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Response of Different *Citrus* Genotypes to Continuous Flooding Conditions

Reza Partiya[1*], Reza Fotouhi Ghazvini[2], Reza Fifaei[3] and Mahmood Ghasemnezhad[2]

1. Former master student of horticulture, Department of Horticultural Science, University of Guilan, Rasht, Iran

2. Department of Horticultural Science, Faculty of Agricultural Science, University of Guilan, Rasht, Iran

3. Faculty of Citrus and Subtropical Fruits Research Center, Ramsar, Iran

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Abstract

Hypoxia is a potential threat to various horticultural crops in lands prone to flooding. *Citrus* is mostly known as a sub-tropical crop that is often exposed to environmental stresses. In order to evaluate response of six different *citrus* genotypes, including sour orange, rough lemon, Trifoliate orange, Troyer citrange and two local genotypes labeled; CRC1 and CRC2 to flooding conditions, an experiment was carried out in factorial experiment based on a completely randomized design with two treatments including flooded and control plants and three replications. Flooding stress significantly decreased leaf chlorophyll content, and plant total fresh and dry weights ($P\leq 0.05$). Flooding caused a significant increase in foliar concentration of proline in CRC1 and CRC2 ($P\leq 0.05$). Guaiacol peroxidase activity was significantly increased in Trifoliate orange. CRC2 and sour orange showed a significant increase in superoxide dismutase activity ($P\leq 0.05$). The longest survival period in continuous flooding condition was observed in Troyer citrange and Trifoliate orange (more than 60 days); while sour orange was the most sensitive genotype (less than 30 days). The best thriving genotype at the end of recovery period was Troyer citrange, while sour orange showed the least ability to re-establish. The results suggest that among the studied genotypes, Troyer citrange and Trifoliate orange are able to resist for longer periods of flooding exposure. Troyer citrange had the highest capacity to re-establish after being flooded to their critical surviving point. Furthermore, CRC2 tolerated anoxic condition and recovered more successfully than the other sensitive genotypes.

Keywords: anoxia, biochemical traits, guaiacol peroxidase, superoxide dismutase, water-logging

Introduction

Soil flooding is one of the major abiotic stresses that can affect growth, development and survival of wild and horticultural plants. As predicted by climate change models, more and more flooding incidents are expected to happen globally. Waterlogging mainly causes hypoxic conditions around the root system of plants, affecting a variety of factors- e.g. water and nutrient uptake, carbohydrate mobilization, reactive oxygen species (ROS) metabolism, production of superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$) and the peroxidation of membrane lipids (Wu et al., 2013; Pucciariello et al., 2014; Yang et al., 2015). In addition to the detrimental effects of flooding stress on photosynthetic system

* Corresponding author, Email: reza.partiya@gmail.com

and the resulting over-production of ROS, restoring the plant from the pressure exerted by the stress may even reinforce the oxidative damage (Hossain et al., 2009).

With different degree of sensitivity, many species are sensitive to waterlogging. Some species have developed special mechanisms and adaptations in order to cope with anaerobic conditions of soil. These mechanisms include some physiological, biochemical and molecular responses, all of which contributes to some changes in anatomical and morphological characteristics of plants (Martínez-Alcántara et al., 2012). The responses of plants facing such hypoxic conditions are different and controlled by many factors, including genetic specifications, age of the plant, characteristics of flooding water and time and duration of exposed to waterlogging conditions. Growth of many species that are tolerant to hypoxic conditions are slowed down when their root systems sense the anaerobic conditions of the soil, which produce signals which affect the stomatal conductance. Contrary to sensitive species, tolerant species resume growth after the soil restored to normal aerobic conditions (Nicolás et al., 2004).

Citrus genotypes have no specific adaptations in response to flooding stress, but there are some differences in the level of tolerance to hypoxic conditions. In an experiment, among the three studied genotypes, Carrizo citrange showed the most tolerance while Cleopatra mandarin showed the most sensitivity and Citrumelo exhibited an intermediate tolerance toward flooding. Considering the ability of the plants to recover after prolonged periods of flooding, Carrizo citrange was able to fully recover compared to other studied genotypes upon long-term exposure to detrimental flooding conditions (Arbona et al., 2007). In another experiment on the responses of two *Citrus* genotypes to flooding and drought stresses, proline concentration in root was elevated as a result of exposure to both stresses. Based on water relations and gas exchange traits, Cleopatra mandarin was more tolerant to short-term (9 days) flooding stress than Carrizo citrange (García-Sánchez et al., 2007).

