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Effects of Grafting on Cucumber Growth under Flooding Stress during 15 Days in Vegetative Stage

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ABSTRACT

In this study, the effects of flooding stress on grafted cucumber were examined over time. A factorial experiment based on a complete randomized design was conducted with three plots including 9 plants in each plot. Water status treatments included optimum irrigation and flooding stress for 15 days and three cucumber treatments consisted of grafted cucumber (*Cucumis sativus* var. Isfahan) into *Cucurbita maxima*×*Cucurbita moschata* 'Ferro'= R1 and 'Cobalt'= R2; and Non-Grafted cucumber= NG was used as the control. Some physiological and growth parameters were measured 3 times in the vegetative stage to follow the effect of flooding stress when used on grafted cucumber. The results showed that K concentration decreased by flooding in the non-grafted, while R1 and R2 could efficiently uptake K in the flooding condition. Both decreasing photosynthesis and water potential of the plant were observed in non-grafted cucumber, reducing the transpiration and stomatal conductance, resulting in more water content in plants and increasing the fresh weight of grafted cucumbers. Grafted cucumber, i.e., R1 and R2 effectively maintained their growth under flooding stress, seemingly by using a different mechanism: R1 by increasing water potential for preserving water and R2 by improving photosynthesis, could successfully keep the growth of grafted-cucumbers under the flooding stress.

Keywords: *Cucumis sativus*, Phonological stage, Photosynthesis, Waterlogging, Water potential.

INTRODUCTION

Globally, production of plants face various biotic and abiotic stresses. One of the most critical stresses is water stress, including deficient or excess water (Schwarz *et al*., 2010). Twenty percent more water than the field capacity saturates the soil and is known as waterlogging or flooding (Aggarwal *et al*., 2006), which cause complete submergence of plants (anoxia and hypoxia) (Striker *et al*., 2005). Moreover, flooding is an ecological concern as it seems that the frequency and severity of flooding may increase in future, due to continuing climate changing scenario (Wright *et al*., 2017). Flooding could happen with heavy rainfalls or by excess irrigation of farmlands, exceeding the surface and subsurface drainage capabilities, especially on poorly drained soils (Stanley *et al*., 1980; Sullivan *et al*., 2001). Worldwide, waterlogging is frequently observed in nearly 10% of the lands under irrigation. The other form of waterlogging happens in a hydroponic culture that makes a lack of air with water saturating the substrate. This waterlogging, which is ignored, might be happening in the greenhouses of vegetables and flowers.

Damaging impacts of anoxia and hypoxia are cytoplasmic pH drop, the storage of reactive oxygen species, and toxic metabolites, which result in the low growth

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and poor yield (Subbaiah and Sachs, 2003). In flooding conditions, enzymes of the fermentation pathway are produced. These enzymes produce the alanine fermentation, ethanol, lactic acid sulfides, soluble Fe and Mn, ethanol, acetaldehyde, and acetic and formic acid (Fiedler *et al*., 2007).

Enhancing resistance of fruit vegetables to the various biotic and abiotic stresses is induced by the environment and grafting (Colla *et al*., 2010). For this purpose, sensitive plants may be grafted onto the tolerant ones as rootstock having vigorous root system without altering fruit quality. As per literature available, the resistance of the bitter melon (*Momordia charanthia* L. CV. New Known) to flooding improved through grafting it onto luffa (*Luffa cylinder Roem* Cv. Cylinder) (Liao and Lin, 1996). It was also observed that grafted plants have more growth, higher yield, and better physiologically adaptation to the stresses than the non-grafted and self-grafted plants. Use of grafting in cucumbers for greenhouse cultivation has utmost importance and is developing fast. Nevertheless, so far, few studies have been carried out regarding the effects of grafted cucumbers under flooding stresses. Therefore, testing grafting as a way to decrease the harmful effects of a flood is necessary. Therefore, regarding the lack of information on the physiological and growth changes of grafted and non-grafted cucumbers under flooding stresses, this research focused on the physiological and growth parameters of this plant after 5,10, and 15 days under flooding.

