ISSN 1808-3765

WATER DEFICIT AFFECTS GAS EXCHANGE IN PEACH TREES CULTIVAR CHIMARRITA GRAFTED ONTO DIFFERENT ROOTSTOCKS

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1 ABSTRACT

The aim was to investigate the effect of the water deficit on the gas exchange of Chimarrita plants grafted onto five different rootstocks (Tsukuba 1, Tsukuba 2, Tsukuba 3, Aldrighi 1, and UFPel 0402 Selection), that is, five ‘Chimarrita’/rootstocks combinations. The factorial scheme consisted of two water conditions (control and water deficit) and eight days of evaluation (0, 1st, 2nd, 3rd, 4th, 6th, 7th, and 9th, the latter two were the recovery period). Gas exchange was assessed with the aid of the portable CO2 infrared analyzer. Results were subjected to the analysis of variance ($p \leq 0.05$) by using Tukey’s test in order to compare the means between treatments at each day of stress for each ‘Chimarrita’/rootstocks combination. It was found that the different ‘Chimarrita’/rootstocks combinations showed a distinct physiological behavior regarding the initial water-deficit tolerance. Plants consisting of the combination ‘Chimarrita/Aldrighi 1’ had the highest initial water-deficit tolerance. The reduction in the net assimilation rate in face of the water deficit is not only related to the stomatal limitation, suggesting that there may also be non-stomatal limitations. Seventy-two hours after providing irrigation back to plants, all combinations evaluated showed an overall resilience of the physiological performance.

Keywords: Prunus persica, abiotic stress, stomatal conductance, net assimilation rate.

RICKES, L. N.; KLUMB, E. K.; SILVA, C. D. S. e; BACARIN, M. A.; BIANCHI, V. J. DÉFICIT HÍDRICO AFETA AS TROCAS GASOSAS EM PESSEGUEIRO CULTIVAR CHIMARRITA ENXERTADA SOBRE DIFERENTES PORTAENXERTOS

Recebido em 25/01/2016 e aprovado para publicação em 21/02/2017

DOI: http://dx.doi.org/10.15809/irriga.2017v22n1p140-153

2 RESUMO

O objetivo foi avaliar o efeito do déficit hídrico sobre as trocas gasosas em plantas da cultivar Chimarrita enxertada sobre cinco diferentes portaenxertos (Tsukuba 1, Tsukuba 2, Tsukuba 3, Aldrighi 1 e Seleção UFPel 0402), ou seja, cinco combinações 'Chimarrita'/portaenxertos. O esquema fatorial foi composto de duas condições hídricas (controle e déficit hídrico) e oito dias de avaliação (0, 1º, 2º, 3º, 4º, 6º, 7º e 9º, os dois últimos sendo período de recuperação). As trocas gasosas foram avaliadas com um analisador portátil a infravermelho de CO_2. Os resultados foram submetidos a análise de variância ($p \leq 0{,}05$), utilizando-se o teste de Tukey para comparação de médias entre os tratamentos em cada dia de estresse de cada combinação 'Chimarrita'/portaenxertos. Verificou-se que as diferentes combinações de 'Chimarrita'/portaenxertos apresentaram comportamento fisiológico diferencial para a tolerância inicial ao déficit hídrico. Plantas compostas pelas combinações de enxertia entre 'Chimarrita'/Adrighi 1' foram as que apresentaram a maior tolerância inicial ao déficit hídrico. A redução da taxa assimilatória líquida perante o déficit hídrico, não está relacionada principalmente à limitação estomática, sugerindo-se que ocorrem também limitações não estomáticas. Após 72h do retorno da irrigação das plantas, todas as combinações avaliadas demonstraram total capacidade de recuperação do desempenho fisiológico.

Palavras-chave: *Prunus persica*, estresse abiótico, condutância estomática, taxa assimilatória líquida.

3 INTRODUCTION

Amongst the major species of agronomic interest belonging to the genus *Prunus*, the peach tree is considered the most important for the world economy. In the State of Rio Grande do Sul (RS), peach cropping is an important agricultural activity and the State accounts for the production of approximately 137 thousand tons/years, about 60% of the national production of this fruit (IBGE, 2015).