Therefore, this study was conducted in order to evaluate the biochemical and morphological responses of six *Citrus* genotypes, including two Iranian local genotypes, and also their ability to re-establish after long-term exposure to soil hypoxic conditions, to introduce more tolerant rootstocks for regions prone to waterlogging and soil flooding conditions.

Materials and Methods

Plant material and growth conditions

The study was conducted at the Citrus and Subtropical Fruits Research Center, Ramsar, Iran, 2016. The plant material included one year old seedlings of six different *Citrus* genotypes, including *C. aurantium* L. 'sour orange', *C. jambhiri* Lush. 'rough lemon', *Poncirus trifoliata* Raf. 'Trifoliate orange' and *C. sinensis* (L.) Osb. × *P. trifoliata* (L.) Raft. 'Troyer citrange' and two local genotypes labeled CRC1 and CRC 2. The growing condition of the greenhouse was 26/21^{o}C day/night and the relative humidity was kept between 60-70%. The seedlings were transplanted into 2.5 l pots filled with a mixture of equal perlite and sand, 1:1 (v/v) and were irrigated two times a week with 500 ml of tap water and supplemented once every two weeks with 500 ml of a nutrient solution (Table 1). The plants were trained as a single shoot and all the lateral shoots were trimmed away weekly.

Table 1. The concentration of nutrient elements (mM/500 ml) in tap water

Nutrient element	NO_3	NH_3	P_2O_5	K_2O	Fe	Mn	Zn	B	Cu	Mo
Concentration (Molar)	1.5	4.5	1.25	1.9	0.025	0.014	0.007	0.04	0.003	0.0008

Treatments

After the time needed for acclimation to the new condition, the plants were flooded continuously in four-liter pots so that the seedling pots were completely submerged and the water level was 2 cm above the potting media. The pots were covered by sheets of aluminum foil to prevent algal bloom. Meanwhile the control group was regularly watered. The flooded condition continued until half of the flooded plants showed explicit leaf damage including vein yellowing, wilting and curling. Reaching this stage of damage, intermediate leaves of each plant were collected and immediately frozen in liquid nitrogen, and ground using a mortar and pestle then kept at -80°C for further analysis (Arbona et al., 2008; Hossain et al., 2009; Rodríguez-Gamir et al., 2010). Three flooded plants of each genotype were taken to the recovery state in order to study the post-stress morphologic responses of the plants and their ability to survive and re-establish their root system.

Plant fresh and dry weight

To determine the impact of flooding stress on the physical characteristics of the genotypes, the plants were carefully removed from the pots and dissected into roots, shoots and leaves. The roots were fully cleaned out. The exact weight of each part was recorded by a digital balance (0.001g) and then they were put into paper packets and dried in an oven at 70°C for 48 hours. At the end the dry weight was obtained.

Chlorophyll content

The total chlorophyll content of the leaf tissue was determined by the use of 80% ice-cold acetone as described by Arbona et al. (2008). The absorbance value at 663.2 and 646.2 nm was read and used in formulas mentioned by Lichtenthaler and Buschmann (2001) for calculation of total chlorophyll content (Formulas 1-3).

$$C_a(\mu g/ml)= 12.25A_{663.2}-2.79A_{646.2} \quad (1)$$

$$C_b(\mu g/ml)= 21.5A_{646.2}-5.10A_{663.2} \quad (2)$$

$$C_{(x+c)}(\mu g/ml)= (1000A_{470}-1.82c_s-85.02c_b)/198 \quad (3)$$

Protein and enzyme extraction

Enzymes and proteins were extracted with cold enzyme extraction buffer composed of 0.2% polyvinyl polypyrrolidone (PVP), 0.1 mM EDTA, and 50 mM potassium phosphate (pH 7.8). For this purpose one milliliter of the above mentioned buffer was added to 0.2 g of frozen leaf tissue and centrifuged at 14000 rpm for 15 min at 4°C. The resulting supernatant was then pipetted into test tubes and stored in ice (Boyer, 2012; Slabbert et al., 2014).