MATERIALS AND METHODS

The experiment was conducted in the greenhouse with an average temperature of 30-35 °C. This study was carried out as a factorial experiment based on a Complete Randomized Design (CRD) with three replications, including 9 plants in each plot. Treatments were water status included optimum irrigation at Field Capacity (FC) and flooding situation for 15 days; flooding was applied by waterlogging of the pot. The pot's surface was covered to prevent evaporation. Cucumber scion was (*Cucumis sativus)* accession Isfahan, which is available in Isfahan Province in Iran by grafting into pumpkin rootstocks 'Ferro' (R1) and 'Cobalt' (R2), and Non-Grafted (NG) cucumber was used as a control. *Cucumis sativus* accession Isfahan is an endemic cucumber that has a low yield but good quality and very desirable for consumers. On the other hand, 'Ferro' (R1) and 'Cobalt' (R2) are hybrids variety with high yield. Scion seed was sown 10 days before rootstock seed in cocopeat: perlite 1:1mixture. Scion plants were cut beneath the true leaves, and rootstock was cut above the first true leaves. Whole grafting was used, and grafted plants were transferred to a recovery greenhouse with high relative humidity. Seedlings were kept for 2 weeks in recovery condition, then, gradually adapted to the production greenhouse condition. Grafted plants were transferred to 5 L pots including soil and after a week, the water treatment was applied. Chemical fertilizer NPK (20, 20, 20) was used every 10 days using 0.005 mg L^{-1}. Plants were conducted to wire above the greenhouse. All the parameters were measured every 5 days, except antioxidant, phenol, and K concentration that were measured at the end of the experiment. During the study period, every 10 days, gas exchange parameters (photosynthesis rate, transpiration, stomata conductivity, and intercellular CO_2 of stomata) were determined for the youngest fully expanded leaf for 3 replications per treatment by a calibrated portable gas exchange system (LCi, ADC Bioscientific Ltd., UK) from 10:00 to 11:00 am on a clear day. Furthermore, the same leaves were used for chlorophyll index measurement using a chlorophyll meter (SPAD-502 plus, Minolta, Japan). Mesophyll conductance (mmol CO_2 m^{-2} s^{-1}) was calculated by dividing the photosynthetic rate by the sub-stomatal CO_2 concentration (Ahmadi and Siosemardeh, 2005). Total phenolic content was determined using the Folin-Ciocalteu. The results were expressed in gallic acid equivalents (mg 100 g^{-1} fresh weight) using gallic acid (0–0.1 mg mL^{-1}) standard curve (Singleton and Rossi, 1965).

Antioxidant was measured and expressed as gallic acid (equivalents l g gallic acid g^{-1}) with UV-VIS spectrophotometer (UV 160A-Shimadzu Corp., Kyoto, Japan). (Koleva *et al*., 2002). K concentration was determined by atomic absorption (Model PFP7-Japan) after digestion with HCl (Murillo-Amador *et al*., 2007).

Shoots were excised from the roots using a steel blade, then, root and shoot fresh weight was measured. Tissues were oven-dried to a constant weight at 70°C, then, the dry weight of each sample was measured (Boughalleb and Hajlaoui, 2011). Catalase activity was determined spectrophotometrically (UV 160A-Shimadzu Corp., Kyoto, Japan) by following the decrease of absorbance of H_2O_2 at 240 nm as described by Khoshbakht *et al*. (2018). Peroxidase (POD, EC 1.11.1.7) activity was measured using guaiacol as a substrate. The increase in absorbance at 470 nm due to the guaiacol oxidation was recorded for 3 minutes. One unit of Peroxidase (POD) activity indicates the amount of enzyme that catalyzes the oxidation of 1.0 μM of guaiacol in 1 minute (Khoshbakht *et al*., 2018). Chlorophyll fluorescence was measured in the dark and light-adapted leaves between 9:00–11:00 hour, with a portable fluorometer (OS- 30, USA). After 30 minutes of dark adaptation, Fv/Fm was calculated (Genty *et al*., 1989). Furthermore, the same leaves were measured for chlorophyll index with a chlorophyll meter (SPAD-502, Minolta Corp., Ramsey, NJ, USA). The leaves of the harvested seedlings were cut into discs of uniform sizes, weighted for Fresh Weight (FW) and immediately floated on distilled water at 25°C in the darkness. After 12 hours, the Turgid Weight (TW) was measured, and the discs were dried in an oven at 80°C for 48 hours. The Relative Water Content (RWC) was determined by the following Equation (1) (Dhopte and Manuel, 2002):

$$\text{LRWC (\%)} = (FW-DW)/(TW-DW)\times 100 \quad (1)$$

Total soluble protein content was measured according to Bradford (1976) using Bovine Serum Albumin (BSA) as a protein standard with Uv-vis spectrophotometer (UV 160A-Shimadzu Corp., Kyoto, Japan) at 595 nm. Data were analyzed with Statistix 8 (Tallahassee FL, USA), and significant differences between means were separated by the Least Significant Difference (LSD) test at P≤ 0.05.

