



Short-term root flooding and recovery conditions—affected photosynthetic process of common bean plants

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Abstract Photosynthetic process of common bean plants (*Phaseolus vulgaris* L.) was evaluated from short-term root flooding and recovery conditions. Common bean plants (BRS Expedito genotype) were grown in single plastic pots (1 L), containing soil as substrate. At the early reproductive stage (R1), distilled water was added up to 20 mm above the soil surface to flood the root system of plants for 1 day. The flooding was maintained by fitting a second pot without holes. After 1 day of flooding, the pots without holes were removed to drain water and recover the plants. Control plants were kept under normoxia. Chlorophyll *a* fluorescence transient, gas exchange, glycolate oxidase, antioxidative enzymes, reactive oxygen species (ROS) and lipid peroxidation were measured in leaves upon flooding (1 day) and recovery (1, 3 and 7 days) conditions. Root flooding (1 day) induced decrease (two-fold) in CO₂ assimilation rate and did not recover even after 7 days of normoxic conditions was re-established, besides limited transpiration rate and decreases in stomatal

conductance. Moreover, the continuous light energy absorption by chlorophylls induced an increase in fluorescence and heat and impaired the connectivity between photosystems I and II, leading to ROS formation. The antioxidative enzyme system induced upon flooding and recovery conditions did not deal efficiently with ROS, which led to oxidative damage (lipid peroxidation) in leaves of common bean. Therefore, short-term root flooding impairs photosynthetic process recovery of common bean plants upon re-establishment of normoxic conditions.

Keywords Antioxidative enzymes · Oxidative damage · *Phaseolus vulgaris* L. · Photosynthesis · Waterlogging

1 Introduction

Climate changes are increasing flooding events since the 1950's worldwide (Bailey-Serres et al. 2012; Limami et al. 2014; Pedersen et al. 2017), especially in the 28 million hectares of flood-prone located in Cerrado and lowlands in the South of Brazil (Jackson and Colmer 2005), due to increases in heavy rainfall (IPCC 2014). Currently, the lowland areas are mainly occupied with rice cropping system (Garcia et al. 2020), and food production by small farmers, such as common bean (*Phaseolus vulgaris* L.). Common bean

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provides as much as 30% of the daily human dietary protein (Celmeli et al. 2018), however, this crop is extremely sensitive to flooding conditions (Posso et al. 2018).

The lowland soil characteristics influence the duration of flooding stress, since oxygen has a 10^4 -fold lower diffusion in water than in air (Armstrong et al. 1994). Low oxygen availability around the root system shift aerobic to anaerobic respiration (Limami et al. 2014) and induces a metabolic reorganization for energy synthesis and consumption (Pedersen et al. 2017). Low ATP synthesis occurs through glycolytic reactions (Pasteur effects), maintained by fermentative reactions upon oxidative phosphorylation inhibition (van Dongen and Licausi 2015; Pucciarello and Perata 2017). The end products of fermentation and low ATP availability lead to cytosol acidification (Yang et al. 2014), which results in aquaporin inhibition (Tournaire-Roux et al. 2003). Upon aquaporin inhibition, water uptake by roots and hydraulic transport to the shoot are impaired (Aroca et al. 2012), leading to a decrease in photosynthetic rates (Santos Jr. et al. 2015; Posso et al. 2018; Garcia et al. 2020), and changes in the dynamic dissipation of the photosynthetic energy in common bean (Posso et al. 2018). However, when normoxic conditions are re-established in the root zone, its extent effect on shoot may increase the CO_2 assimilation rates that was affected by flooding, as shown by soybean genotypes after 7 days of root flooding (Garcia et al. 2020).

