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RESEARCH ARTICLE

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# Morphological and physiological responses and some *WRKY* genes expression in cherry rootstocks under salt stress

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## Abstract

*Aim of study:* To determine morphological, physiological and molecular responses of cherry rootstocks under salt stress condition.

*Area of study:* Konya, Turkey.

*Material and methods:* A pot trial was conducted to assess moderate salt stress (35 mM NaCl) effects on cherry rootstocks (CAB-6P, MaxMa 14 and Mazzard). We have evaluated many morphological and physiological parameters and analyzed *WRKY* genes (*WRKY25, WRKY33* and *WRKY38*) under salinity conditions.

*Main results:* All rootstocks survived with slight leaf burn under salinity conditions and the plant growth and physiological parameters, except membrane permeability, decreased in all rootstocks. The membrane permeability increased with salinity and the lowest increment in the membrane permeability (12.17%) was in MaxMa 14, while CAB-6P and Mazzard showed higher levels of increases reaching 46.81 and 56.42%, respectively. Furthermore, the expression of *WRKY25, WRKY33* and *WRKY38* genes was significantly increased by salinity. The rankings of the *WRKY* genes expression levels among control rootstocks were: MaxMa 14 < CAB-6P < Mazzard.

*Research highlights:* CAB-6P, MaxMa 14 and Mazzard rootstocks were found relative salt-tolerant at the moderate salinity levels and there is a cross-talk between physiological and molecular responses. Mazzard had higher tolerance to salinity shown in molecular responses. The study possesses importance for plant physiologists and cherry growers as it showed how cherry rootstocks respond to salt stress.

**Additional keywords:** plant physiology; Prunus; salinity; transcription factors.

**Abbreviations used:** DW (dry weight); EC (electrical conductivity); FW (fresh weight); LRWC (leaf relative water content); RGR (relative growth rate); SPAD (soil plant analysis development); TW (turgor weight).

**Authors' contributions:** Conceived, designed and performed the experiments: AE and SA. Analyzed the data: SA, AE and YK. Contributed reagents/materials/analysis tools: YK and SA. Wrote the paper: SA. All authors read and approved the final manuscript.

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## Introduction

Salinity exhibits many constraints in fruit growing and limits fruit yield and quality. Salt load in plant body affects growth rate and plant morphology (Munns, 1993; Moya *et al.*, 1999; Massai *et al.*, 2004). Although *Prunus* species, including cherry (*Prunus avium* L.), are known as sensitive to salt stress (Gucci & Tattini, 1997), a significant part of cherry growing is accomplished in salt-affected areas such as in arid, semi-arid and coastal areas. Growing of cherry plants under salinity causes many malignant effects on plants including increases in reactive oxygen species (ROS), decreases in mineral uptakes and plant growth, and is responsible for enormous economic losses (Niu *et al.*, 1995; Foyer & Noctor, 2000; Yin *et al.*, 2010). Managers for cherry must deal with reduced growth, nutritional imbalances and specific ion toxicities caused by salt stress.

One strategy to increase cherry plant growth, fruit yield and quality under salinity conditions is to use

rootstocks with superior salinity tolerance. Thus, selecting rootstocks which can maintain economic yield and fruit quality under salinity is an important strategy for diminishing the salinity damages. Rootstocks posses influences on tree vegetative growth through affecting mineral nutrition, water use and/or uptake and hormonal balance (Olien & Lakso, 1986; Kamboj *et al.*, 1999). Salt-tolerant cherry rootstocks can be utilized in cherry growing under salinity conditions. Discrepancy of tendency among *Prunus* rootstocks against salt stress can be screened by evaluating morphological parameters such as relative growth rates of rootstock and scions. Higher plant growth constitutes a major improvement in stress tolerance and reduces deleterious effects of the stress. Therefore, it is necessary to have precise knowledge of plant growth responses to salt stress in order to overcome the problems.