Guaiacol peroxidase

The activity of peroxidases was determined by a modification of Chance and Maehly (1955) method. The reagents included 45 mM guaiacol and 225 mM hydrogen peroxide. The assay was carried out at room temperature and the changes in absorbance at 470 nm were recorded at 10 second intervals for a minute using a PG Instruments T80+ spectrophotometer. The maximum slope of the resulting curve was used to determine the enzyme activity expressed as the increase in absorbance min^{-1} per gram fresh weight of the leaves (Ballester et al., 2006). The extinction coefficient of tetraguaiacol is 26.6 cm^{-1} mM^{-1} and was used to determine the concentration of the enzyme present in the substrate.

Superoxide dismutase activity (SOD)

To determine the presence and activity of superoxide dismutase, its ability to inhibit the photoreduction of nitro blue tetrazolium chloride (NBT) was assayed. The reaction mixture was composed of 1.3 µM riboflavin, 13 mM methionine, 63 µM NBT, 0.1 mM EDTA and the appropriate amount of enzyme extract. The mixtures with a control tube, lacking the enzyme extract, were evenly illuminated in uniform

glass test tubes for 15 minutes. Also at the same time an identical tube with above mentioned solution kept in a dark shelf as blank. One unit of the enzyme was defined as the amount of SOD that inhibited 50% of NBT photoreduction and determined at 560 nm. The activity of superoxide dismutase was then determined with the following formula (4) (Giannopolitis and Ries, 1977; Wu et al., 2013).

$$\textit{The activity of SOD}\ (U/mg) = \frac{100 - \left[\frac{100 - (\text{OD control} - \text{OD sampel})}{\text{OD Control}} \times 100\right]}{50} \quad (4)$$

Soluble proteins

The method developed by Bradford (1976) was employed to quantify soluble proteins present in the leaf tissue extract. Protein reagent composed of Coomassie Brilliant Blue G250 (100 mg) dissolved in 50 ml 95% ethanol, 100 ml 85% phosphoric acid diluted to a final volume of one liter. The assay mixture contained 0.1 ml tissue extract added to 5 ml of the reagent. After mixing the solution by inversion, the absorbance of each sample was determined at 595 nm. The standard curve was obtained by different concentrations of bovine serum albumin (0, 200, 400, 600, 800, and 1000 mg). The concentration of protein in each absorption point was determined by the following formula (5).

Y (absorption) =0.0009X (protein concentration) +0.0651 (R^2=0.98) (5)

Proline content

As mentioned by Bates et al. (1973), to determine the free proline, one ml of 3% aqueous sulfosalicylic acid was added to 0.2 g of leaf tissue in a test tube. The test tube was then centrifuged at 14000 rpm for 5 min. The acid-ninhydrin reagent was prepared by warming 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid. One ml of the supernatant of the centrifuged extract, one ml of the acid-ninhydrin and one ml of glacial acetic acid was reacted in a test tube for one hour at 100°C and the reaction was terminated in ice-cold water. Two ml of toluene was added to the test tube and stirred for 15-20 seconds. The absorbance of the upper phase, containing toluene and the chromophore was then read at 520 nm using toluene as blank. The presence of proline was then calculated with a standard curve by the following equation (6).

Y (absorption) = 0.0086X (proline concentration) + 0.0128 (R^2=0.99) (6)

Recovery and survival

To investigate the survival capability of the studied *Citrus* genotypes and their ability to regenerate root system after the detrimental effects of continuous waterlogging condition, three flooded plants of each genotype were drained and restored to normal condition. After 30 days from the start of recovery period the plants were taken out of the pots and their root system were photographed and studied.

Statistical analysis

The experiment was based on a completely randomized, factorial design including two watering treatments; flooding and control groups, and six genotypes with three replications. Data were statistically analyzed by two-way ANOVA at a significance level of $p \leq 0.05$, and the means were compared by the Tukey's multiple range test ($p \leq 0.05$).