RESULTS

Growth Parameters Changes in Flood during 15 Days

Shoot fresh weight slightly increased during the 15 days. Shoot fresh weight in flooding was less than the control in 5 days, but it increased in R1 and R2 after 10 and 15 days compared with NG. The increase in dry shoot weight was less than shoot fresh weight during 15 days. Shoot dry weight under flooding was less than the control in 5 days. Shoot dry weight under flooding was the same as the control in R1 and R2 after 10 days. Shoot dry weight was the same as the control in flooding after 15 days and increased in R2. It seems that R1 kept the fresh and dry weight in the same condition as the control, and R2 was more efficient and improved the fresh and dry weight even in flooding stress. Root fresh weight under flooding was higher or equal to the control during 15 days and it was higher in R2 after 10 days, but increased in R1 after 15 days. Root dry weight was the same or less than the control at flooding, and although it was higher in R2 until 10 days, the highest was observed in R1 after 15 days. It seems that flooding had more deleterious effect on the dry weight of root rather than the fresh weight of root during 15 days [Figure 1 (a-d)].

Chlorophyll and Photosynthesis Changes under Flooding

The photosynthesis rate was lower under flooding stress and increased in R1 and R2 after 10 and 15 days. R2 had a higher photosynthesis rate in 5, 10, and 15 days.

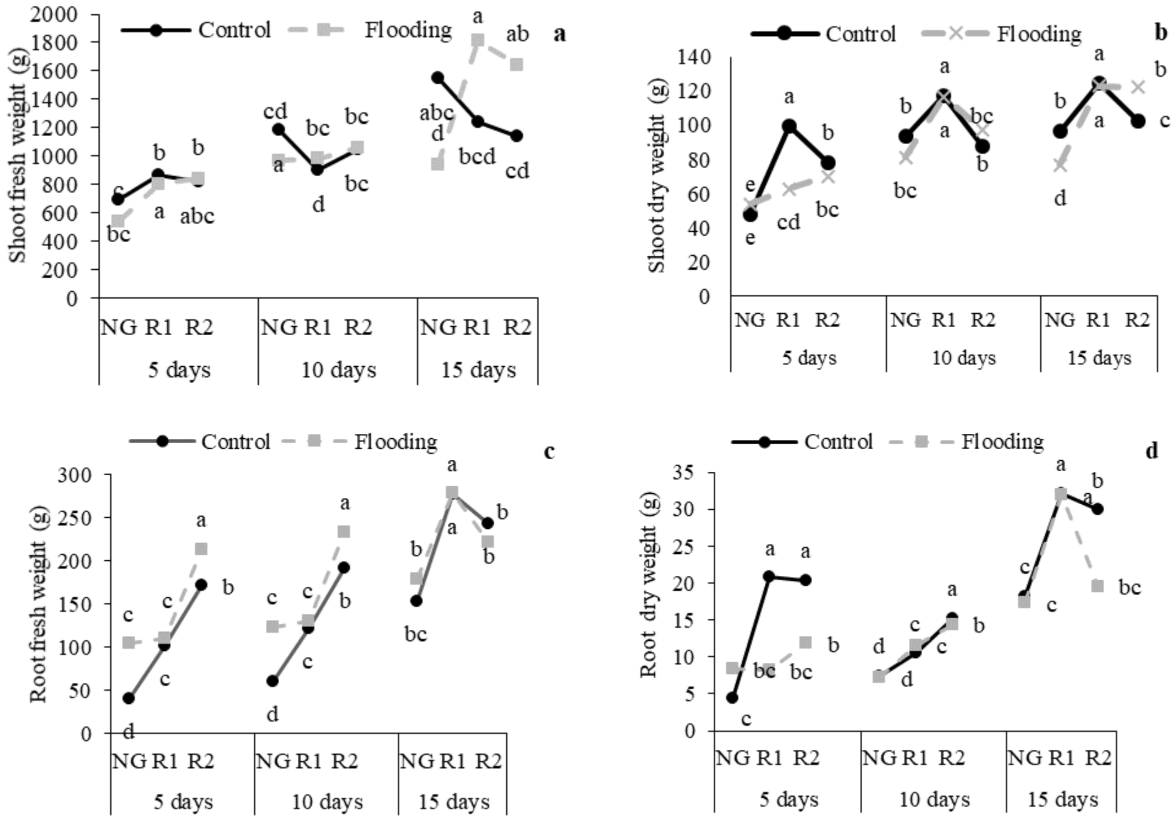

Figure 1. The effect of flooding and rootstock on shoot fresh weight (a), shoot dry weight (b), root fresh weight (c) and root dry weight (d) during 15 days (Non-Grafted cucumber= NG, Ferro= R1 and Cobalt= R2). NG, R1, and R2 under flooding stress were compared on days 5, 10 and 15 with LSD.