Many physiological responses against salt stress have been discussed in many woody plants such as apple (Yin *et al.*, 2010), pear (Wu & Zou, 2009) and peach (Massai *et al.*, 2004). The tolerance mechanisms of plants against salinity highly integrate plant growth and physiological behaviours including the decrease in stomatal conductance (Aras & Eşitken, 2019), plant water status (Garcia-Legaz *et al.*, 2008) and the increase in membrane permeability (Aras *et al.*, 2015). Rootstock salt tolerance should be evaluated by taking into account many physiological parameters as well as plant growth. Besides physiological responses, the molecular changes in plants possess importance. The molecular mechanism underlying plant salt tolerance is not still fully clarified. Many genes take part in plant stress tolerance and the induction of the genes occurs at the transcription level (Agarwal *et al.*, 2011). Transcriptional control is regulated by transcription factors (TFs). Thus, TFs play a crucial role in the plant stress tolerance. Many families of TFs such as WRKY, bZIP, NAC, ERF have been identified in plants and a number of studies have shown that TFs are highly and rapidly induced or repressed by many abiotic stress factors and possess a role in the regulation of plant responses (Bielsa *et al.*, 2016; Sohrabi *et al.*, 2017; Bankaji *et al.*, 2019). Among TFs, *WRKY* TFs, are the members of zinc finger superfamily TFs (Babu *et al.*, 2006), and interconnect networks to regulate stress responses (Zhao *et al.*, 2017). The WRKY proteins are identified by their DNA-binding domain which has the conserved domain and bind to W-box (TTGACC/T) in the target gene promoters (Rushton *et al.*, 2010; Chi *et al.*, 2013).

There is increasing evidence to suggest that several *WRKY* genes are involved in the response to many stresses. Salt-inducible *SIWRKY3* affected salt stress response in tomato plants (Hichri *et al.*, 2017). Similarly, Bao *et al.* (2018) demonstrated that *PcWRKY33* is involved in *Polygonum cuspidatum* plant tolerance against salinity. Some studies revealed that *WRKY25* is altered under salinity, cold and heat stress (Jiang & Deyholos, 2009; Li *et al.*, 2009). Similarly, *WRKY33* is involved in plant defense against pathogens in *Arabidopsis* (Lai *et al.*, 2011). Moreover, *WRKY38* participates in defense against cold and drought stress in barley (Mare *et al.*, 2004). Li *et al.* (2011) revealed that positive cross-regulation of *WRKY25*, *WRKY26*, and *WRKY33* reflects a synergistic interaction and expression of the genes increased plant thermotolerance in *Arabidopsis thaliana*.

Considering the magnitude of salinity worldwide and the economic value of cherry plants, the current study was undertaken to evaluate the role that *WRKY25*, *WRKY33* and *WRKY38* TFs play in salt stress response in Mazzard, MaxMa 14 and CAB-6P cherry rootstocks through the regulation of plant growth and physiological functions. Therefore, we wished to investigate the responses of cherry rootstocks to salt stress at morphological, physiological and molecular level, which would indicate tolerance of plants against salinity.

## Material and methods

The study was conducted in the greenhouse of Selcuk University, Konya, Turkey during April-September of 2015 and 2016. Uniform saplings of 1-year-old Mazzard (*Prunus avium* L.), MaxMa 14 (Mahaleb × Mazzard) and CAB-6P (*Prunus cerasus* L.) rootstocks were grown in 13 L pots filled with the mixture of soil, peat and perlite in a volumetric proportion of 1:3:1 respectively. Until the start of the experiment, all plants were irrigated with tap water and then during four months the treated plants were watered with a fertilizer solution containing 35 mM NaCl (the growing media's salinity of the plants exposed to salt stress maintained in a range of 2.0-2.5 mS $cm^{-1}$ EC (electrical conductivity) through applying 35 mM NaCl) and the untreated control plants were watered with a fertilizer solution without NaCl. We used 35 mM NaCl, because this salt concentration is appropriate for moderate salinity in horticulture plants shown in many studies (Romero-Aranda *et al.*, 2001; Kaya *et al.*, 2002; Sotiropoulos, 2007). Excess solution was allowed to drain from the pot. The plants were stressed during four months. The experiment was carried out following a randomized complete plot desings with three replications and 5 plants per replication.

