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RESEARCH ARTICLE

Photosynthetic activity in the rootstock of hybrid peach trees submitted to water restriction and flooding

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ABSTRACT

This study aimed to evaluate the effect of flooding and water restriction (drought) on the photosynthetic activity in plants of the hybrid rootstock ‘GxN-9’ (*Prunus dulcis* Mill. × *Prunus persica* L. Bastsch), grown in a greenhouse. Chlorophyll fluorescence and gas exchange were analyzed in plants of approximately 12 months. The reduction of photosynthetic activity in both the stresses were associated with decreased carboxylation efficiency, but without affecting the internal concentration of carbon dioxide. The JIP-test parameters revealed changes in the energy flux processes demonstrating that in terms of flooding the rootstock GxN-9 the rate of photosynthetic performance significantly decreases, resulting in a considerable decrease in net assimilation rate**.** The JIP-test analysis was efficient in determining the thresholds mainly of the flood response, proving to be useful in exploration of the photosynthetic activity as a physiological marker of the stress tolerance.

Key words: Abiotic stress; chlorophyll *a* fluorescence; gas exchange; JIP-test; *Prunus dulcis* Mill. × *Prunus persica* L. Bastsch.

INTRODUCTION

Water stress is an important environmental factor that affects photosynthesis, affecting plant growth (Akcay et al., 2010). Plants respond to this stress through adaptive mechanisms allowing the photochemistry and photosynthesis biochemistry phases, supporting adverse conditions (Oukarroum et al., 2007).The photosynthetic capacity is gradually reduced in water stress conditions as a consequence, there is a decrease in the utilization of light energy, causing only part of the energy absorbed by the photosynthetic apparatus which is used for ATP and $NADPH_2$ production increasing photoinhibition (Oukarroum et al., 2007).

Drought has a profound impact on ecological systems and agriculture, since the plant reactions to stress differ at various organizational levels, depending on the intensity and duration of stress, species or developmental stage (Shao et al., 2008). On the other hand, flooding imposes restrictions on the cultivation of many species, due to low availability of oxygen to the root system (Drew, 1997), which can be harmful or even lethal to plants because it blocks the transfer of oxygen and other gases between the soil and atmosphere (Steffens and Sauter, 2010).

The action of these types of stress can induce profound changes in plant metabolism and its effect can be assessed by analyzing different parameters. The analysis of chlorophyll *a* fluorescence allows monitoring changes in the photosynthetic apparatus without destroying the plants, being a highly sensitive technique (Tsimilli-Michael and Strasser, 2008; Falqueto et al., 2010). Analysis of the kinetic emission of chlorophyll *a* fluorescence, by using the JIP-test (Strasser and Strasser, 1995) enables a better understanding and interpretation of chlorophyll *a* fluorescence data, since the light energy absorption by the antenna system of the photosystem II (PSII) reduce the final electron acceptor of the photosystem I (PSI).

The genus *Prunus* includes species that produce fruit of great commercial importance (such as the peach, almond, plum, apricot and cherry) used as rootstocks and/or ornamental. Successful cultivation of stone fruit species in southern Brazil relies on some important requirements, including the use of seedlings with guaranteed quality, for achieving high orchard productivity. Furthermore, this region may, during periods of drought or excessive rain, which combined with soils deficient in retaining water and/or drainage, compromise the plant production. Thus, for the successful implementation of fruit species the use of rootstocks tolerant to these stresses is necessary.

This study aimed to evaluate the effect of flooding and water stress (drought) on the photosynthetic activity in plants of the rootstock hybrid 'GxN-9' (*Prunus dulcis Mill. Bastsch* × *Prunus persica L.*) to identify parameters that may be markers of tolerance of these stresses.

MATERIAL AND METHODS

Seedlings of hybrid rootstock 'GxN-9' (*P. dulcis* Mill. × *P. persica* L. Bastsch), obtained through asexual reproduction by tissue culture at the Laboratory of Cell Culture and Plant Tissue (Botany Department - Federal University of Pelotas), were transplanted to polyethylene vessels with a volume of 10 L, containing commercial substrate (Plantmax®), and regularly supplied with nutrient solution (Hoagland and Arnon, 1950). The seedlings were kept in a greenhouse, and when they reached approximately 12 months, were treated with: (1) flooding, which consisted of maintaining water depth of approximately 25 mm above ground level and (2) water deficit which consisted of the suspension of irrigation and (3) control, which was irrigated daily. The parameters of chlorophyll *a* fluorescence transient gas exchange were evaluated in the first, fourth, seventh and tenth days for flooded plants and in the second, fifth, eighth and eleventh for the plants in water deficit.