Chlorophyll index was lower in R2 after 5 days, and in R1 after 15 days flooding; with no significant changes in other treatments. The result of the chlorophyll index showed that the decrease of photosynthesis could not be related to chlorophyll changes. On the other hand, the CO_2 of internal stomata increased for R1 and R2 in 10 and 15 days. Mesophyll conductance increased with grafting in R1and R2 at 5, 10, and 15 days, in the control; it was increased by flooding at 5 days too. Mesophyll conductance decreased by grafting in flooding stress at 10 and 15 days. Transpiration increased during the time in all plants and at each measurement time. Transpiration decreased in R1 and R2 compared with NG. Stomatal conductance decreased during 15 days; it was higher in R1 and R2 in 5 and 10 days, but decreased in 15 days compared with NG [Figure 2 (a-f)].

Antioxidant, Potassium Concentration, Phenol Changes and Shoot Water Potential and Enzymes under Flooding

Catalase did not change after 5 and 10 days. Catalase increased in the control in R1 and R2. Peroxidase decreased in grafted cucumber and increased in flooding treatment; total protein was highest for R1 in flooding. Protein decreased in R1 and R2 in the control treatment at 5, 10 and 15 days (Figure 3).

Shoot water content was highest in R1 on days 5, 10, and 15. Shoot water content was lower in the control compared with flooding over 15 days, but it was not significantly different between rootstocks. Shoot water potential in the control was higher than flooding on days 5 and 10 and was the same