## Morphological measurements

Rootstock diameter at 10 cm from the soil and shoot diameter at 10 cm from the trunk and branch junction were measured with a digital caliper. Shoot length was measured with a ruler. The measurement of relative growth rate was taken for plant biomass as the whole plant (root+shoot). The weights of plant biomass were taken and recorded. Plants were weighted before planting as initial plant biomass and at the end of the study plants were weighted as the final plant biomass. The relative growth rate (RGR) was calculated using the equation given below (Del Amor & Marcelis, 2003):

$$RGR = 100 \times [(\ln X_{t2} - \ln X_{t1}) / (t_2 - t_1)]$$

with $t_2$ = end of the NaCl treatment period, $t_1$ = start of the NaCl treatment period, $X_{t2}$ = final plant biomass weight, $X_{t1}$ = initial plant biomass weight.

Root and shoot dry weights were measured after drying the plant material at 70ºC for 48-72 hours. The value of root:shoot in dry weight was calculated as the dry weights of root/shoot.

## Physiological measurements

Chlorophyll (Chl) was measured with a Minolta SPAD-502 chlorophyll meter (Minolta Camera Co). For this determination, the adaxial side of the leaves was placed in the instrument and major veins were avoided. Stomatal conductivity and leaf temperature were measured with a leaf porometer (SC-1 porometer, Decagon Devices).

For the measurement of membrane permeability (electrolyte leakage), the procedure of electrolyte leakage based on Lutts *et al.* (1996) was used to assess membrane permeability. Electrolyte leakage was measured using an EC meter (Jenway EC meter). Mature leaves were taken from each plant and cut into 1 cm segments. The leaf samples were then placed into the individual stoppered vials containing 10 mL of distilled water after three washes with distilled water for removing surface contamination. These samples were incubated at room temperature (25ºC) on a shaker (100 rpm) for 24 h. EC of bathing solution ($EC_1$) was measured after incubation. The same samples were then placed in an autoclave at 120ºC for 20 min and the second measurement ($EC_2$) was taken after cooling solution to the room temperature. The electrolyte leakage was calculated as $EC_1/EC_2$ and expressed as percentage.

For the leaf relative water content (LRWC), the leaves were collected from the young fully expanded leaves. Individual leaves were first detached from the stem and then weighted to determine their fresh weight (FW). In order to determine turgid weight (TW), the leaves were floated in distilled water inside a closed petri dish. Leaf samples were weighted periodically, after gently wiping the water from the leaf surface with the tissue paper until a steady state was achieved. At the end of imbibition period, the leaf samples were placed in a pre-heated oven at 72ºC for 48 h, in order to determine their dry weight (DW). The values of FW, TW, and DW were used to calculate LRWC using the equation given below (Smart & Bingham, 1974):

$$LRWC(\%) = [(FW-DW)/(TW-DW)] \times 100$$

## Molecular analyses

The expression level of *WRKY25*, *WRKY33* and *WRKY38* genes was determined in the leaves of cherry rootstocks. These genes are involved in stress tolerance in many plants (Mare *et al.*, 2004; Jiang & Deyholos, 2009; Li *et al.*, 2009; Birkenbihl *et al.*, 2012). The leaves were frozen in liquid nitrogen immediately after the experiment and stored at −80ºC until analysis. Total RNA was extracted from the leaves using a modified isothiocyanate method (Strommer *et al.*, 1993). DNase treatment of each total RNA sample was carried out by DNA-Free kit (Invitrogen) to remove any residual genomic DNA. The concentration of RNA was determined with Qubit RNA assay kit (Invitrogen) using the Qubit 2.0 fluorometer. RNA integrity was checked by visualization on a 2% (w/v) formaldehyde agarose gel. The total RNA isolated from the leaves was used for the quantitative real-time polymerase chain reaction (RT-qPCR). The synthesis of first strand cDNA was performed in a total volume of 20 µL containing 1 µg of total RNA, 20 pmol of oligo(dT)18, 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 0.5 mM each dNTP, 1 U RNase inhibitor, 200U MMLV Reverse Transcriptase (Clonetech), and DEPC-treated water. RT-qPCR analyses were performed using Maxima SYBR Green/ROX qPCR Master Mix kit following the manufacturer's protocol in a Real-Time PCR System (Applied Biosystems). The reaction mixture (20 µL) contained 10 µL Maxima SYBR Green/ROX qPCR Master Mix (2×), 1 µL of each forward and reverse primer (10 µM) (Table 1), 6 µl ddH2O and 2 µl cDNA (25 ng). The following program was used for RT-qPCR: 95ºC for 5 min followed by 40 cycles of 95ºC for 30 s, 60ºC for 60 s, 72ºC for 60 s. StepOne™ Software v2-2-2 (Applied Biosystems) program was utilized to analyze the data. The relative changes in gene expression levels were calculated with the 2−ΔΔCT method as described by Schmittgen & Livak (2008). β-actin gene (forward 5'-GAGACCTTCAACACCCCAGCC-3', reverse