Results

The flooding period (based on showing visual sign of explicit leaf damage to half of the plants) was 26 days for Sour orange; 28-30 days for rough lemon, CRC1 and CRC2; and more than 60 days for Trifoliate orange and Troyer citrange. The results indicated that interactions between flooding stress and genotypes on plant fresh and dry weight, total chlorophyll content, proline, total protein level, activity of peroxidases and superoxide dismutase were significant ($P \leq 0.05$).

Fresh and dry weights

The continuous long-term flooding reduced the total fresh weight and consequently the total dry weight of genotypes. Figure 1 shows that the total fresh weight of CRC1, CRC2, Rough lemon and Sour orange significantly decreased while this decrease was not significant for Trifoliate orange and Troyer citrange. CRC1, CRC2, and Sour orange experienced a significant decrease in dry weight while the decrease in dry weight for Trifoliate orange, Troyer citrange and Rough lemon was not significant (Fig. 2).

Chlorophyll content

Trifoliate orange, Troyer citrange, Sour orange and CRC1 genotypes, showed a significant decrease in leaf tissue chlorophyll content in waterlogged condition compared to control ones, but the decrease in CRC2 and Rough lemon was not significant (Fig. 3).

Proline content

The proline content of the leaves of CRC1, CRC2, was significantly higher in flooded plants. Whereas in the other genotypes, Trifoliate orange and Troyer citrange, the level of proline was not significantly different between the two groups (Fig. 4).

Peroxidase activity

Flooded plants of Trifoliate orange showed an increase, while CRC1 showed a decrease for peroxidases activity. In other genotypes peroxidases activity was not significantly different between control and flooded plants (Fig. 5).

Superoxide dismutase

In response to flooding condition, superoxide dismutase activity was significantly increased in leaves of CRC2 and Sour orange but the increase in the activity of this enzyme was not significant in the other genotypes (Fig. 6).

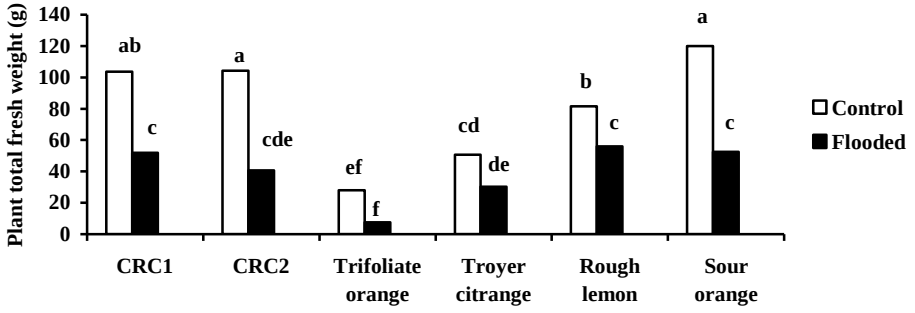

Fig. 1. Total fresh weight in control and flooded plants. Means with at least one similar letter are not significantly different (P≤ 0.05) based on Tukey test

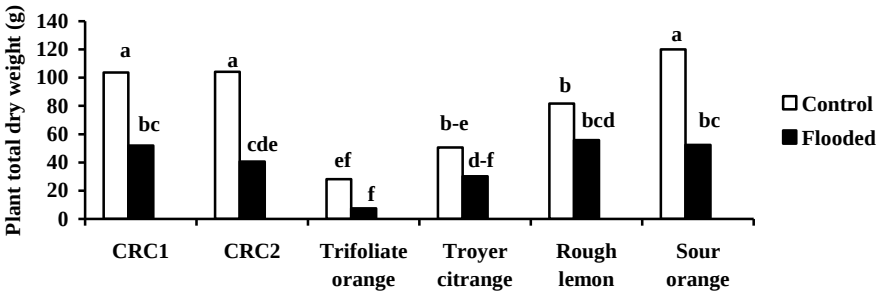

Fig. 2. Total dry weight in control and flooded plants. Means with at least one similar letter are not significantly different (P≤ 0.05) based on Tukey test