Taking into account that the pre-harvesting and harvesting season of the fruit in Southern Rio Grande do Sul State takes place between November and January, this period coincides with the highest shortage of water resources throughout the years due to the decreased rainfall (TIMM et al., 2007a,b). Along with the high irradiance and high temperatures, such stresses affect negatively the quality and yield of fruit farms, since the number of farmers who use some irrigation system is still limited (TIMM et al., 2007b).

The water deficit causes changes in the plant behavior and the irreversibility of the negative effects of the stress depends on the genotype, duration, intensity, and developmental stage of the plant, in addition to the several interactions soil-plant-atmosphere as well as other weather conditions (SHAO et al., 2008).

Such a condition may induce responses at all levels of cell organization, though in a physiological level, it affects most of the processes involving gas exchange, stomatal conductance, transpiration, osmotic, and leaf water potential, accelerated senescence, and leaf abscission (JIMÉNEZ et al., 2013).

The stomatal closure is the main mechanism to control gas exchange under water stress, leading to a decrease in net photosynthetic rate, respiration, ion absorption, nutrient metabolism, among others (CHAVES et al., 2009). Due to the decreased photosynthetic ability under water stress conditions, a lower light intensity is used in the photosynthesis, thus, plants cannot use all the light energy to produce ATP and $NADPH_2$, resulting in the photoinhibition (OUKARROUM et al., 2007, MARTINAZZO et al., 2013).

The decrease in stomatal conductance leads to a decrease in the intercellular concentration of CO_2 (C_i) as well as the transpiration. The C_i is a parameter which demonstrates the availability of carbon dioxide for the photosynthesis, and the reduction of C_i values may indicate the occurrence of stomatal limitation (CHAVES et al., 2009) so that the stomatal and mesophyll resistance reduce the concentration of CO_2 which can affect the chloroplasts. The water deficit may also affect the production and use of photoassimilates by inducing a lower assimilation of CO_2 and mobilizing reserves for growth and maintenance of plants under stress. The maintenance of root growth in plants under water stress and under low photosynthetic activity may be due to the preferential targeting of newly-produced photoassimilates to roots and also by mobilizing reserves from other organs to roots (MAGALHÃES FILHO et al., 2008).

The proper supply of water to peach trees is very important when plants leave numbness, which coincides with pollination of flowers and effective fructification. At this phase, the lack of water may increase floral abortion or even limit the ratio of cell division in fruits. Another important phase is during the fast growth of fruits mainly two or three weeks before maturation in which the adequate supply of water contributes significantly to increase the size of fruits (fruit filling). Another important phase is after harvesting fruits, as the plant needs to maintain the uptake of nutrients and keep on the good production of photoassimilates. Those will be stored and later used to support budding and flowering in the next growth season (TIMM et al., 2007a).

In this context, the search for rootstocks presenting differential levels of water-deficit tolerance and improved effectiveness in absorbing water from the soil is the key to reducing losses in the yield and quality of peach fruits (JIMÉNEZ et al., 2013). The differences in the ability of the root system of rootstocks in absorbing water may be responsible for variations in the degree of drought tolerance of scion cultivar (CARBONNEAU, 1985). This ability may provide drought tolerance, which has been demonstrated in other woody species, such as vine (SOUZA; SOARES; ALBUQUERQUE, 2001), almond tree (ROUHI et al., 2007), apple tree (WANG et al., 2012), and citrus (SUASSUNA et al., 2014).

The study of the processes involved in the physiological and biochemical responses of peach trees under water deficit by analyzing the photosynthetic apparatus of plants may provide a better understanding and ease the screening procedures to select more tolerant rootstocks (JIMÉNEZ et al., 2013). Thus we aimed to evaluate the effect of the water deficit on gas exchange parameters of scion cultivar Chimarrita grafted onto five different *Prunus persica* rootstocks.

4 MATERIAL AND METHODS

In this study, we used 3-year-old peach trees cultivar scion Chimarrita grafted onto five *Prunus persica* rootstocks (Tsukuba 1, Tsukuba 2, Tsukuba 3, Aldrighi 1, and Selection UFPel 0402). Plants were removed from the nursery in the second year after grafting and transplanted to 25-litter polyethylene pots filled with soil from the nursery as a substrate classified as dystrophic red-yellow argisol (STRECK et al., 2008).

After achieving one year and four months growing in pots, the plants were used for the experiment, which was conducted in a greenhouse during the month of December, 2013, in Campus Capão do Leão, at University Federal of Pelotas.