For the determination of chlorophyll *a* fluorescence transient, we used four mature leaves not detached of the three plants, totalizing 12 readings. The leaves were adapted to the dark for 30 minutes. The fluorescence intensities were measured using a portable fluorometer Handy-PEA (Hansatech Instruments Ltd., King's Lynn Norfolk, PE 30 4NE, UK) following the issuance of a saturating pulse of red light of approximately 3,000 μmol m^{-2} s^{-1}. The JIP-test (Strasser and Strasser, 1995; Tsimilli-Michael and Strasser, 2008) was employed to analyze the chlorophyll *a* fluorescence transient. The following data from the original measurements were used: maximal fluorescence intensity (F_M); fluorescence intensity at 50 μs (considered initial fluorescence - F_0); fluorescence intensity at 300 μs; fluorescence intensity at 2 and 30 ms (the J- and I- step, denoted as F_J and F_I, respectively). The JIP-test parameters were calculated with the use of the software 'Biolyzer' - Laboratory of Bioenergetics, University of Geneva, Switzerland (courtesy of Dr. R. Strasser).

The gas exchanges were measured in mature leaves, fully expanded, located in the middle third of each branch using a portable infra-red CO_2 analyzer (model LI-6400XT LI-COR Inc., Lincoln, NE, USA). The readings were taken on three plants and three leaves per plant, totaling nine leaves per treatment and collection. Measurements were taken between 9:00 and 11:00 am, with the CO_2 concentration in the chamber of 380 mol mol^{-1} and photon flux density of 1,200 μmol m^{-2} s^{-1}, using the light source LI- COR 6400-02, coupled to the measuring chamber (LI-COR 6400-08, LI-COR Inc., Lincoln, NE, USA).

The experiment was conducted in a completely randomized design in a factorial arrangement (3×4) with three treatments (water regimes) and four collections, and the averages compared by the Tukey test ($p < 0.05$).

RESULTS

In relation to specific fluxes by reaction center (RC), it was observed that the flux absorption (ABS/RC), from the capture (TR_0/RC) and especially, energy dissipation per reaction center (DI_0/RC) increased relative to the control for both stresses (Figure 1A and B). However, the electron transport flux (ET_0/RC), reduction of the final electron acceptor (RE_0/RC) and total number of electron carriers per reaction center (EC_0/RC) had decreased compared to the control.

For JIP-test parameters that describe the quantum yields (Figure 1A and B) revealed that: (*i*) photochemical quantum yield (ϕ_{Po} or TR_0/ABS,) showed no effect in the water stresses studied, (*ii*) the quantum yield of electron transport (ϕ_{Eo} or ET_0/ABS,) was more affected only by the flooding with the lowest values observed at day 10, (*iii*) the quantum yield of electron transport from Q_A^- to the final electron acceptor of the PSI (ϕ_{Ro} or RE_0/ABS) was the parameter that showed the greatest reduction compared to the control in both stresses, and for flooded plants the lowest values were obtained after 10 days of flooding, while for plants exposed to water deficit the lower values were obtained from the eighth day of water restriction.

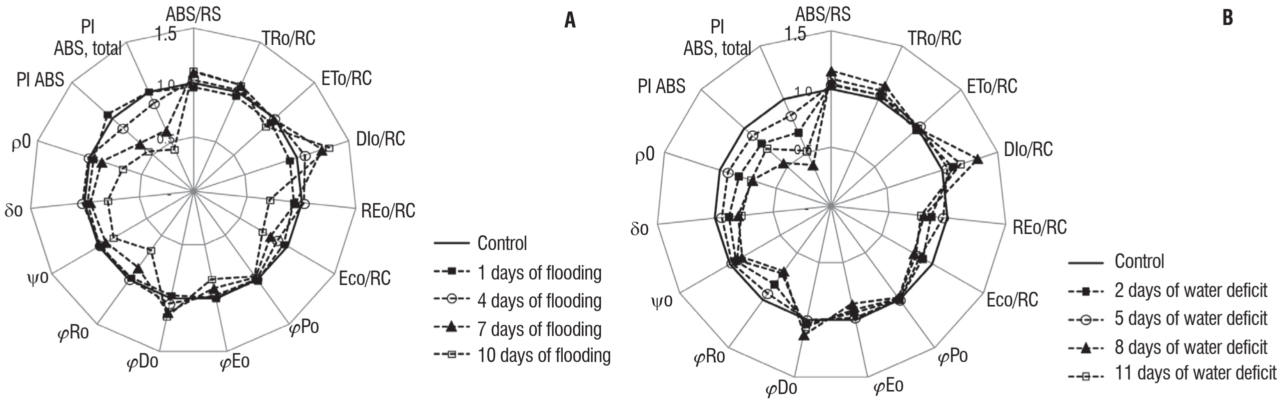

Figure 1. JIP-test parameters calculated from the chlorophyll *a* fluorescence transient in the plant rootstock hybrid 'GxN-9' (*Prunus dulcis* Mill. Bastsch × *Prunus persica* L.) subjected to flooding (**A**) and water deficit (**B**) for different periods. For each parameter, values were normalized using the respective control as a reference.