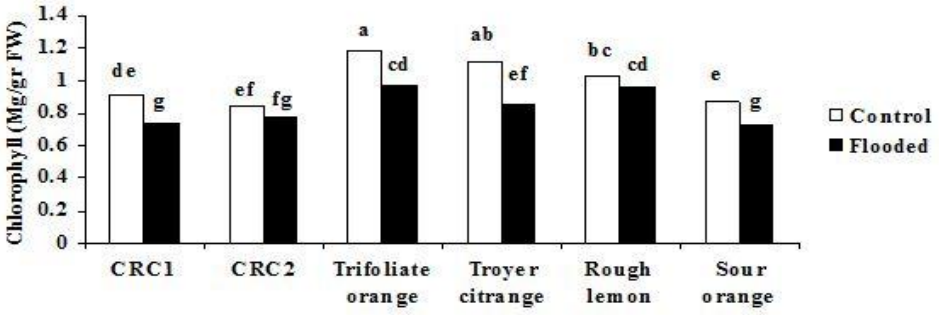

Fig. 3. Leaf total chlorophyll content in control and flooded plants. Means with at least one similar letter are not significantly different (P≤ 0.05) based on Tukey test

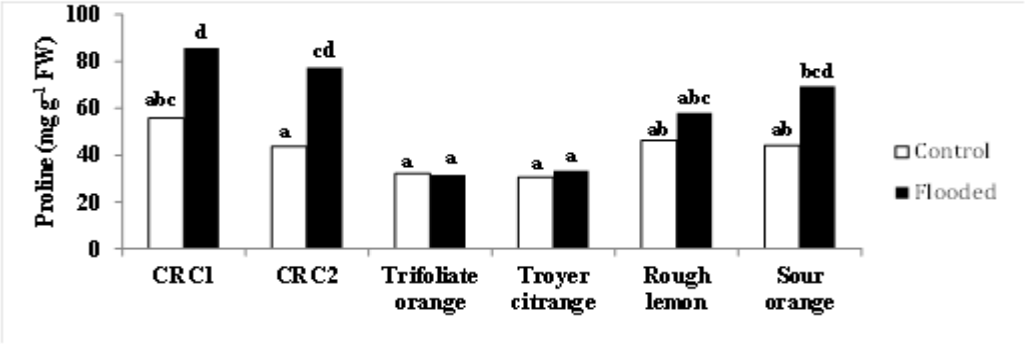

Fig. 4. Proline content of leaves of flooded and control genotypes. Means with at least one similar letter are not significantly different (P≤ 0.05) based on Tukey test

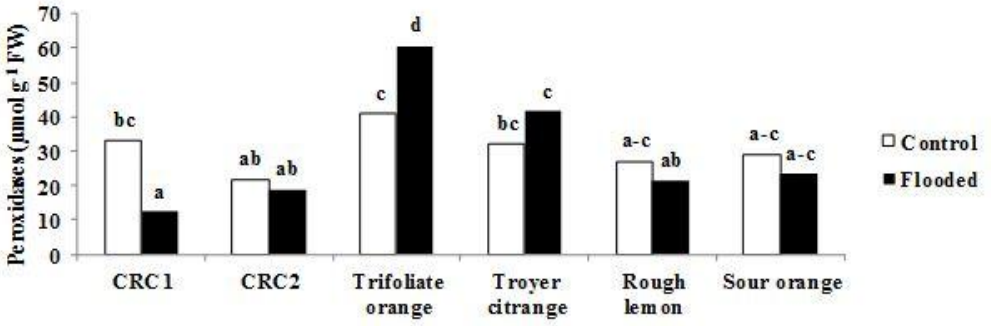

Fig. 5. The activity of peroxidases in leaves of flooded and control plants. Means with at least one similar letter are not significantly different (P≤ 0.05) based on Tukey test