Plants were subjected to the following treatments: (i) stress by water deficit, consisting of the full suspension of irrigation from the beginning of the experiment on 19/12/2013, and (ii) plants irrigated daily with 5 litters of water for each pot/combination, preserving field capacity (control). Plants belonging to the treatment by water deficit were kept under stress conditions for six days and, afterwards, subjected to normal irrigation conditions for three days (characterizing the recovery period).

The parameters of gas exchange were assessed: zero, one, two, three, four, and six days of stress, and the seventh and ninth days featured the recovery period. The evaluations were carried out in fully expanded, mature leaves located in the middle third of each branch by using a portable infrared CO_2 analyzer (Model LI-6400XT LI-COR, Inc., Lincoln, NE, USA). Readings were performed in two leaves per plant located in different branches between 9:00 a.m. and 11:00 a.m. with a CO_2 concentration of 380 µmol mol^{-1} in the chamber and photon flow density of 1.200 µmol m^{-2} s^{-1}, by using the light source LI-COR 6400-02 coupled with the camera (LI-COR, Inc., Lincoln, NE, USA LI-COR 6400-11).

During the experiment, the CO_2 net assimilation rate (A, µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), intercellular concentration of CO_2 (C_i, µmol CO_2 mol^{-1}) and the transpiration rate (E, mmol H_2O m^{-2} s^{-1}), for each combination of plants cultivar 'Chimarrita'/rootstock, were assessed.

The experiment was conducted in a completely randomized design with four replicates per treatment in a 2 x 8 factorial, two water conditions (control and water deficit) and eight evaluations (0, 1st, 2nd, 3rd, 4th, 6th, 7th, and 9th). Each combination of grafting between cultivar Chimarrita and different rootstocks was individually assessed. Results were subjected to the analysis of variance ($p \leq 0.05$) and the Tukey test was used in order to compare the means between treatments of each day of stress for each treatment ('Chimarrita'/rootstock) through the System of Statistical Analysis – Win Stat – Version 2.0 (MACHADO & CONCEIÇÃO, 2003).

5 RESULTS AND DISCUSSION

The net photosynthetic rate in control plants cultivar Chimarrita grafted onto different rootstocks ranged from 7.9 to 14.5 µmol CO_2 m^{-2} s^{-1}, exhibiting higher values at the beginning of the experiment and decreasing up to its end (Figures 1A, 2A, 3A, 4A, and 5A). However, the values recorded were within a range consistent to that described for different species of *Prunus*, e.g. studies by Romero et al. (2004), who reported an assimilation of up to 14 µmol CO_2 m^{-2} s^{-1} in adult plants of *Prunus dulcis* in field conditions in Spain. De Herralde; Biel; Savé (2003) recorded values between 14.8 and 20.3 µmol CO_2 m^{-2} s^{-1} in almond trees (*P. amygdalus*) and Rieger; Bianco; Okie (2003), values above 10 µmol CO_2 m^{-2} s^{-1} in *Prunus persica*. In rootstocks of the hybrid 'GxN-9' or 'Monegro' (*P. dulcis* x *P. persica*), Martinazzo et al. (2011) reported values of up to 12.6 µmol CO_2 m^{-2} s^{-1}.

Under water-deficit condition, plants cultivar Chimarrita had a similar decrease for the values of A and g_s up to the sixth day of stress, regardless the rootstock. On the first and second day after the beginning of the water deficit, the different combinations of 'Chimarrita'/rootstocks had a similar behavior to their controls, except for 'Chimarrita' plants grafted onto 'Aldrighi 1' (Figure 4A and B) that had a less pronounced reduction in the values of A and g_s on the second day of water deficit (11.95 µmol CO_2 m^{-2} s^{-1}, 0.28 mol H_2O m^{-2} s^{-1}, respectively) with values higher than the other combinations of graftings.

The most striking reductions in the values of A and g_s under water stress occurred from the third day on, reaching minimum values on the fourth and sixth day. The opposite was observed by Messchmidt et al. (2015) in plants cultivar Mirabolano 29-C under water-deficit conditions whose A and g_s values were 47% and 84%, respectively, lower than control plants on the first day of evaluation reaching values close to zero on the eighth day.