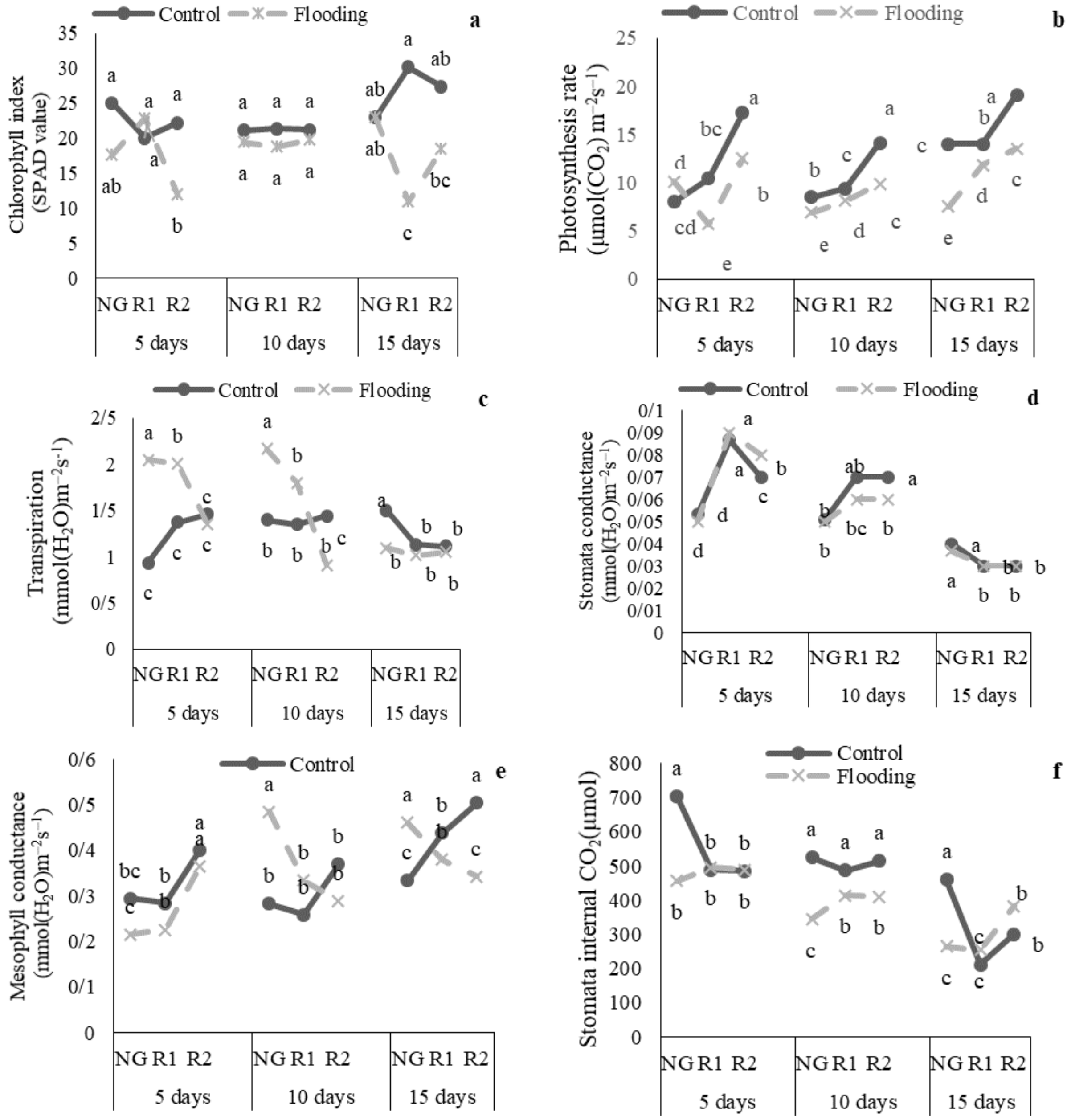

Figure 2. The effect of flooding and rootstock on Chlorophyll index(a), Photosynthesis rate (b), Transpiration (c), Stomatal conductance (d), Mesophyll conductance (e), Stomata internal CO_2 (f) during 15 days (Non-Grafted cucumber= NG, Ferro= R1 and Cobalt= R2). NG, R1, and R2 in flooding stress were compared on days 5, 10, and 15 with LSD.

in 15 days. It was higher in R1 after 10 and 15 days, and it was higher in R1 and R2 after 5 days (Figure 4).

Phenol content increased in R2 and was higher in flooding in R1 and NG; conversely, antioxidant activity decreased in R2 and there were no significant difference between flooding and the control treatments. K concentration decreased in NG and R1 by flooding (Figure 5).

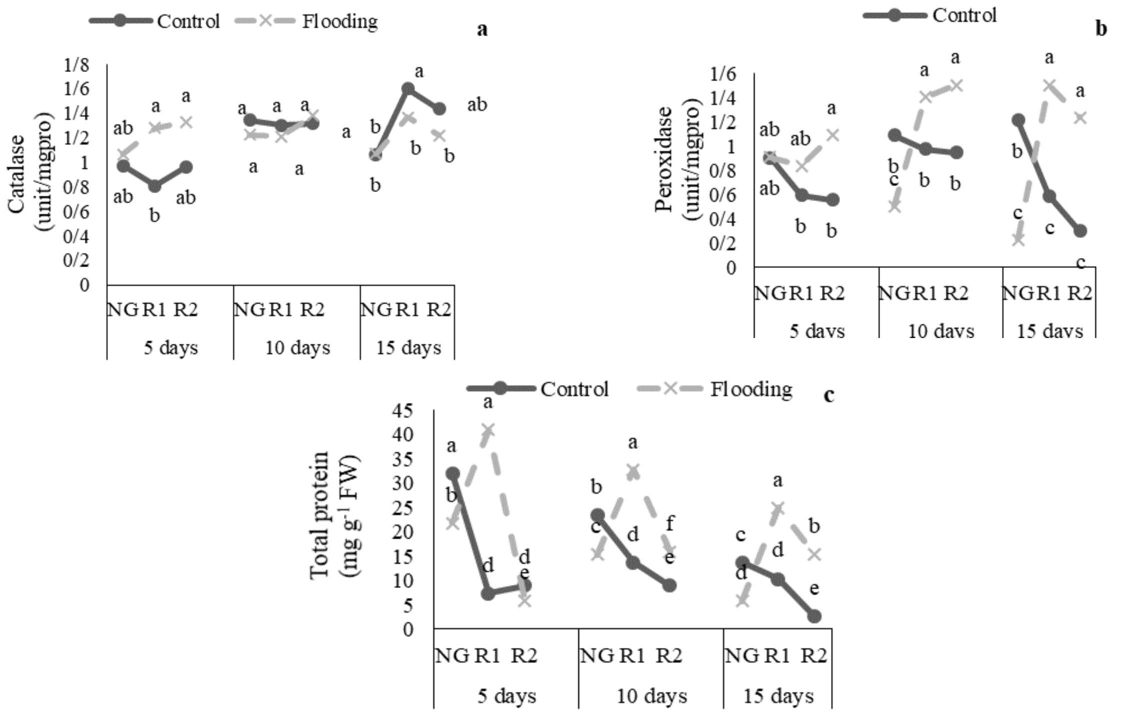

Figure 3. The effect of flooding and rootstock on catalase (a), peroxidase (b), total protein (c) during 15 days (Non-Grafted cucumber= NG, Ferro= R1 and Cobalt= R2). NG, R1, and R2 in flooding stress were compared on days 5, 10 and 15 with LSD.

