration decrease of *Lagenaria* and interspecific-grafted plants were lower, than the non-grafted and self-grafted plants. The decrease were most obvious in the case of non-grafted plants. The transpiration of the self-grafted plants were still higher at the end of the experiments than the non-grafted ones, their transpiration level showed similarity to the *Lagenaria* and Interspecific-grafted plants in experiment 2014. It can be concluded that the grafting per se may enhance the stress tolerance of the plants. The transpiration of interspecific-grafted plants was stronger in the II. treatment compared to I. treatment for a longer period during both experiment. It could be a physiological response to militate the osmotic stress and maintain the water uptake.

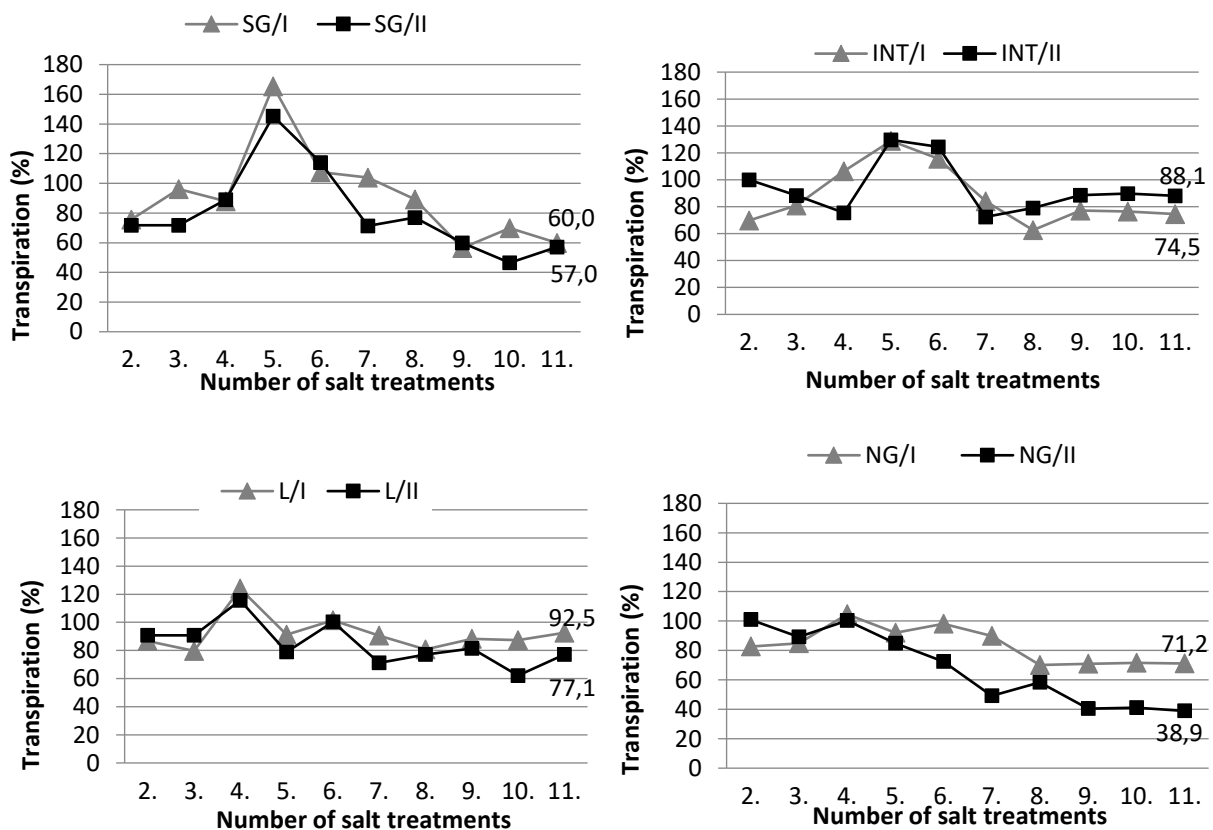


Figure 2: Effect of salt treatments on the transpiration of plants in the fitotron experiment (2012)