**Table 1.** Primers used for the the quantitative real-time polymerase chain reaction (RT–qPCR) assays.

| Gene | Accession | Forward primer | Reverse primer |
|---|---|---|---|
| *WRKY25* | - | ATCTGATGAGTTTCCCGAAG ATGGA | GGAGGAGTAGCTTTAGTTTTTGAG |
| *WRKY33* | XM_021970745 | ATCGCTGTGCCTCTCTCTCT TACAT | GGTTGCTTGAATTTTTTTTCTG |
| *WRKY38* | XM_021976344 | ATGGCCTTTTGACAGATTT CTGGAT | GAGTTTCTGCAATATATGAGTTGA |

5'-GACTTCGAGCAAGAGATGGCC-3') (CACT1, GenBank FJ560908) was used as a reference gene in the qRT-PCR reactions.

### Statistical analyses

Statistical analyses were performed with the statistical software package SPSS, version 20.0. Average of the data belonging two consecutive years was analyzed. The means were compared by the Duncan multiple range test at the 5% level of significance. Furthermore, interactions were considered in the analyses.

## Results

The cherry rootstocks were exposed to the moderate salinity at 35 mM for 4 months at the vegetative stage and relative damage to the indices of stress and the plant growth, physiological responses and *WRKY* gene expression levels were evaluated.

### The plant growth

Within the four months of salinity, NaCl reduced the plant growth of the cherry rootstocks (Table 2). Rootstock diameter was reduced by 12.48, 10.90 and 8.06% in salt-treated plants of CAB-6P, MaxMa14 and Mazzard, respectively as compared with the control. Shoot diameter was reduced by 5.42, 12.96 and 8.13% in salt-treated plants of CAB-6P, MaxMa14 and Mazzard, respectively. Moreover, the shoot lengths of the plants decreased by 12.00 and 16.18% in salt-treated plants of CAB-6P and MaxMa14, respectively. A lower reduction of shoot length (7.21%) was observed in NaCl-treated Mazzard rootstock as compared to the other rootstocks. There was no statistical difference in the RGR of the plant biomass. The root:shoot rate in dry weight decreased by 20.90 and 21.91% in salt-treated plants of CAB-6P and MaxMa14, respectively. In Mazzard, the root:shoot rate in dry weight decreased by 14.91% .

### The physiological response

There were considerable differences in the pattern of the SPAD (soil plant analysis development) value in response to the salt stress (Table 3). The SPAD value declined by 16.03 and 26.78% in salt-treated plants of CAB-6P and Mazzard, respectively and a lower reduction level was observed in MaxMa 14 (11.41%). The stomatal conductivity was reduced by 4.64, 11.60 and 3.76% in salt-treated plants of CAB-6P, MaxMa14 and Mazzard, respectively. The leaf temperature was not significantly affected. The membrane permeability was considerably affected among rootstocks as compared with the control. The lowest increase in the membrane permeability (12.17%) was in MaxMa 14, while CAB-6P and Mazzard showed higher levels of increases reaching 46.81 and 56.42%, respectively. There were similar decreases in LRWC among rootstocks. The reductions in LRWC were 6.16, 4.35 and 5.02% in salt-treated plants of CAB-6P, MaxMa14 and Mazzard, respectively as compared with the control values.

**Table 2.** Effects of salinity on morphological responses of cherry rootstocks.

| Treatments | Rootstocks | Rootstock diameter (mm) | Shoot diameter (mm) | Shoot length (cm) | Biomass RGR | Root:shoot in dry weight |
|---|---|---|---|---|---|---|
| CAB-6P | Control | 17.30 a | 6.08$^{NS}$ | 33.83 a | 4.23$^{NS}$ | 0.598 a |
| | 35 mM NaCl | 15.14 b | 5.75 | 29.77 b | 4.20 | 0.473 b |
| MaxMa 14 | Control | 18.52 a | 8.25 a | 39.10 a | 4.23$^{NS}$ | 0.502 a |
| | 35 mM NaCl | 16.50 b | 7.18 b | 32.77 b | 4.14 | 0.392 b |
| Mazzard | Control | 9.17$^{NS}$ | 3.81 a | 23.41 a | 4.62$^{NS}$ | 0.912 a |
| | 35 mM NaCl | 8.43 | 3.50 b | 21.72 b | 4.58 | 0.776 b |

RGR: relative growth rate. Means separation within columns by Duncan's multiple range test. $p<0.05$, NS: non significant.