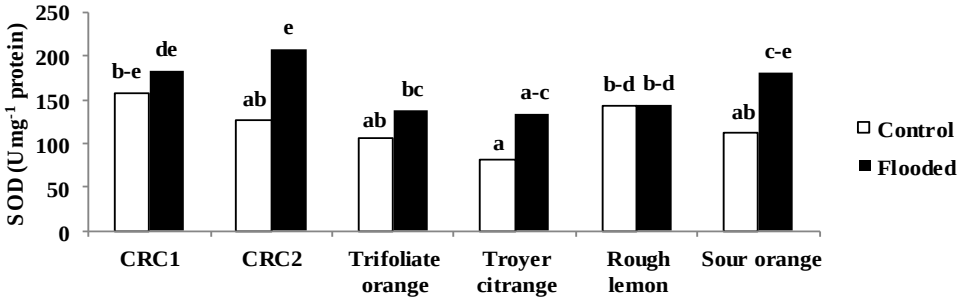

Fig. 6. Superoxide dismutase activity in leaves of flooded and control plants. Means with at least one similar letter are not significantly different (P≤ 0.05) based on Tukey test

Total protein content

According to Fig. 7, flooding stress decreased total protein level in all genotypes. The highest content of total proteins was observed in control groups of Trifoliate orange and Troyer citrange. The total protein content of leaf tissue was significantly decreased in CRC1, Trifoliate orange, and Troyer citrange.

Correlation analyses

Pearson's correlation analysis showed a strong statistically significant correlation between proline content and peroxidase activity of leaves in flooded genotypes (r= -0.74, P<0.01) (Table 2). Moreover, proline and protein content of leaves in flooded genotypes showed a statistically significant correlation (r= -0.76, P<0.01). The correlation between superoxide dismutase activity and total protein content of the leaves was also significant (r=-0.87, P<0.01). Another significant correlation was between proline content of the leaves and the activity of superoxide dismutase in leaf tissue of flooded plants (r=0.81, P<0.01). Total chlorophyll and protein content of the leaf tissue showed a positive statistically significant correlation (r= 0.67, P<0.01), while the correlation between superoxide dismutase and total chlorophyll content was negative (r= -0.61, P<0.01).

Recovery and survival

The visual inspection of the flooded genotypes showed detrimental non-reversible damage to vascular cylinder of the primary root in CRC1 and Sour orange. Meanwhile other studied genotypes showed degrees of damage to secondary root system but the main root was intact. Among the genotypes, Troyer citrange showed a well-established root system after 30 days of recovery period in spite of longer period of flooding stress (60 days). Meanwhile, CRC1 and Sour orange showed the least root regeneration though they experienced shorter period of flooding. On the contrary, CRC2 ended up with a comparatively better root system (Fig. 8).

Table 2. Pearson`s correlation coefficients for proline, superoxide dismutase (SOD), peroxidases and total proteins. (= P<0.01, ns= not significant)**

	Proline	SOD	Peroxidases	Proteins
SOD	0.81**			
Peroxidases	-0.74**	-0.43^{ns}		
Proteins	-0.76**	-0.87**	0.44*	
Total Chlorophyll	-0.55**	-0.61**	0.45*	0.67**

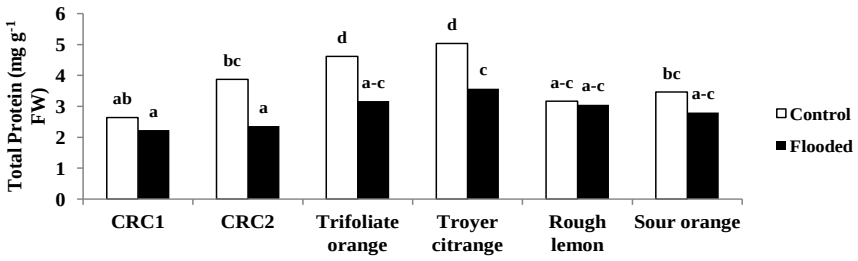

Fig. 7. Total protein content in leaves of flooded and control plants. Means with at least one similar letter are not significantly different (P≤ 0.05) based on Tukey test.

Fig. 8. The ability of genotypes to regenerate root system after 30 days of recovery from flooding.