4.5 Photosynthesis activity

The photosynthesis activity of the plant did not decrease significantly on the 8th day from the first salt treatment in the greenhouse experiment but it did in case of the II. salinity treatment compared to control in the fitotron experiment in 2014. The photosynthesis activity of non-grafted plants showed the most obvious decrease compared to the other grafting combination in all experiment (Figure 3.). The salinity treatments significantly affected the photosynthesis activity of the plants 18 day (13 day- Soroksár experiment) after the initialization of the salinity treatment in all experiment. However the statistical analysis showed significant differences in all grafting combinations in the II. treatment compared to control, the photosynthesis activity of the interspecific-grafted plants were still twice bigger than all the other grafting combinations'. Our findings are parallel to the study by COLLA et al. (2012) where the salinity stress did not decreased the photosynthesis activity of cucumbers grafted onto interspecific rootstocks as much as the non-grafted ones.

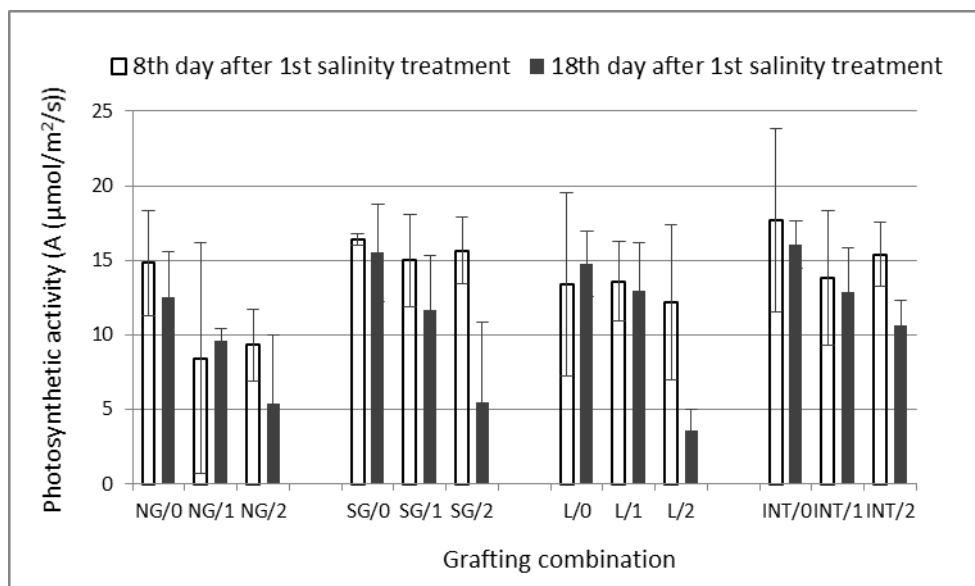


Figure 3: Effect of salt treatment on photosynthesis activity in the greenhouse experiment 8 and 18 day after the initialization of the salinity treatments

4.6 Water potential

The leaf water potential were significantly reduced by salinity. The biggest reduction were observed in the non-grafted and *Lagenaria*-grafted plants in the fitotron experiments (although statistical difference were observed in case of *Lagenaria*-grafted plants only). The salt treatment only slightly decreased the leaf water potential of interspecific-grafted plants in the fitotron experiment, but significantly decreased in the greenhouse experiment.

4.7 Antioxidant capacity of leaves

Concerning the FRAP of leaves significant difference in salt treatments, grafting combination and leaf level were observed. The FRAP was higher in developing leaves than in basal leaves. The II. salt treatment significantly increased the FRAP in every grafting combination except *Lagenaria*-grafted plants. The most conspicuous increase is shown in case of self-grafted plants. In case of *Lagenaria* and interspecific-grafted plants the II. salt treatment did not result in increased FRAP compared to the I. salt treatment.