**Table 3.** Effects of salinity on physiological responses of cherry rootstocks.

| Treatments | Rootstocks | SPAD | Stomatal conductivity (mmol $m^{-2}$ $s^{-1}$) | Leaf temperature (°C) | Membrane permeability (%) | LRWC (%) |
|---|---|---|---|---|---|---|
| CAB-6P | Control | 48.53 a | 554 a | 33.9$^{NS}$ | 18.50 b | 88.7 a |
| | 35 mM NaCl | 40.75 b | 528 b | 34.5 | 27.16 a | 83.3 b |
| MaxMa 14 | Control | 46.18 a | 474 a | 33.2$^{NS}$ | 19.13 b | 89.6 a |
| | 35 mM NaCl | 40.91 b | 419 b | 33.3 | 21.46 a | 85.7 b |
| Mazzard | Control | 39.42 a | 672$^{NS}$ | 34.4$^{NS}$ | 17.19 b | 83.6$^{NS}$ |
| | 35 mM NaCl | 28.86 b | 647 | 34.8 | 26.89 a | 79.4 |

SPAD: soil plant analysis development. LRWC: leaf relative water content. Means separation within column by Duncan's multiple range test. $p<0.05$, NS: non significant.

### The molecular response

The expression levels of *WRKY25*, *WRKY33* and *WRKY38* in response to salt stress in leaves were analyzed by qRT-PCR. The results showed that the expression of the genes was induced by salinity (Fig. 1). Under NaCl stress, remarkable differences in transcripts abundance were detected among rootstocks and the genes displayed similar expression patterns. The maximum expression of *WRKY25* was in Mazzard (4.4-fold of the control) followed by MaxMa 14 (4.3-fold of the control). The greatest increases in the expression level of *WRKY33* were 5.8-fold, 5.7-fold and 5.6-fold in Mazzard, MaxMa 14 and CAB-6P, respectively. A similar expression pattern was observed in *WRKY38* gene. The highest expression level of *WRKY38* was in Mazzard and MaxMa 14 (5.2-fold of the control).

To explore the possible defense mechanisms which *WRKY25, WRKY33* and *WRKY38* may be involved in, we performed interaction analyses between molecular and physiological parameters under salt stress (Table 4). Negative correlative values have been found between three genes (*WRKY25, WRKY33* and *WRKY38*) and SPAD (r=-0.862, $p\leq0.05$; r=-0.848, $p\leq0.05$ and r=-0.864, $p\leq0.05$, respectively) and LRWC (r=-0.869, $p\leq0.05$; r=-0.850, $p\leq0.01$ and r=-0.864, $p\leq0.05$) values. Moreover, positive correlation between the genes and membrane permeability (r=0.914, $p\leq0.01$; r=0.924, $p\leq0.01$ and r=0.912, $p\leq0.01$) was determined.

## Discussion

### Morphological responses

Cherry is an important temperate zone fruit tree grown in salted-areas due to poor drainage, excessive fertilization and coastal areas. Salt stress has many malignant effects on plants and may cause dysfunction in photosynthetic process (Munns & Tester, 2008). The main symptom of NaCl damages is leaf-tip necrosis (Wahome *et al.*, 2001). In the current study, all rootstocks survived with slight leaf burn under 35 mM NaCl irrigation for four months. In our experiment, we applied 35 mM NaCl solution to the cherry rootstocks at moderate salinity stress in order to probe into the changes in the plant growth and physiological responses. Our study demonstrated that the plant growth in cherry rootstocks was influenced by salinity, and the extent of the reduction in the growth was dependent on the rootstock. At the end of the experiment, for determination of physiological responses plant leaves were used to determine the salinity effects on plant because leaves are more vulnerable than roots in terms of the accumulation of $Na^+$ and $Cl^-$ in higher levels (Tester & Davenport, 2003).