Discussion

Based on the results, long term continuous flooding and the subsequent hypoxia, imposed a strong oxidative stress on the *Citrus* genotypes. Genotypes responded differently to this situation, Troyer citrange and Trifoliate orange were able to endure waterlogging for a considerably longer time than other genotypes and this could be due to the different nature of *Citrus* and *Poncirus* genera, the latter possessing adaptations making possible surviving temperate zones, as it has origins in higher latitudes compared to *Citrus* genus (Krueger and Navarro, 2007).

Flooding stress dramatically decreased plant growth compared to the control plants. In an experiment Wu et al. (2012) reported a significant reduction in plant height, stem diameter, and shoot as well as root fresh weights in flooded plants not inoculated with an arbuscular mycorrhizal fungus as a defense to oxidative damage. They also documented that prolonged 37 day waterlogging condition, significantly inhibited plant growth, - i.e. root and shoot fresh weights. All these findings are in consistent with the result obtained from present study. Considering the negative impact of flooding on plant growth, Troyer citrange and Trifoliate orange showed the least decrease in plant fresh and dry weights (Fig. 1, and Fig. 2). One the first mechanisms involved in the responses of *Citrus* seedlings to hypoxic condition is stomatal responses in order to minimize water loss. As a result the photosynthetic rate is decreased and CO_2 assimilation is impaired, hence the decrease in plant dry weight would not be inevitable (Martínez-Alcántara et al., 2012). Flooding stress imposes large injury to the root system of plants in the form of drastic decay of plants underground organs due to lack of oxygen. Vu et al. (1991) reported a reduction of fibrous roots in two rootstocks, sour orange and rough lemon. However, the extent of this destruction was more prominent in sour orange than rough lemon.

Leaf damage and chlorophyll loss are common responses resulted from long-term soil flooding. Arbona et al. (2008) reported a decrease in chlorophyll content of plants, while this reduction was different among the studied genotypes as the Carrizo citrange showed no significant change following 20 days of continues flooding compared to Citrumelo and Cleopatra mandarin. In another experiment studying flooding impact on chlorophyll content of sweet orange trees grafted on rough lemon

and sour orange, the leaves grafted on sour orange had more chlorophyll loss compared to those grafted on rough lemon (Vu et al., 1991). The same pattern is seen in the present study in which chlorophyll content in sour orange is significantly reduced in flooded plants while it was not significantly different between flooded and control plants of rough lemon and CRC2 (Fig. 3).

Proline accumulation, as a non enzymatic defense system, is a common response in plants exposed to adverse environmental conditions, but the extent of this accumulation is different among genotypes. In a study on the responses of three *Citrus* genotypes to waterlogging stress, citrumelo and Cleopatra mandarin showed an increase in proline level of leaves compared to control plants while in Carrizo citrange this factor was unchanged with respect to controls. It has been proposed that there is a positive correlation between stress pressure imposed and sensed by plant and proline accumulation response. Moreover, the role of proline in scavenging reactive oxygen species in flooding stress is minimal because higher levels of this osmolyte is associated with an earlier increase of oxidative damage, senescence and as a result lower tolerance (Arbona et al., 2008). Considering this rationale, the stress pressure which was exerted by continuous flooding on CRC1, CRC2, sour orange and rough lemon, was higher compared to what happened in Trifoliate orange and Troyer citrange for which showed no significant difference in proline content between two groups of flooded and control plants. García-Sánchez et al. (2007), in a study on the responses of two *Citrus* rootstocks to flooding and drought stress, reported that while the proline content of the two genotypes were different in control plants, flooded plants had similar values of proline as control ones. Considering the protective role of proline in stressed plants and their detoxifying role in ROS scavenging, these protective functions seems negligible in *Citrus*, as more sensitive genotypes accumulated more proline in their leaf tissue (Arbona et al., 2008). The results of this study is also in agreement with this hypothesis, as more flooding sensitive genotypes demonstrated more proline content while Troyer citrange and Trifoliate orange kept their proline content as low as the control group. Furthermore, considering the strong negative correlation between proline content and the guaiacol peroxidase activity ($r=-0.9$, $P<0.01$) and the ability of the two mentioned genotypes to cope with waterlogged conditions, it seems that the activity of this enzyme is of more importance in enabling *Citrus* survive such condition than the role of proline.