4.8 Polyphenol content of leaves

The polyphenol content of leaves changed in relation to the FRAP. As the FRAP level increased due to the salt treatments the polyphenol content of the leaves increased as well. The polyphenol content of the developing leaves was significantly higher than the basal leaves (except in case of *Lagenaria*-grafted plants). The II. salt treatment caused significant increase in all grafting combinations except interspecific-grafted plants (both leaf level) and *Lagenaria*-grafted plants (basal leaves). The polyphenol content of the leaves of interspecific and *Lagenaria*-grafted plants increased in I. treatments compared to control but II. salt treatment did not cause further remarkable growth. Moreover in case of developing leaves of interspecific-grafted and basal leaves of *Lagenaria*-grafted plants the polyphenol content decreased in II. salt treatment compared to I. salt treatment. REZAZADEH et al. (2012) found similar tendency in case of artichoke, which is considered a salt tolerant plant. The lower salinity dose resulted in increased polyphenol content in leaves, but it decreased by higher salinity dose. On the contrary, the I. salt treatment caused decrease in polyphenols compared to control, but the II. salt treatment resulted in significant increase in case of non-grafted and self-grafted plants.

4.7 Cl⁻ content of the plants

The Cl⁻ content of the leaves and roots augmented in parallel with the salinity dosage. Comparing the graft combination the elevation was higher in the roots but lower in the leaves of the interspecific-grafted plants. It indicates that the interspecific rootstock has chlorid retention to some extent.

4.10 Na⁺ content of the plants

Based on our results it can be stated that the squash rootstocks have sodium retention, since the elevation of Na⁺ content in the leaves of the *Lagenaria* and interspecific-grafted plants were negligible comparing to self-grafted and non-grafted ones (Figure 4.). On the contrary the Na⁺

content of the roots were higher in the squash-grafted plants (Figure 5.). In addition grafting per se may have a slight Na⁺ retention effect itself because the Na⁺ content in the leaves of self-grafted plants were lower in the control and the lower salinity treatment compared to non-grafted plants. Na⁺ retention of interspecific rootstock was confirmed by other studies as well (ROMERO et al., 1997., ORSINI, 2013.)