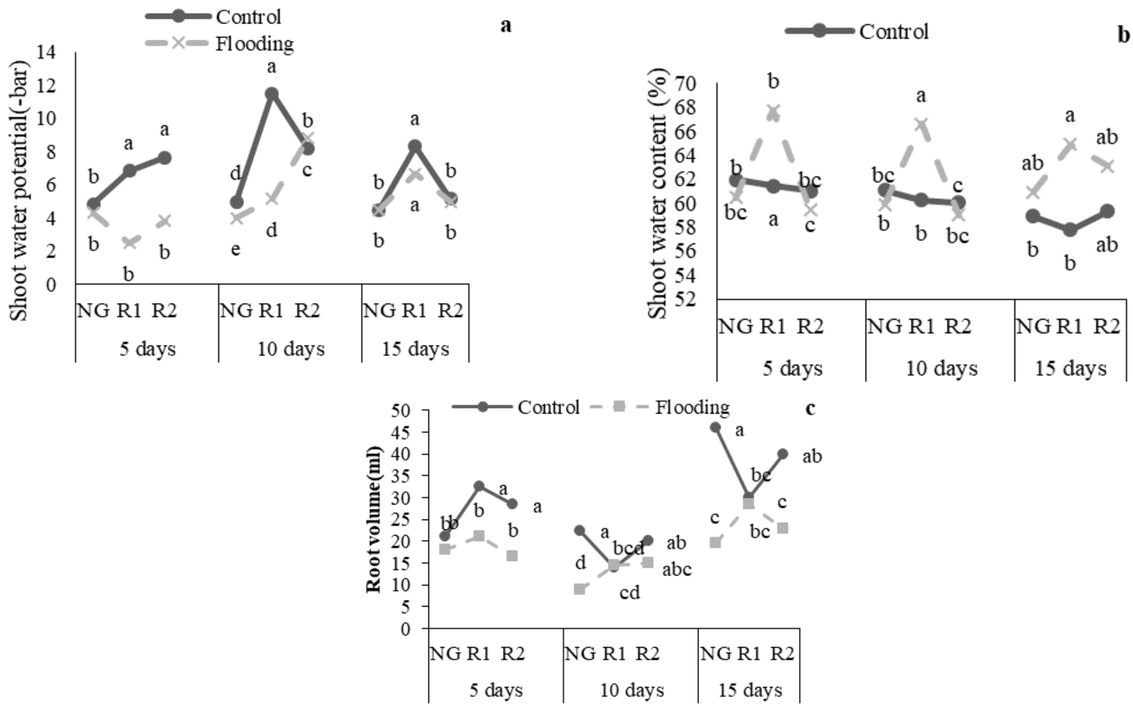

Figure 4. The effect of flooding and rootstock on shoot water potential (a) and shoot water content (b), Root volume (c) during 15 days (Non-Grafted cucumber= NG, Ferro= R1 and Cobalt= R2). NG, R1, and R2 in flooding stress was compared in each 5, 10 and 15 days with LSD.