Table 1. Net photosynthetic rate (A, μmol CO_2 m^{-2} s^{-1}), internal CO_2 concentration (Ci, μmol CO_2 mol^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}), stomatal conductance (gs, mol H_2O m^{-2} s^{-1}) and efficiency use water (EUA) of the rootstock hybrid 'GxN-9' (*Prunus dulcis* Mill. Bastsch × *Prunus persica* L.) submitted to water deficit and flooding for different periods.

Tratments		**Collection (1)**			
		1	**2**	**3**	**4**
A (μmol CO_2 m^{-2} s^{-1})	Control	11.01 aB(2)	11.67 aA	12.25 aA	12.69 aA
	Water Deficit	10.46 abB	11.14 aA	9.87 abB	7.94 bB
	Flooding	13.35 aA	12.83 aA	8.59 bB	7.79 bB
Ci (μmol CO_2 mol^{-1})	Control	298 aB	297 aA	278 aA	294 aA
	Water Deficit	307 aB	303 aA	302 aA	305 aA
	Flooding	297aB	290 aA	194 aB	244 aB
E (mmol H_2O m^{-2} s^{-1})	Control	6.57 aA	5.82abB	5.16 bA	6.40 aA
	Water Deficit	6.96 aA	5.42 bB	4.93 bcA	4.52 cB
	Flooding	7.00 aA	6.55 aA	2.08 cB	3.10 bC
gs (mol H_2O m^{-2} s^{-1})	Control	0.439 aB	0.442 aA	0.412 aA	0.468 aA
	Water Deficit	0.506 aA	0.462 abA	0.389 bcA	0.319 cB
	Flooding	0.547 aA	0.446 bA	0.099 cB	0.139 cC
EUA	Control	1.68 bB	2.01 abA	2.54 aB	1.99 abB
	Water Deficit	1.50 bB	2.05 aA	1.99 aB	1.74 abB
	Flooding	1.91 bA	1.97 bcA	4.29 aA	2.65 bA
Carboxylation efficiency (A/Ci)	Control	0.037 aB	0.040 aA	0.044 aA	0.043 aA
	Water Deficit	0.034 aB	0.037 aA	0.033 aB	0.026 bB
	Flooding	0.045 aA	0.045 aA	0.045 aA	0.033 bB

(1) For treatment of flooded collections correspond to 1, 4, 7 and 10 days after onset of stress; for the treatment of water deficit samples corresponding to 2, 5, 8 and 11 days after the start of stress. (2) Significant differences are indicated by different lower case letters in column and upper case letters in line by Tukey's test at $P < 0.05$.

The parameters that describe the energy flux efficiency (ET_0/TR_0 - ψ_0; RE_0/ET_0 - δ_0 and RE_0/TR_0 - ρ_0) presented different behavior between the types of stress. For plants subjected to flooding, a sharp reduction was observed after 10 days of flooding in the three parameters. In contrast, for the plants under water deficit it was gradually reduced in response to the stress period.

The total performance index PI_{ABS} and PI_{ABS} showed different behaviors for the two plants under stress. Since, for the flooded plants, the reduction in both parameters occurred only after the fourth day of stress (Figure 1A), whereas plants subjected to stress by water restriction, the reduction was observed after the second day of drought (Figure 1B).

For the flooded plants, the net assimilation rate of CO_2 (A) and stomatal conductance (gs) showed a sharp reduction in the control after seven days, with no change in internal CO_2 concentration, which remained similar to the control. The transpiration rate (E) was lower at seven days of flooding and increased at 10 days, but it was less than the first and second days of flooding. The carboxylation efficiency (A/Ci) differed from control only at 10 days of flooding (Table 1).

Water restriction resulted in a net photosynthetic rate decrease, which reached values close to flooding in the last collection, and stomatal conductance decreased with the stress elapse. The transpiration rate followed the behavior of the stomatal conductance and internal CO_2 concentration remained similar to the control plants. The carboxylation efficiency decreased with the passing of the stress days, reaching values lower than the control at 11 days of water deficit (Table 1).