In control plants of ‘Chimarrita’ grafted onto ‘Tsukuba 2’ (Figure 2A and B) and ‘Selection UFPel 0402’ (Figure 5A and B) we observed the lowest values of A and g_s throughout the evaluation period. Furthermore, on the second day of water shortage, it was the combination that recorded the lowest means for the analyzed parameters, which allows us to suggest that these rootstock induces a low tolerance to such stress especially compared to the responses induced by ‘Tsukuba 3’ and ‘Aldrighi 1’. The rootstock ‘Selection UFPel 0402’ has the lowest vigor in relation to all others analyzed (data not shown), which could be one of the factors associated to the induction of the lowest values of A and g_s in cultivar Chimarrita.

For the different combinations ‘Chimarrita’/rootstocks, both for A and g_s in the 72-hour recovery period, plants reached very similar values to their respective controls, except for data of A from the combination ‘Chimarrita/Tsukuba 1’ that had a photosynthetic rate 25% lower in relation to the control.

In *Prunus* spp. plants, Messchmidt et al. (2015) found that the water deficit caused a reduction of 43% in A values on the eighth day of stress in comparison to the values of control plants. When subjected to the recovery, A of plants previously exposed to water deficit reached similar values to control three days after the reposition of water in soil, corroborating the results of the present study. According to Torrecillas et al. (1996), the fast recovery after a stress condition might be related to an increased tolerance to water deficit, however, such an effect should be further investigated in the case of the ‘Selection UFPel 0402’, due to lower A and g_s patterns induced in cultivar scion.

As it was observed in the present study, Rouhi et al. (2007) found the same behavior in *Prunus persica* and *Prunus lycioides* plants where g_s had a pronounced decreasing trend under water deficit induced by PEG 6000, yet such decrease in g_s did not reflect in an increase in the efficient use of water, evidencing that there was no stomatal limitation.

Figure 1. CO_2 net photosynthesis rate *(*A), stomatal conductance (*g*s), transpiration (E), and intercellular concentration of CO_2 (*Ci*) of peach trees scion cultivar ‘Chimarrita’ grafted onto rootstock ‘Tsukuba 1’ subjected to water deficit for six days. Arrows indicate recovery period (7=24h and 9=72h, respectively). Means compared within each day, and the asterisks indicate significant difference by Tukey test at 5% probability (n = 4). Bar indicates standard deviation of the mean.

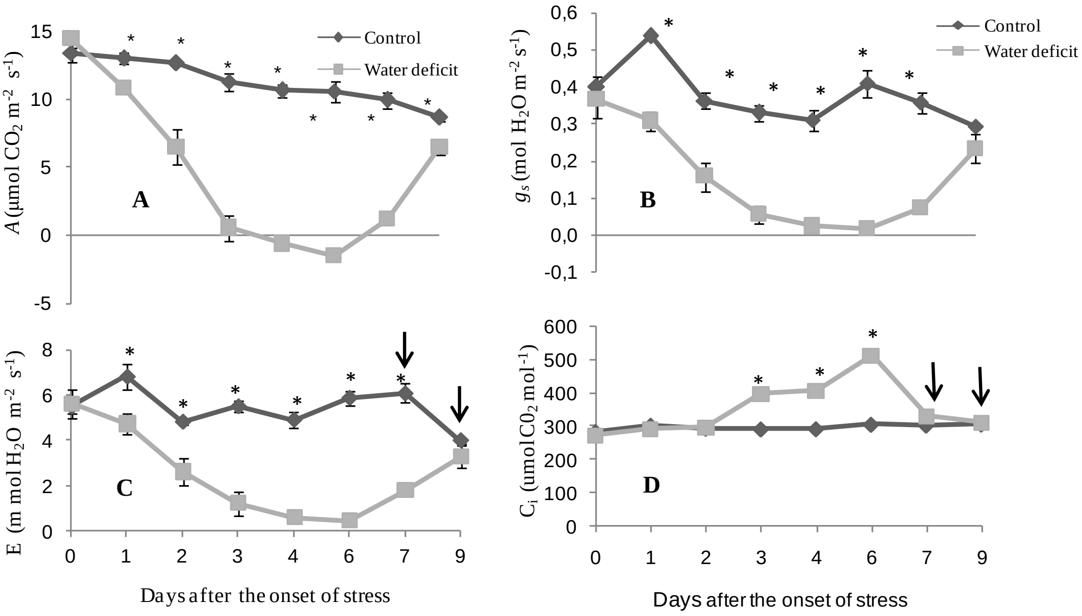


Figure 2. CO_2 net photosynthesis rate (*A*), stomatal conductance (g_s), transpiration (E), and intercellular concentration of CO_2 (C_i) of peach trees scion cultivar ‘Chimarrita’ grafted onto rootstock ‘Tsukuba 2’ subjected to water deficit for six days. Arrows indicate recovery period (7=24h and 9=72h, respectively). Means compared within each day, and the asterisks indicate significant difference by Tukey test at 5% probability (n = 4). Bar indicates standard deviation of the mean.