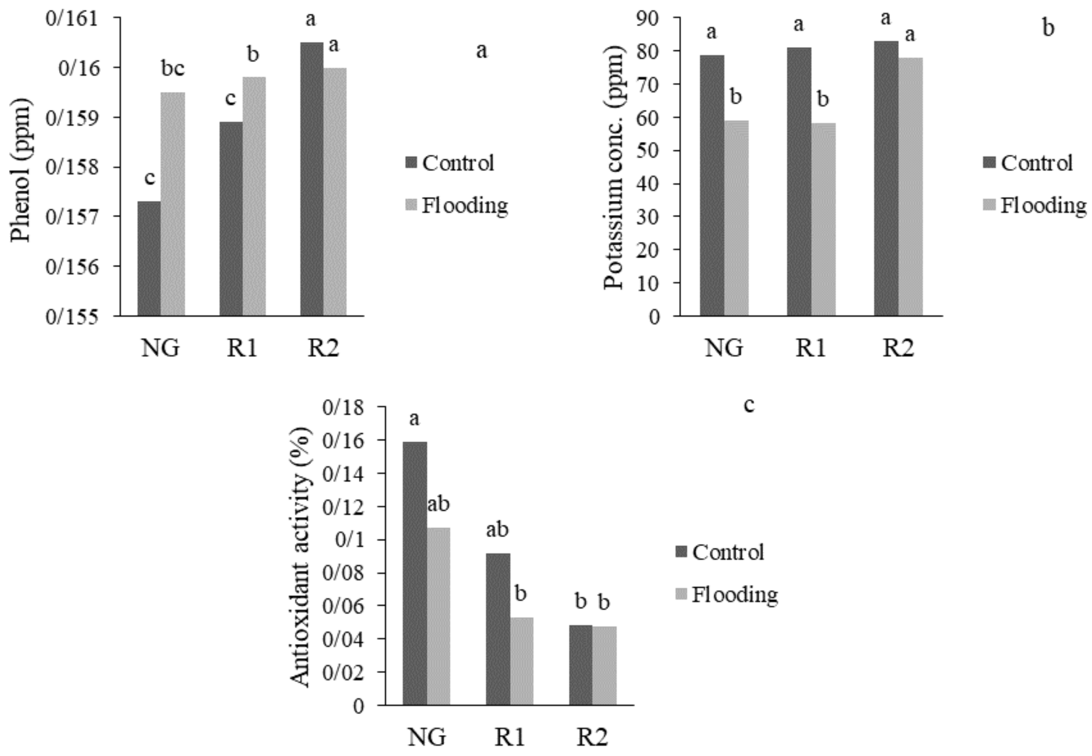

Figure 5. The effect of flooding and rootstock on phenol (a), K concentration (b) and antioxidant activity(c) at 15th day (Non-Grafted cucumber= NG, Ferro= R1 and Cobalt= R2).

DISCUSSION

Stomatal closure negatively affects water relations, mineral nutrient uptake and transport, carbohydrate and hormone relationships, photosynthesis, and respiration, consequently, yield is reduced (Bacanamwo and Purcell, 1999; Barrick and Noble, 1993). In contact with these results, it seems that, compared with non-grafted cucumber, the grafted cucumber decreased transpiration to minimize the harmful effects of flooding stress. This protection was more significant after 10 days of flooding by reducing stomatal conductance. On the other hand, mesophyll conductance was higher in grafted-cucumbers under flooding stress after 5 and 10 days, but decreased after 15 days. Conclusively, according to our results and those suggested in the literature, decreasing photosynthesis under the flooding conditions in cucumbers can result for the following reasons:

1) Increasing ABA in the root and its transfer to leaves, which can close stomata (Pessarakli, 2010);

2) Decreasing the hydraulic conductance of the root, which results in decreasing water potential which is seen in non-grafted cucumbers and stomata closing (Pessarakli, 2010);

3) A decrease in the Rubisco activity, which can occur under flooding stresses and results in decreasing the photosynthesis rate, which is also probable in cucumbers, but was not tested here (Pessarakli, 2010);

4) Flooding decreases leaf area, thereby decreasing the photosynthesis rate, dry weight, and yield in clover, it may happen in cucumber (Neocleous, 2015; Pessarakli, 2010);

5) It seems that K uptake decreases by decreasing water absorption potential of the shoot, so, K concentration decreases in

gourd cells of stomata and stomata closure occurs, which is seen in non-grafted cucumber.

It can be concluded that, under flooding stress, transpiration and shoot water potential decreased, especially after 5 days, but also after 15 days, as the stomata closure and transpiration reduction was more effective, the water potential reduction was less than its decrease after 5 and 10 days in R1 and R2. Water potential decrease was 70.83% and 70.37% in R1 and R2 after 5 days, 55.06% and 18.36% after 10 days, and 6% and 3.23% after 15 days.

Previous works showed that chlorophyll fluorescence decrease with flooding results in damage of PSII and photosynthesis reduction. On the other hand, the damaged light reaction system was evident. This may have led to a loss in chemical energy provided by light reaction systems, which are thought to be used for the production of active oxygen species under stressful condition, in which the chemical energy is not used for the CO_2 fixation. Thus, when waterlogging was prolonged, production of active oxygen species was retarded, resulting in the reduction of activities of protective enzymes (Zhao *et al*., 2014; Guidi and Soldatini, 1997). The CO_2 reduction in stomata was less than transpiration, so, it can be concluded that grafted plants can manage CO_2 loss by decreasing the stomata conductance, compared with H_2O loss; indeed, it can help plants keep more efficient photosynthesis via keeping water potential and hydraulic conductance in plants. R2 was apparently the most capable till 10 days, but when flooding was continued for 15 days, its ability was the same as that of R1, although more efficient than non-grafted plants. On the other hand, catalase and peroxidase activity in R2 was more effective even after 15 days of flooding and kept the cucumber capability more efficient.