Decrease in plant growth is a consequence of salinity (Yin *et al.*, 2010) and in woody plants, the growth response to salinity varies with rootstocks (Maas, 1993). The decrease of plant growth in the presence of salt stress could also be due to the presence of the toxic ions $Na^+$ and $Cl^-$ (Hu & Schmidhalter, 2005). Many studies showed the stunting effects of salt stress on the growth of temperate zone fruit species (Massai *et al.*, 2004; Sotiropoulos *et al.*, 2006; Yin *et al.*, 2010; Koc *et al.*, 2016a,b). Depressed plant growth after an increase in soil salinity may be due to the osmotic effect of the salt around the roots. A sudden increase in soil salinity causes water loss in leaves (Munns & Tester, 2008). Salinity also causes a remarkable decrease in plant dry weights (Chartzoulakis & Klapaki, 2000). Najafian *et al.* (2008) have reported that in almond rootstocks, plant height, stem diameter, root and shoot fresh and dry weights decrease with the increase in salinity. Moreover, the RGR values were similar to those reported for many plants under salinity stres (Ruiz *et al.*, 1997; Fernandez-Garcia *et al.*, 2004; Massai *et al.*, 2004). Differences in biomass RGR observed among the *Prunus* rootstocks in response to salt stress may be related to the growth characteristics of the rootstocks.