It is well-known that the extent effects of root flooding on the shoot are related to impairment on photosynthesis due to a reduction in CO_2 intake (Ahmed et al. 2002; Bansal and Srivastava 2015). Although photosynthesis is hampered, light energy is continuously absorbed by photosynthetic pigments, which force plants to deal with the excess of light to avoid both the over-reduction of the electron transport chain (ETC) (Bräutigam and Gowik 2016; Hanawa et al. 2017) and reactive oxygen species (ROS) production, such as hydrogen peroxide (H_2O_2) and anion superoxide ($\text{O}_2^{\cdot-}$) (Gill and Tuteja 2010; Bräutigam and Gowik 2016; Hanawa et al. 2017; Mitler 2017; Ivanov et al. 2018). ROS is reported to be scavenged by an efficient induction of antioxidative enzyme system (Blokхина and Fagerstedt 2010a, b), to avoid oxidative damage that lead to lipid peroxidation (Gill and Tuteja 2010; Mitler 2017). Besides, photorespiration is also reported to be an alternative sink

drain of electrons in plants with C3 metabolism (Bräutigam and Gowik 2016; Posso et al. 2018).

Several authors have reported a rapid recovery of plants upon re-oxygenation, however, such experiments have employed other substrate than soil, such as vermiculite (Justino and Sodek 2013; Posso et al. 2020), commercial substrate (Kissmann et al. 2014), or even hydroponic system (Borella et al. 2017). The characteristics of these substrates allow rapid and fast drainage and oxygen re-establishment in the root zone, increasing the recovery mechanism of plants. However, besides the negative effects caused directly by the course-time of flooding, especially in flood sensitive common bean (Posso et al. 2018), we hypothesized that the soil of lowlands may take more time to reduce the excess of humidity upon drainage, due to its structural characteristics, which could maintain the soil waterlogged for a longer time (high moisture). Besides, as far as the moisture takes time to decline in the soil, the metabolism of root from common bean plants takes time to recovery and may extent the effects to the shoot.

Responses to flooding and the re-oxygenation vary from species to species depending on severity, exposure time, genotype, stage of development, organ, and tissue (van Dongen and Licausi 2015; Pucciarello and Perata 2017; Borella et al. 2017; Posso et al. 2018), which may be different on shoot tissues of common bean from the roots subjected directly to flooding conditions. Studies of recovery plants from flooding conditions remain scarce, especially the extended recovery effects on the shoot of common bean subjected to short-term root flooding. Therefore, this work aimed to evaluate the effects of short-term root flooding and recovery conditions on the photosynthetic process of common bean plants.

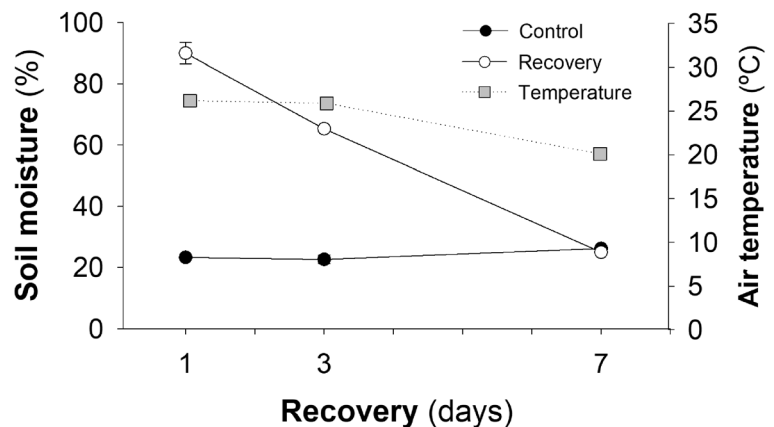
2 Material and methods

2.1 Plant material and growth conditions

Common bean plants (*Phaseolus vulgaris* L.) of BRS Expedito (Antunes et al. 2007) were obtained from seeds. After germination, one plant was grown in a single plastic pot (1 L) with holes to maintain the drainage and avoid water accumulation in the soil. Plants were grown in Hydromorphic Eutrophic Planosol soil [pH (water) = 5.3, CTC = 4.8 cmol_0