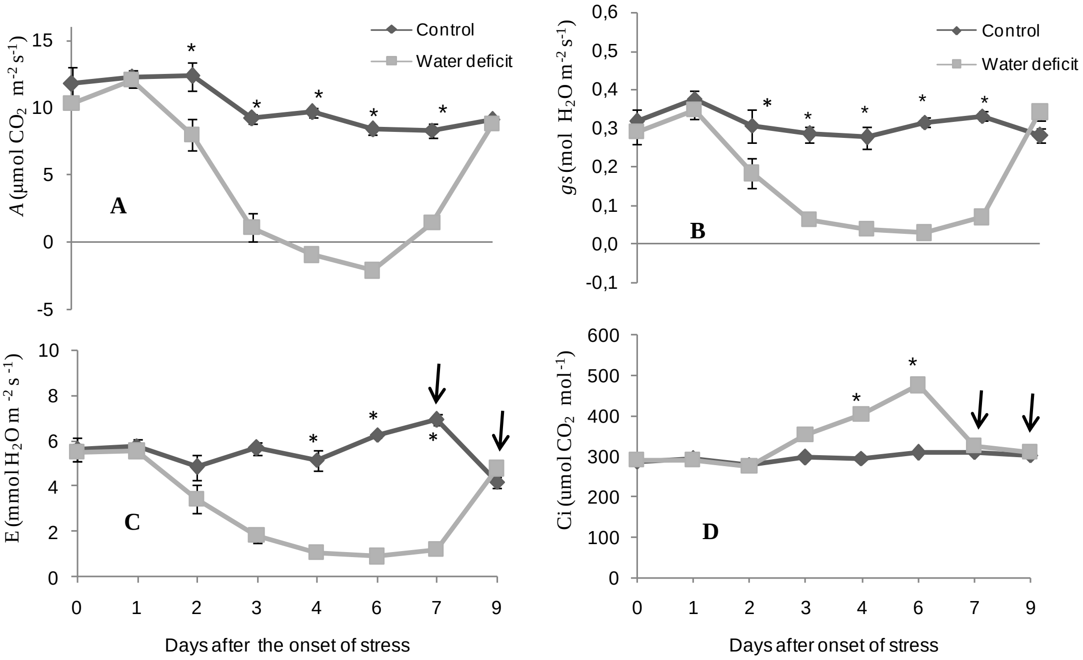


Figure 3. CO_2 net photosynthesis rate (*A*), stomatal conductance (g_s), transpiration (E), and intercellular concentration of CO_2 (C_i) of peach trees scion cultivar 'Chimarrita' grafted onto rootstock 'Tsukuba 3' subjected to water deficit for six days. Arrows indicate recovery period (7=24h and 9=72h, respectively). Means compared within each day, and the asterisks indicate significant difference by Tukey test at 5% probability (n = 4). Bar indicates standard deviation of the mean.