Linkermer *et al*. (1998) showed that plants gave different responses to flooding in different growth stages and were more sensitive in the first stage of growth. Flooding decreased the yield by 44% in wheat as a result of photosynthesis reduction and lower level of water potential in plants, which both were observed in non-grafted cucumbers in this study. It seems that decreasing the transpiration and stomatal conductance can make the plants keep more water and thus increase the fresh weight of the grafted plants, but the dry weight is more affected by flooding stresses. Excess water in the soil affects plant roots directly and the shoots indirectly (Henshwa *et al*., 2007).

Shoot potassium content declines substantially (e.g., several-fold) in plants exposed to prolonged waterlogging (Board, 2008). Reports for roots are more controversial, ranging from significant decline to no change or even increase in root K^+ content (Smethurst *et al*., 2005). The most likely explanation for the latter controversy comes from the complexity of root to shoot nutrient translocation in flooded plants, and its strong dependence on the duration of the treatment and species specificity.

Noteably, Henshwa *et al*. (2007a) observed that flooding for up to 4 weeks did not result in plant death. There have been few studies on the effect of the length of time of flooding on cucumbers. Our results showed that flooding lasting for 2 weeks did not affect the growth of grafted cucumbers.

There are different reports about antioxidant changes under flooding condition. Pessarakli (2010) reported a reduction in the activities of antioxidant enzymes, including APX, SOD, GR, and CAT in mung beans. The activities of CAT, SOD, and GR were unaffected by waterlogging in tomatoes (Keatinge *et al*., 2014). In cucumbers, antioxidant activity did not change by flooding and was comparatively lower in grafted cucumbers than non-grafted ones. However, the antioxidant enzymes like peroxidase efficiently increased by flooding in R1 and R2. According to Rivero *et al*. (2001), heat stresses lead to the production and accumulation of phenolic compounds in the watermelon, and prevents oxidation. Phenolic accumulation might be due to a reduction in the rate of oxidation reaction, or the production of Glutamate stimulated during the process or an increase in the protease enzyme activity. In the present experiment, phenolic compounds played a more significant part in rootstocks than the antioxidant under flooding stresses because they increased by flooding more than the antioxidants in the grafted cucumbers.

CONCLUSIONS

In grafted cucumbers, rootstocks continued water absorption to maintain the water status of cucumbers in good conditions by keeping the root production under the flooding conditions. Thus, because of the prolonged better photosynthetic performance and more nutrient absorption, the water status and photosynthesis was kept well and, consequently, the growth was not greatly affected. R1 seems more capable of producing catalase and peroxidase to help cucumber continue ROS scavenging. Seemingly, cucumber grafted to ‘Cobalt’ (R2) can endure flooding after 10 days, and less after 15 days.

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اثر پیوند بر رشد خیار تحت تنش غرقابی به مدت 15 روز در مرحله رویشی

م. حقیقی، و س. خسروی

چکیده

در این پژوهش، اثر تنش غرقابی بر خیار پیوندی با گذشت زمان مورد بررسی قرار گرفت. یک آزمایش فاکتوریل در قالب طرح کاملا تصادفی با سه پلات، شامل نه گیاه در هر پلات طراحی شد. تیمار وضعیت آبیاری شامل آبیاری مطلوب و تنش غرقابی به مدت 15 روز و خیار (*Cucumis sativus* var. Isfahan) پیوند شده بر *Cucurbita maxima* × *Cucurbita moschata* رقم فرو (R1) و کبالت (R2) و خیار غیرپیوندی (NG) که به عنوان تیمار شاهد استفاده شد. برخی از پارامترهای فیزیولوژیکی و رشدی در سه زمان از مرحله رویشی اندازه‌گیری شد تا اثر تنش غرقابی بر خیار پیوندی مشخص شود. نتایج نشان داد که غلظت پتاسیم با تنش غرقابی در خیارغیرپیوندی کاهش می‌یابد در حالیکه R1 و R2 در شرایط تنش غرقابی موجب جذب پتاسیم می‌گردد. کاهش فتوسنتز و پتانسیل آب گیاه در خیار غیرپیوندی مشاهده شد، همچنین کاهش تعرق و هدایت روزنه‌ای منجر به افزایش میزان آب در گیاهان و افزایش وزن تر خیارهای پیوندی گردید. خیار پیوندی یعنی R1 و R2 به طور موثری رشد خودشان را تحت تنش غرقابی حفظ کردند و به نظر می‌رسد که هر یک مکانیسم متفاوتی داشتند، R1 با افزایش پتانسیل آب حفظ آب و R2 با بهبود صفات فتوسنتزی قادر به حفظ رشد مطلوب خیارهای پیوندی در شرایط تنش غرقابی شدند.