DISCUSSION

The interpretation of the chlorophyll a fluorescence transient reveals changes in the energy flux processes, to reduce the pool of plastoquinone (PQ) and plastoquinol (PQH_2) until the final PSI acceptors.

The increase in ABS/RC, measured by the apparent size of the antenna (total absorption or total chlorophyll per active center reaction), could mean that: (a) a fraction of the RC is inactive, or (b) the antenna that provides excitation energy for active RC, increased in size (Yusuf et al., 2010). Whereas, the total number of electron carriers per reaction center (EC_0/RC) decreased, the increase in ABS/RC can be linked to the fact that the antenna system transferred excitons to the active reaction centers, but dissipation of excitation energy as heat, occurred.

The heat dissipation can be confirmed by the increase in the energy dissipation flux by RC (DI_0/RC) and quantum yield to dissipation (ϕ_{D0}). This dissipation refers to the loss of the energy absorbed by PSII, through heat, fluorescence emission, or even, energy transfer to other systems (Strasser et al., 2000). The energy dissipation at the system antenna level reduces the proportion of energy absorbed in the photochemistry phase and the over-excitation risks related to the flux of electrons (Bussotti et al., 2011).

The increase in DI_0/RC and ϕ_{D0} was accompanied by stomatal closure, two types of stress were observed. Thus, one can propose that limiting the CO_2 availability for the photosynthesis biochemical reactions, reduce the flux of energy in the electron transport chain in order to regulate the power reducing formation of and prevent photoinhibition.

The photochemical quantum yield ($\phi_{P0} = TR_0/ABS = F_V/F_M$) did not demonstrate to be a stress indicator, since it showed constant values over the following days of flooding and drought, corroborating with recent reports, citing that this parameter is not a good indicator for water stress (Oukarroum et al., 2007). A slight decrease in the photochemical quantum yield during the drought was observed by Oukarroum et al. (2007), accompanied by a decline in the performance index (PI_{ABS}) and significant changes in other JIP-test parameters. When watered, PI_{ABS} presented values which suggest these do not cause irreversible damage to the photosystems. The water deficit effect is reversible, since ϕ_{P0} remains unchanged (Oukarroum et al., 2007). However, changes in the photosynthetic apparatus can be confirmed by changes in the performance levels (PI_{ABS} and $PI_{ABS,total}$) (Strasser et al., 2000, 2004; Tsimilli-Michael and Strasser, 2008). These parameters are excellent stress indicators as they allow the full interpretation of the absorption system and energy utilization in the photosynthetic electron transport chain (Oukarroum et al., 2007).

The fluorescence in leaves adapted to the dark, can reveal the potential photosynthesis efficiency. However, the decline of some parameters such as PI_{ABS} may be related to a decrease in the photosynthetic rate because, in favorable conditions, the efficiencies "potential" and "real" tend to be related (Bussotti et al., 2011).

The total performance index ($PI_{ABS,total}$), which measures the performance until the final electron acceptor of the PSI (Tsimilli-Michael and Strasser, 2008), it decreased considerably over the days of flooding. The reduction in PSI density and decrease in final electron acceptor (ferredoxin and $NADP^+$) causes an imbalance in which occurs between the electrons sent through the electron transport chain to recipients which are beyond the PSI (Bussotti et al., 2011).

The decrease in the net photosynthetic rate at eight days of water restriction, as well as at later stages, was dependent on stomatal closure. Photosynthesis in drought-stressed plants is strongly inhibited by stomatal closure, which limits the diffusion of CO_2 to the chloroplasts, causing decrease in internal CO_2 concentration (Oukarroum et al., 2009).

The decrease in net photosynthetic rate after seven days in flooded conditions was dependent on the decrease in stomatal conductance, which strongly limited the influx of CO_2 in the mesophyll and therefore also restricted water loss through transpiration. Changes in gs depend on hydraulic factors of the stomatal cells (water content, water potential and turgor pressure), including the water transport through membranes in addition to metabolic processes (eg, those related to ABA) (Lawlor and Tezara, 2009).

The reduction of the carbon dioxide assimilation, 10 days after flooding and 11 days of water deficit was characterized by decreases in carboxylation efficiency in the same period. The reduction of the reducing power and energy carrier molecules may be one explanation for the decrease of such parameters.

CONCLUSIONS

There is a differential response for 'GxN-9' plants due to the flooding and water deficit. The JIP-test analysis was efficient in determining the greatest stress level under flooded conditions and, together with the gas exchange analysis, it can be concluded that 'GxN-9' is more sensitive to excess water in the root system in relation to water deficit.

The reduction of photosynthetic activity is associated with decreased carboxylation efficiency, even if the conditions imposed by the stresses do not affect the internal carbon dioxide concentration.

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