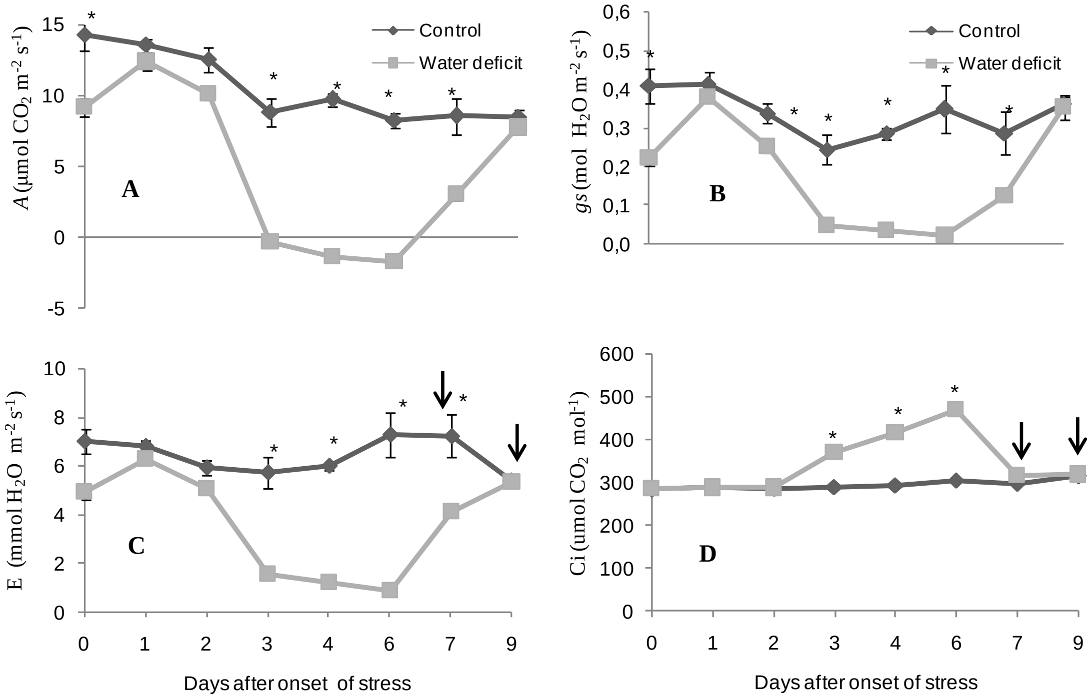


Figure 4. CO_2 net photosynthesis rate (*A*), stomatal conductance (g_s), transpiration (E), and intercellular concentration of CO_2 (C_i) of peach trees scion cultivar ‘Chimarrita’ grafted onto rootstock ‘Aldrighi 1’ subjected to water deficit for six days. Arrows indicate recovery period (7=24h and 9=72h, respectively). Means compared within each day, and the asterisks indicate significant difference by Tukey test at 5% probability (n = 4). Bar indicates standard deviation of the mean.

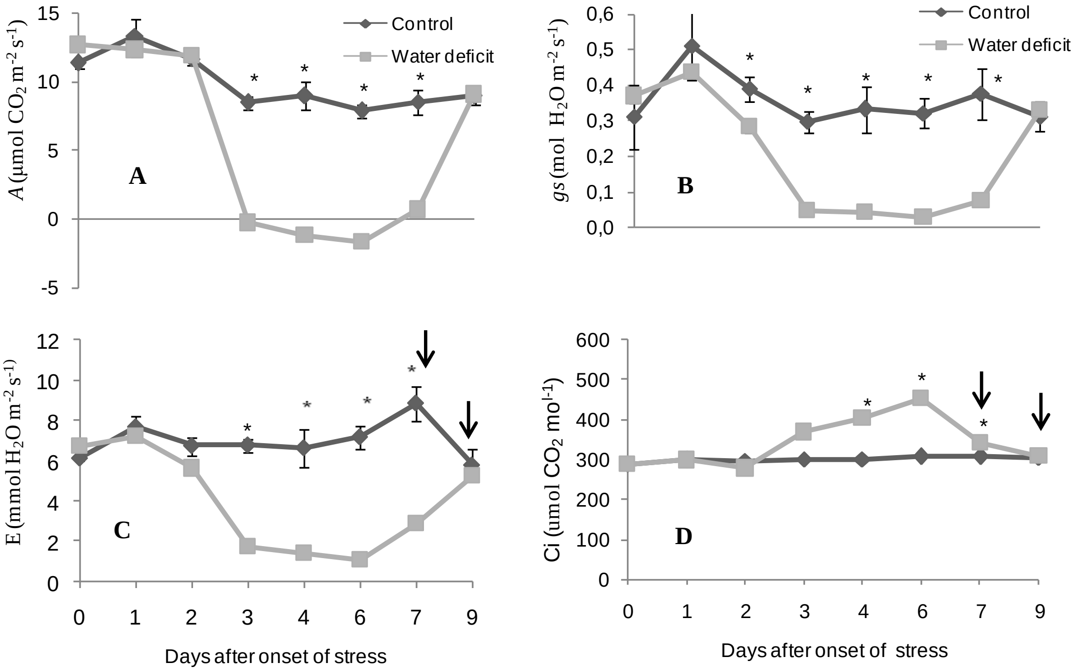
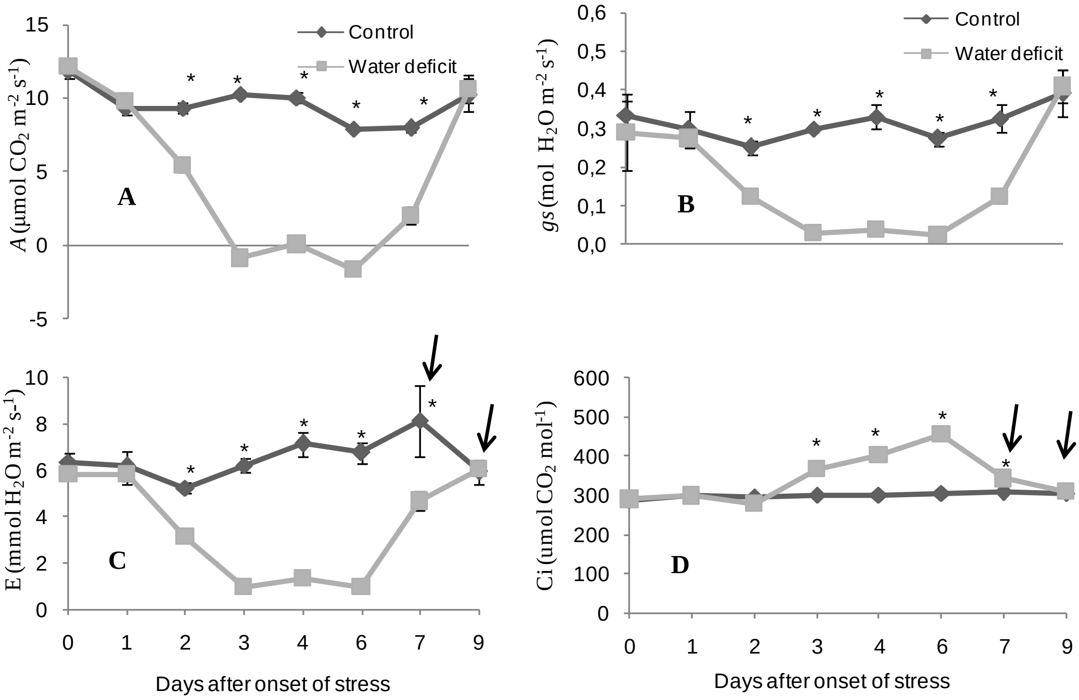