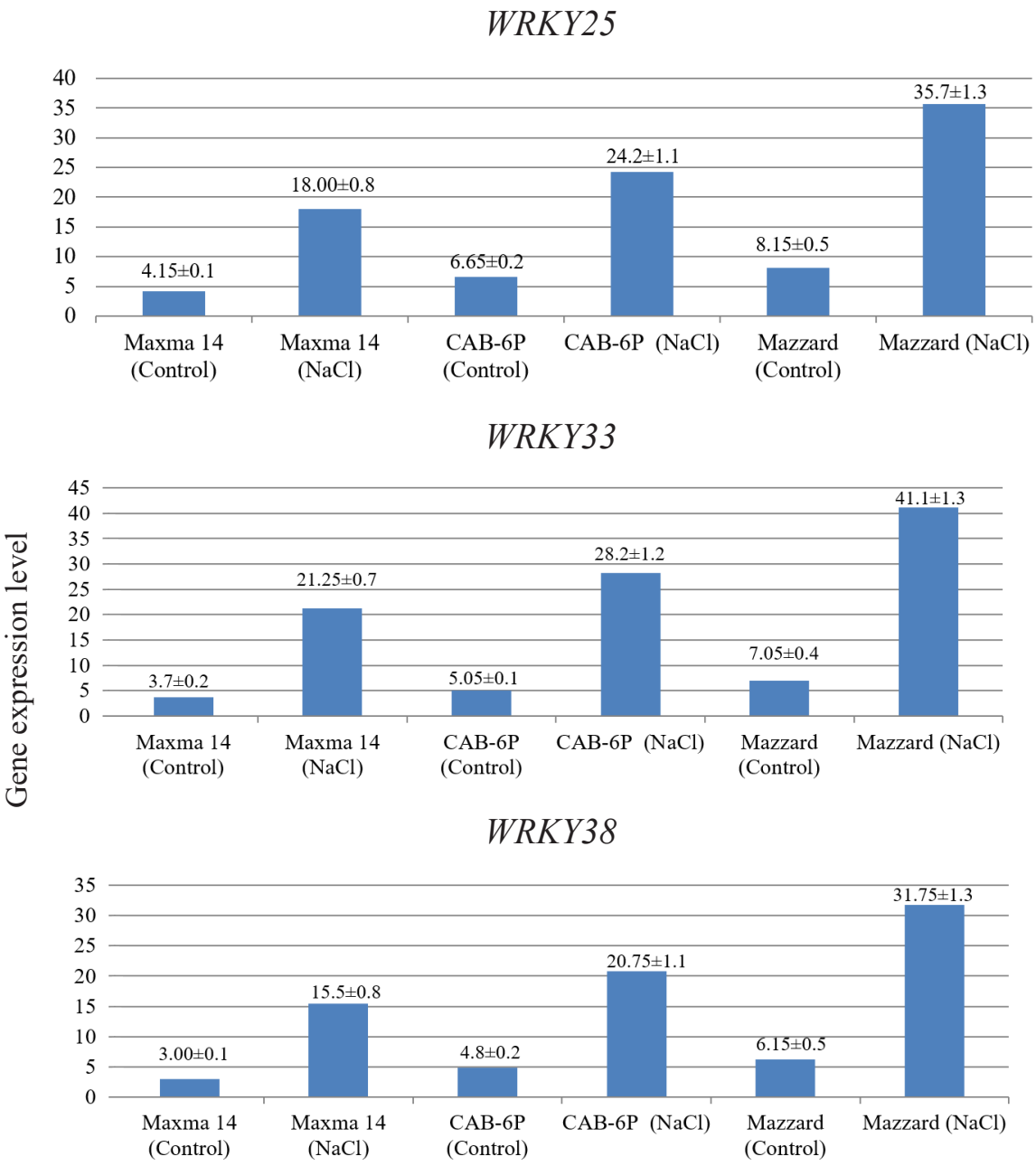


**Figure 1.** Expression levels of *WRKY* genes: *WRKY25* (A), *WRKY33* (B) and *WRKY38* (C). The values and standard errors are written on the columns.

**Table 4.** Interaction between *WRKY* gene expression levels and physiological parameters.

| | SPAD | Stomatal conductance | Leaf temperature | Membrane permeability | LRWC |
|---|---|---|---|---|---|
| *WRKY25* | -0.862* | 0.23 | 0.596 | 0.914* | -0.869* |
| *WRKY33* | -0.848* | 0.174 | 0.554 | 0.924** | -0.850* |
| *WRKY38* | -0.864* | 0.222 | 0.584 | 0.912* | -0.864* |

SPAD: soil plant analysis development. LRWC: leaf relative water content. *significant at $p \leq 0.05$, ** significant at $p \leq 0.01$.

Mazzard rootstock favored a high biomass investment in plant, thus increased its growth. Furthermore, salt stress reduced the root/shoot ratio. There were significant differences in terms of the reductions of plant biomass production among rootstocks and shoot growth was less affected than the root growth, so that the root:shoot ratio in dry weight decreased. Therefore, the salinity in *Prunus* may alter the pattern of dry matter distribution favoring the shoot growth. Apparently, salt stressed *Prunus* rootstocks partitioned more carbon to the shoots during the salt stress period. In the plant growth model, carbon is supplied to the root from the shoot by phloem transport (Marschner *et al.*, 1996). Therefore, carbon may be allocated in shoot of the cherry rootstocks under salinity conditions.

## Physiological responses

Salt stress decreased SPAD value (relative chlorophyll content) in all rootstocks, similarly to what has been described previously (Murkute *et al.*, 2006). The decline in chlorophyll content may be due to an increase in chlorophyll degradation and/or a decrease in chlorophyll synthesis. Our data demonstrated that the moderate salt stress slightly decreased chlorophyll content of CAB-6P and MaxMa rootstocks as compared to the Mazzard rootstock (Table 2).

A small decrease in stomatal conductivity could have a protective effect against stress (Chaves *et al.*, 2009). The decrease in stomatal conductivity reduces transpiration in order to maintain plant turgor (Mahouachi, 2009). In the current study, stomatal conductance slightly decreased in all rootstocks as compared to their control. The reduction in the plant growth under salinity may be attributed to both stomatal limitation and SPAD value reduction. Current study indicates that the cherry rootstocks tend to close their stomata under salinity stress. The reductions in stomatal conductance and SPAD value led subsequently to a decrease in the plant growth. Moreover, there is a close relationship between stomatal conductance and plant growth. The highest decreases in shoot diameter (12.96%), shoot length (16.18%), RGR of biomass (2.12%) and root/shoot ratio (21.91%), as well as stomatal conductivity (11.60%) were determined in MaxMa 14 among the rootstocks.

The increase in the membrane permeability as a result of oxidative damage may be considered as an indicator of the degree of injury under stresses (Li *et al.*, 2010). MaxMa 14 exhibited the lowest increase in membrane permeability (12.17%) among the rootstocks when compared with the control. The increases in membrane permeability in response to salinity were obtained in previous studies (Yin *et al.*, 2010; Sabra *et al.*, 2012). Similar to our results, the decrease in LRWC due to the presence of NaCl at certain concentrations has been reported for various plants such as lemon (Aras *et al.*, 2015), loquat (Garcia-Legaz *et al.*, 2008) and strawberry (Karlidag *et al.*, 2009). In the current study, it is considered that the changes in soil water potential by salinity were less reflected to LRWC in MaxMa 14 among the rootstocks.