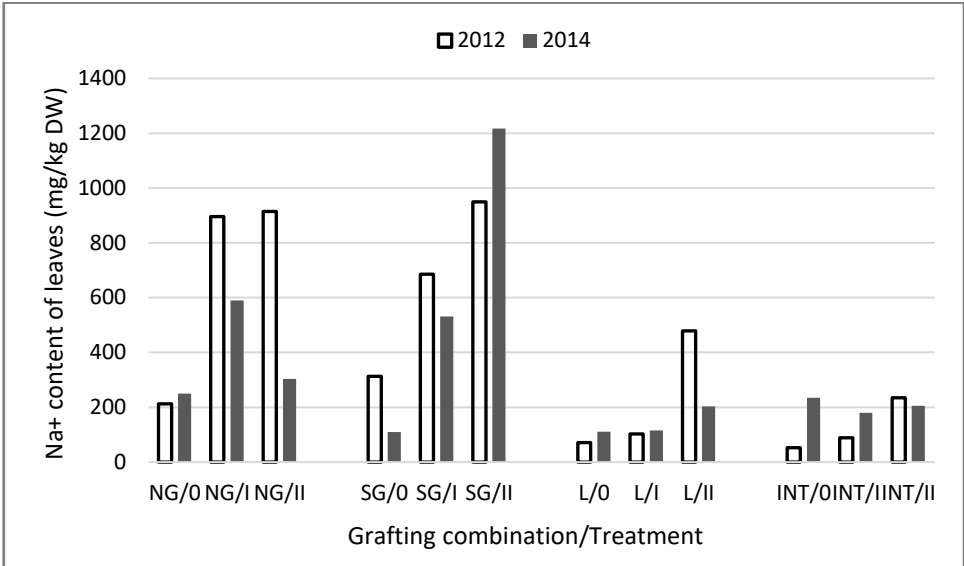


Figure 4: Na⁺ content of leaves in the fitotron experiments

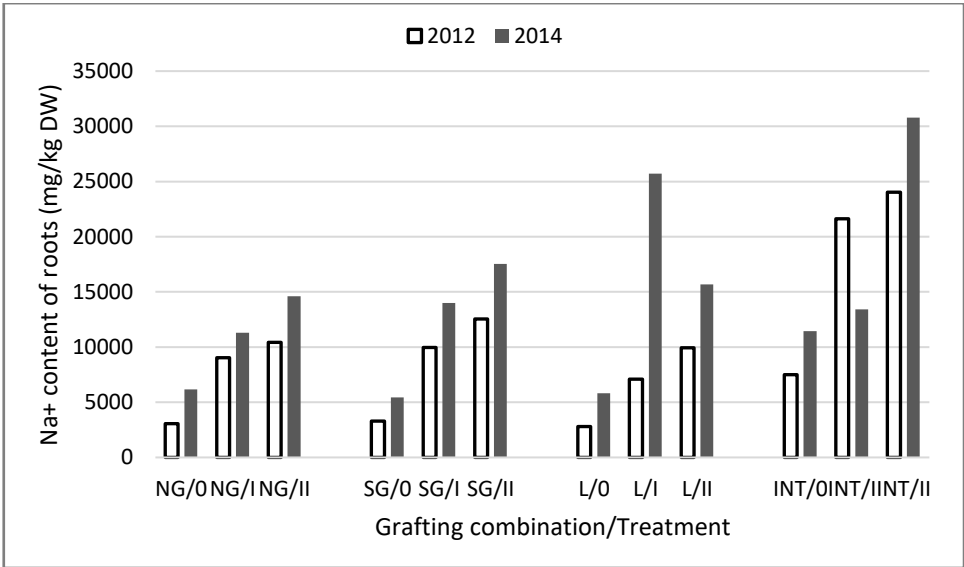


Figure 5: Na⁺ content of roots in the fitotron experiments

4.11 Mg, K és Ca content of the plants

The K^+ content dropped in the roots while it is increased slightly in the leaves parallel to the elevation of the NaCl dosage. Comparing the grafting combinations there were no difference in the K^+ content of the leaves, but the K^+ content were higher in the roots of *Lagenaria*-grafted plants.

The salinity treatments or the grafting combination did not cause significant difference in the Ca^{++} and Mg^{++} content of the plants.

4.12 The correlation of the measured parameters

The correlation analysis of the measured parameters revealed that the fresh weight and dry weight of the shoot, stem, leaves and root strongly correlate ($p < 0,01$). The water potential of the leaves changes parallel with the shoot and stem fresh weight but does not correlate to the root and leaves fresh weight and dry weight, although cohere to the leaf area. Furthermore it is in strong relation with the photosynthesis activity ($p < 0,01$). The photosynthesis activity of the plants on the 8th day of the salinity treatments correlates to the dry weight and fresh weight of all plant parts except the root, but the photosynthesis activity of the plants on the 18th correlates to the fresh weight of the plant parts only (except the root). The results of both photosynthesis activity measurements revealed coherence to the leaf area ($p < 0,05$) and transpiration ($p < 0,01$).

4.13 New scientific results

- 1) Based on my experiments it can be stated that grafting per se can enhance the salinity tolerance since the self-garfted watermelons accumulated less Na^+ to their leaves and retained more in their roots compared to non-grafted plants, furthermore only a slighter decrease were observable in the measured parameters compared to non-grafted plants.
- 2) The reduction of the stomata density of the interspecific-grafted watermelon leaves indicate a better salinity tolerance
- 3) Based on the results of the fitotron experiments salinity treatment slightly enhanced the growing of shoot and root in case of interspecific-grafted watermelon.
- 4) Based on the transpiration measurements of the fitotron experiments it was found that the transpiration of the interspecific grafted watermelon plants was enhanced by the salinity dosage, that is how the plants have maintained the water and nutrient uptake.
- 5) It was found that the root of interspecific rootstock has a Cl^- retention effect at some extent.

- 6) Kísérleti eredményeim alátámasztották, hogy nem csak az interspecifikus alanynak, hanem a *Lagenaria* alany gyökerének is van Na⁺ visszatartó tulajdonsága.
- 7) Our experiments confirmed that besides the root of interspecific rootstock the *Lagenaria* rootstock has Na⁺ retention as well.

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