Figure 5. CO_2 net photosynthesis rate (*A*), stomatal conductance (g_s), transpiration (E), and intercellular concentration of CO_2 (C_i) of peach trees scion cultivar ‘Chimarrita’ grafted onto rootstock ‘Seleção UFPel 0402’ subjected to water deficit for six days. Arrows indicate recovery period (7=24h and 9=72h, respectively). Means compared within each day, and the asterisks indicate significant difference by Tukey test at 5% probability (n = 4). Bar indicates standard deviation of the mean.



Regarding values of intercellular concentration of CO_2 (C_i) and transpiration (E), all combinations ‘Chimarrita’/rootstocks had a drastic decrease in plants subjected to water deficit (Figures 1C and D, 2C and D, 3C and D, 4C and D, and 5C and D). However, there was an exception for C_i data from the third day up to the sixth day of stress, which coincides with the behavior observed for *A* e g_s, C_i values for plants under stress have always been higher than the control in all combinations of ‘Chimarrita’/rootstocks during the third, fourth, and sixth day and even in the 24-hour recovery period.

For all plants subjected to water deficit, the trend of E coincided with the reductions observed in *g*s throughout the experimental period, reaching the lowest values on the sixth day, showing an increased E from the first recovery day (24h), when returning the water supply to plants under stress, equalizing E values to the control in the 72-hour recovery period.

According to Suassuna et al. (2014), such direct relation between E and g_s is expected due to the partial closure of stomata, which narrows both the output of water vapor and the input of CO_2 into the cell. Furthermore, the intensity of E varies as with the action of internal factors, such as light, moisture, temperature, and water availability in soil.

Under water-deficit condition, the direct relationship of the reduction in the C_i due to the stomatal closure has been demonstrated, causing decreases in the assimilation of CO_2 and the quantum yield of the photosystem II (TEZARA et al., 2003). When there is an increase in C_i by decreasing g_s, as observed in the present study under water deficit, the decrease in *A* indicates that such limitation is not only due to the increase in stomatal resistance, but also to the effect of the water stress on *A*, since a high C_i value associated to low g_s values would indicate a decrease in the efficiency of carboxylation (NI; PALLARDY, 1992), as found in this study (Figures 1, 2, 3, 4 and 5).