dm^{-3} , organic matter = 1.24 dag kg^{-1} (1.24%); $\text{Ca}^{2+} = 2.7 \text{ cmol}_0 \text{ dm}^{-3}$, $\text{Mg}^{2+} = 1.6 \text{ cmol}_0 \text{ dm}^{-3}$, $\text{Al}^{3+} = 0.3 \text{ cmol}_0 \text{ dm}^{-3}$, P (21.1 mg dm^{-3}), K (60.0 mg dm^{-3}), to approach the natural conditions. Each plant was supplied with 20 mL of nutrient solution three times per week (Hoagland and Arnon 1950). At R1 stage (flowering—early reproductive stage; Osorno et al. 2014), plants were subjected to flooding treatment by adding distilled water into the plastic pot up to reach 20 mm of water above the substrate surface. The whole root system was maintained submersed for 1 day [period previously selected due to the sensitivity of common bean plants as reported by Posso et al. (2018)]. The flooding was maintained by fitting a second pot without holes to block the drainage. Control plants were kept under normoxic conditions. After the short-term flooding, the plastic pots were drained by removing the pot without holes to drain water and let the plants to recover for one, three and 7 days. After drainage, the soil moisture was monitored across the experiment up to reach the level of the soil moisture of control plants, which occurred with 7 days of recovery, as well as the air temperature into the greenhouse at the moment of gas exchange analysis was performed, as shown in Fig. 1. The experiment was carried out in a greenhouse under natural light ($\pm 1,000 \text{ } \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) and temperature conditions ($25 \pm 5 \text{ } ^\circ\text{C}$). Leaf samples ($n = 4$, as indicated in the figure legends) were harvested upon flooding (1 day) and recovery 1, 3 and 7 days), and kept frozen ($-80 \text{ } ^\circ\text{C}$) until analysis (all analyses were performed in the fully expanded first trifoliolate leaf).

Fig. 1 Soil moisture and temperature performed during recovery treatment of common bean plants subjected to root flooding conditions. Values represent the mean \pm standard deviation (SD)



2.2 Chlorophyll index and chlorophyll *a* fluorescence

All measurements were taken in the fully expanded first trifoliolate leaf still attached to the plant, collected at 7:00 h. Chlorophyll index was measured with a portable chlorophyll meter (CL-01; Hansatech Instruments, King's Lynn, Norfolk, UK), as described by Cassol et al. (2008). Chlorophyll *a* fluorescence transients were measured with a Handy-PEA fluorimeter (Plant Efficiency Analyser, Hansatech Instruments Ltd, UK), in dark-adapted leaf (30 min), in specially provided clips. One saturating red-light flash (peak at 650 nm) with $3,000 \text{ } \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ was applied to measure the polyphasic fluorescence rise (OJIP) during the first second of illumination (10 μs to 1 s) as described by Strasser et al. (2004). The OJIP-steps (relative variable fluorescence) were analyzed using the JIP-test parameters [see Strasser et al. (2004) and Tsimilli-Michael and Strasser (2008)]. The transients were normalized as relative variable fluorescence: $W_t = (F_t - F_0)/(F_J - F_0)$, $W_{OJ} = (F_t - F_0)/(F_I - F_0)$ and $W_{IP} = (F_t - F_I)/(F_P - F_I)$. Besides, difference kinetics from relative variable fluorescence data were also calculated ($\Delta W = W_{\text{stressed}} - W_{\text{control}}$), which reveals hidden bands between the steps W_{OJ} (K-band; 300 μs) and W_{OK} (L-band; 150 μs) according to Yusuf et al. (2010). The JIP-test parameters are: ABS/RC—energy flux for light absorption per reaction centre (RC); DI_0/RC —The flux of energy dissipated (heat) in process other than trapping per active photosystem II (PSII) per RC; TR_0/RC —Maximum trapped exciton flux per active PSII per RC; ET_0/RC —The flux of electrons

transferred from quinone A (Q_A^-) to plastoquinone (PQ) per active PSII per RC RE_0/RC —Electron flux reducing end electron acceptors at the PSI acceptor side, per RC; ϕ_{P_0} —Maximum quantum yield for primary photochemistry; ψ_{E_0} —Efficiency/probability that an electron moves further than Q_A^- ; ϕ_{E_0} —Quantum yield for electron transport (ET); δ_{R_0} —Efficiency/probability with which an electron from the intersystem electron carriers moves to reduce end electron acceptors at the PSI acceptor side; ϕ_{R_0} —Quantum yield for reduction of end electron acceptors at the PSI acceptor side; PI_{ABS} —Performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors; PI_{total} —Performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors, as described by Tsimilli-Michael and Strasser (2008).