Guaiacol peroxidase which is less studied in plant stress studies than ascorbate peroxidase, also acts as a radical scavenger in plants but this activity is lower than that of superoxide dismutase. The basal activity of this enzyme is also different among genotypes (Amador et al., 2012). In spite of high peroxidase activity in flooded genotypes, plants exhibited a high level of H_2O_2 at the 23rd and 28th day of continuous flooding condition denoting that the activity of peroxidases might be insufficient in scavenging all the accumulated H_2O_2 under waterlogging (Hossain et al., 2009). Concerning the studied genotypes of the present paper, it can be assumed that the significant increase of peroxidase activity in the Trifoliate orange, contributed to its higher resistance to flooded conditions.

In a plant cell, superoxide dismutase (SOD) enzyme is the first step in defense against superoxide radicals, with H_2O_2 as a reaction product which is further removed by the activity of peroxidases and catalase. In a study on the antioxidant responses of citrumelo to waterlogging conditions, Hossain et al. (2009) reported that SOD was among the first antioxidant enzymes showing an early response. Increasing from the first day of flooding and reaching its peak at 18th day, it slowly decreased from this point with the continuation of waterlogged situation. As mentioned earlier,

added to their lethal effect on cells, hydrogen peroxide can inactivate different forms of SOD isoforms resulting in an increase in H_2O_2 concentration and a decline in SOD activity over time. Moreover, exogenous application of proline not only decreases lipid peroxidation and superoxide radicals but also decreases the activity of SOD in young plants (Balakhnina, 2015). According to a study on the effects of waterlogging on three *Citrus* genotypes by Arbona et al. (2008), the increase in the activity of superoxide dismutase (SOD) might be related to the accumulation of superoxide radical as a result of oxygen deprivation. The result of their investigation on the enzymatic and antioxidant activities in response to flooding indicated that all the studied genotypes experienced an increase in their SOD activity while the onset of this increase and the time reaching the peak of its activity was different among the genotypes. For Cleopatra mandarin this peak happened at day 14 but reached the same level as control ones thereafter. Considering the differences in response among genotypes and the fact that this enzyme might be suppressed over time with the accumulation of hydrogen peroxide, the extent of increase in the activity of SOD for all genotypes except for CRC2 and sour orange was not significant.

The accumulated reactive oxygen species in a cell due to flooding conditions disrupts the cellular redox system causing oxidative damage to lipids, proteins and nucleic acids (Hossain et al., 2009). This adverse effect on the protein content of leaves is noticed by a decrease in protein level in the leaf tissue of all studied genotypes.

The 30 days recovery period proved that Trifoliate orange, Troyer citrange and Rough lemon and CRC2 are genotypes able to reproduce their root system after being flooded for a comparatively long period. According to Syvertsen et al. (1983) Rough lemon having a larger root system and higher stomatal conductivity, can tolerate flooding condition better than Sour orange. The same pattern is seen here as Rough lemon was able to re-establish its root system and recover better than Sour orange after long term soil hypoxia and subsequent recovery period (Fig. 8). Rough lemon as a rootstock is more tolerant to continued flooding for 60 days compared to Sour orange which showed 90 percent death after just 30 days of continued flooding (Vu, J. C. V. et al., 1991).

Conclusions

Flooding stress imposes physiological and morphological changes in plants but the extent of the damage is different among species and genotypes. These differences determine tolerance and survival abilities of genotypes. Moreover, the responses induced by flooding stress are complicated and depends on multiple factors and their interactions. In our study Troyer citrange and Trifoliate orange as inherited a different origin compared to other genotypes, acted differently in their response to hypoxic conditions by accumulating less proline and keeping high guaiacol peroxidase activity. In the recovery period, Troyer citrange and rough lemon among the commercial rootstocks were able to re-establish their root system and survive from the flooding conditions. While sour orange as an example of a sensitive genotype, not only could not tolerate hypoxic conditions for long term but also was not able to recover its root system. Among the studied local genotypes, CRC2, thanks to the protective effects of superoxide dismutase and proline, showed a better tolerance and also a comparatively good ability to re-establish at the end of flooding condition.

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