Another probable consequence of the increased C_i with the stomatal closure is the reduction of *A* due to predominance of non-stomatal limitations with the occurrence of the mesophyllic resistance (RAMANJULU; SREENIVASULU; SUDHAKAR, 1998), supported by the low conductance of the mesophyll which reduces the diffusive ability of CO_2.

The accumulation of this gas in the substomatal chamber promotes the stomatal closure, resulting in an inhibition of the stomatal conductance and a decrease in the activity of enzymes involved in the process of CO_2 fixation, such as the enzyme rubisco in the carboxylative form which would increase its function as oxigenase and, therefore, the photorespiration, resulting in an energy unbalance (SAGE; SAGE; KOCACINAR, 2012). Moreover, it may also be due to lack of space to accumulate more photoassimilates, since the remobilization of the synthesis sites to the storage sites can be compromised due to water deficiency.

In almond trees cultivar America (*P. salicina*) under water deficit, Martinazzo et al. (2013) found that the C_i values reduced throughout the stress period, also followed by a reduction in the photosynthetic activity, which is related to the decrease in the carboxylation efficiency. In rootstock plants 'GxN-9', Martinazzo et al. (2011) found a reduction of the photosynthetic activity from the fifth day under water deficit and it was related to the decrease in the carboxylation efficiency, yet with no significant alteration in C_i.

Jiménez et al. (2013) found that plants of four hybrid *Prunus* rootstocks after 16 and 26 days of water deficit had a decrease in all parameters evaluated: *A*, g_s, E e C_i. Rieger; Bianco; Okie (2003) also found after 24 days a decrease in the parameters *A* (49%) and g_s (83%) in *Prunus ferganensis* plants under the same stress condition.

Other authors have reported that even in short term, the decrease in the photosynthesis may not be attributed only to stomatal limitations (NI; PALLARDY, 1992, RAMANJULU; SREENIVASULU; SUDHAKAR, 1998, ROUHI et al., 2007), which can be noticed in this study through the increase in C_i and, at the same time, decrease in g_s. According to Chaves et al. (2009), such a response may be attributed to a heterogeneous stomatal closure and / or interference of the cuticular conductance. For Ramanjulu; Sreenivasulu; Sudhakar (1998), the relative importance of stomatal in relation to the non-stomatal limitations seems to depend both the intensity and duration of stress and the tolerance of the species/genotype to water deficit, which is generally less important for plants with an increased tolerance to such stress or during softer tensions.

Based on the parameters of gas exchange assessed, it was possible to observe a trend for higher water-deficit tolerance even on the third day of stress in plants grafted onto 'Aldrighi 1' wich is feasibly related to the medium vigor of this genotype (SCHMITZ et al., 2012) followed to an increased volume of the root system (data not shown). It was also found that 'Chimarrita' plants grafted onto 'Tsukuba 3' and 'Selection UFPel 0402' showed faster water-deficit recovery (24h). However, 'Tsukuba 3' seems to induce a higher initial water-deficit tolerance as well as present an increased ability of post-stress recovery, yet such response need further investigation, both at physiological and molecular level, aiming to a better understanding of the interactions of different genotypes under water stress conditions.

Along with the above mentioned, another question to be further investigated is the responses induced in 'Chimarrita' by the rootstock 'Selection UFPel 0402' where we observed the lowest values of *A* and g_s, including control plants, which might be used as parameters indicating a lower water deficit tolerance.

6 CONCLUSIONS

- We can conclude that the different combinations of grafting between rootstocks and the scion cultivar Chimarrita show a differential physiological behavior for the initial water-deficit tolerance. The combinations ‘Chimarrita/Aldrighi 1’ and ‘Chimarrita/Tsukuba 3’ presented an increased initial water-deficit tolerance.
- The decrease in the CO_2 net assimilation rate throughout water-deficit period is not related mostly to the stomatal limitation, suggesting that there may also be non-stomatal limitations.
- The water deficit for up to six days affects parameters related to gas exchange in plants of different combinations of *Prunus* cultivar ‘Chimarrita’/rootstocks, however, irreversible, since after 72h after supplying water back to plants, all the combinations evaluated showed a full ability to recover the physiological performance.

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