2.3 Gas exchange measurements

The portable infra-red CO_2 analyser (model LI-6400XT LI-COR, Inc., Lincoln, NE, USA), was used to measure gas exchange [net assimilation rate (A); stomatal conductance (g_s); transpiration rate (E), and intercellular CO_2 concentration (C_i)], in intact leaves (fully expanded first trifoliate leaf) attached to the plant, measured between 10:00 and 11:00 h. An in-chamber CO_2 concentration of $400 \mu\text{mol mol}^{-1}$ and a photon flow density of $1\,250 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, using the light source LI-COR 6400-02B attached to the measuring chamber ($20 \times 30 \text{ mm}$) with a block temperature control set up at 25°C was employed during the measurements.

2.4 Reactive oxygen species and lipid peroxidation measurements

The generation rate of $O_2^{\cdot-}$ was determined according to Elstner and Heupel (1976) and following the modifications of Li et al. (2010). Leaf tissues ($\pm 0.2 \text{ g}$) were powdered in liquid nitrogen and homogenized in 65 mM potassium phosphate buffer (pH 7.8). The homogenate was centrifuged at $5,000 \times g$ at 4°C for 10 min. The supernatant was mixed with 65 mM potassium phosphate buffer (pH 7.8), 10 mM hydroxylamine and incubated at 25°C for 20 min. Subsequently, 17 mM sulfanilamide and 7 mM α -naphthylamine were added in the reaction and incubated for 20 min at 25°C , afterward, the

absorbance of the solution was recorded at 530 nm . To calculate the generated rate of $O_2^{\cdot-}$, a standard curve was performed with known concentrations of nitrogen dioxide (NO_2). The H_2O_2 and lipid peroxidation levels were determined in leaves ($\pm 0.2 \text{ g}$). The tissues were powdered using a mortar and pestle with liquid nitrogen and homogenized with 0.1% (w: v) trichloroacetic acid (TCA). The homogenate was centrifuged at $12,000 \times g$ at 4°C for 20 min. The supernatant was used to determine the H_2O_2 content according to Velikova et al. (2000) and lipid peroxidation as described by Cakmak and Horst (1991). Lipid peroxidation was determined by using thiobarbituric acid (TBA), which determines malondialdehyde (MDA) as an end-product of lipid peroxidation. The molar extinction coefficient ($\epsilon = 155 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$) was used to calculate the amount of MDA–TBA complex (red pigment).

2.5 Enzyme activity

Glycolate oxidase (GO; EC 1.1.3.1) activity was assayed in leaves ($\pm 0.2 \text{ g}$), as described by Bai et al. (2014). The tissues were ground to a powder using a mortar and pestle with liquid nitrogen and homogenized with 5% polyvinylpolypyrrolidone (PVPP) and 50 mM Tris–HCl buffer (pH 7.8) containing 0.01% Triton X-100 and 5 mM dithiothreitol (DTT). The homogenate was centrifuged at $12,000 \times g$ for 20 min at 4°C . An aliquot of the supernatant was added in a medium reaction containing 50 mM Tris–HCl (pH 7.8), 0.009% Triton X-100 (v/v), 3.3 mM phenylhydrazine HCl (pH 6.8) and 5 mM glycolate (pH 7.0). GO activity was assayed by following the formation of glyoxylate phenylhydrazine at 324 nm recorded at 10 s intervals.