## Molecular responses

Salt stress is known to induce *WRKY* gene expression in many plants such as maize (Li *et al.*, 2013), diploid woodland strawberry (Wei *et al.*, 2016). The identification of the genes involved in the physiological responses would allow a better understanding of the salt stress tolerance regulation. In some cases, *WRKY* genes are down-regulated in salt stress conditions (Bankaji *et al.*, 2019). In our study, the rankings of the *WRKY* genes expression levels among control rootstocks were as follows: MaxMa 14 < CAB-6P < Mazzard. In many plants, *WRKY* genes were reported to be differentially expressed in response to external stimuli, such as salinity (Zhou *et al.*, 2015) and heat stress (Li *et al.*, 2010). The protein product of *WRKY25, WRKY33* and *WRKY38* genes can attach to the promoter regions of the genes related to salt stress response and induce their expressions (Jiang & Deyholos, 2009).

Many studies reveal that TFs such as WRKY, DREB, MYB induce defense mechanisms against stressors in many plants. Transcription factors regulate the expression of stress-related functional genes, which promote stress resistance (Zhu *et al.*, 2019). Shen *et al.* (2017) demonstrated that *PacMYBA* gene increased salt-tolerance in the cherry plant. An increase in the *OsDREB* gene expression was considered to be the reason for the salinity tolerance in rice (Dubouzet *et al.*, 2003). Bielsa *et al.* (2016) also found that enhanced level of bZIP TF gene in *Prunus* rootstocks under drought conditions could be a consequence of the increased stress tolerance. Moreover, Zhu *et al.* (2019) confirmed the important role of *VvWRKY30* in increasing salt stress resistance by regulating the reactive oxygen species-scavenging capacity in grape plant. These studies prompted us to determine the roles of the common *WRKY* genes in *Prunus* rootstocks against salinity.

The changes in gene expressions in rootstocks paralleled with the variations in morphological and physiological parameters. As suggested by our study, Mazzard exhibited more adaptation to salt stress as compared to MaxMa 14 and CAB-6P rootstocks probably because it showed higher gene expressions which might be related with its defense mechanism.

Moreover, avoiding water loss through stomatal closure might be triggerred by *WRKY* gene expressions related with salt-tolerance. It is possible that the increased expression of the *WRKY25, 33* and *38* genes in *Prunus* may be indicative of salt stress tolerance. Moreover, we can conclude that there is a close relationship between the expressions of WRKY genes and morphological and physiological responses.

In woody plants as citrus, some published data confirm that the expression of *WRKY* genes is increased under salt stress conditions. Moreover, this increase in this expression is higher in tolerant rootstocks than in sensitive ones, with similar results to the obtained in this work (Vives-Peris *et al.*, 2018). Regarding with WRKY TFs against salt stress, many studies have been conducted in herbaceous plant but studies on woody plants are still limited. To our knowledge, this study is the first attempt to determine the *WRKY25, WRKY33* and *WRKY38* gene expression levels in *Prunus* rootstocks in response to salt stress. Mazzard which showed more salt-tolerance demonstrated the higher levels of the genes expressions as compared to MaxMa 14 and CAB-6P. These genes can be a good candidate for salt-tolerance molecular markers for the selection of more tolerant genotypes to stress conditions.

In summary, salinity induced many physiological and morphological changes in *Prunus* rootstocks including reduction in the plant growth, SPAD value, stomatal conductance and LRWC, as well as increase in the membrane permeability. The characteristics such as plant growth, stomatal behaviour, and chlorophyll content may be commonly used as criteria for evaluating salt tolerance among plants. The saline-induced decrease in plant growth could be associated with a decrease in LRWC, SPAD value and stomatal conductivity. This study demonstrated that CAB-6P, MaxMa 14 and Mazzard rootstocks had relative salt tolerances at the moderate salinity levels. It is worth noting that the responses of plants to salt stress may differ with rootstocks. Moreover, the results show that there is a cross-talk between physiological and molecular responses.

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