Antioxidative enzyme activity was assayed in leaves ($\pm 0.2 \text{ g}$), as described by Azevedo Neto et al. (2006). The tissues were ground to a powder using a mortar and pestle with liquid nitrogen and homogenized with 5% (w:v) PVPP and 100 mM potassium phosphate buffer, pH 7.8, containing 0.1 mM ethylenediaminetetraacetic acid (EDTA) and 20 mM sodium ascorbate. The homogenate was centrifuged at $12,000 \times g$ (20 min at 4°C). An aliquot of the supernatant was used as a crude enzyme extract for a specific medium reaction. Superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed by monitoring the inhibition of the nitro blue-tetrazolium (NBT)

coloration at 560 nm. Catalase (CAT; EC 1.11.1.6) activity was assayed by monitoring the decline in the absorbance at 240 nm. Ascorbate peroxidase (APX; EC 1.11.1.11) activity was assayed through ascorbate oxidation at 290 nm. Guaiacol peroxidase (GPOD; EC 1.11.1.7) activity was assayed by monitoring the tetraguaiacol production at 470 nm.

2.6 Statistical analysis

Each treatment (flooding or recovery) consisted of a minimum of four biological replicates (the number of biological replicates is indicated in figure legends), in a fully randomized design. The JIP-test parameters (Chl *a* fluorescence) was analysed by normalizations and subtractions, calculated by PEA + software version 1.12 (Hansatech Instruments Ltd, UK). All other data were checked for normality (Shapiro–Wilk) and homogeneity, and then analysed by one-way analysis of variance (ANOVA). When *F* was significant, the treatment means (control and flooding/recovery) were compared by t-test ($p \leq 0.05$). Statistical analyses were performed using the SAS 9.0 statistical software program (SAS Institute Inc. Cary, NC, USA).

3 Results

3.1 Chl index and Chl *a* fluorescence

One day of root flooding did not change the Chl index in comparison to control plants (Fig. 2a). However,

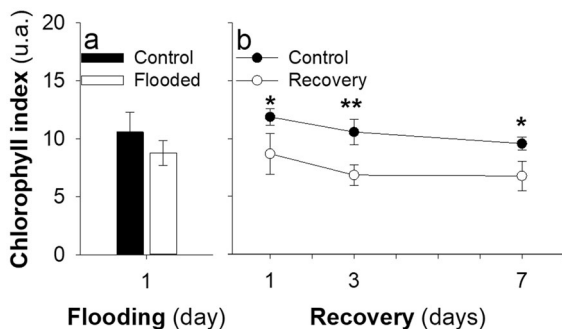


Fig. 2 Chlorophyll index of common bean plants subjected to root flooding (a) and recovery (b) conditions. Values represent the mean \pm standard deviation (SD). Asterisk indicates a significant difference by the t-test ($*p \leq 0.05$, $**p \leq 0.01$ and $***p < 0.0001$; $n = 4$) between control and stressful condition (flooding or recovery), in each period, independently

during the recovery period of short-term root flooding, plants showed a decrease in Chl index ($\sim 35\%$), which did not recover even with 7 days after the drainage of the flooded pots (Fig. 2b).

The measurement of Chl *a* transients from the dark-adapted leaves of common bean plants (50 μ s up to 1 s; on a logarithmic time scale) and presented as relative variable fluorescence during flooding and recovery, respectively (Fig. 3a; b), demonstrated in the plots a typical polyphasic Chl *a* fluorescence OJIP (W_t) transients (OJIP steps marked in the plot). A slight increase in fluorescence on the J-step was observed in plants under flooding conditions (Fig. 3a). During the recovery time of plants, an increase in fluorescence emission at J-step was shown (Fig. 3b). Moreover, the structural and functional aspects of the photosynthetic machinery (energy distribution in the photosynthetic apparatus) are presented in Fig. 3c, d. During 1 day of flooding (Fig. 3c), there was an increase in ABS/RC (energy flux for light absorption) DI_0 /RC (heat dissipation) and TR_0 /RC (electron trapping). Oppositely, there was a decrease in performance indexes (PI_{ABS} and PI_{total}). During the recovery, plants showed an increase in ABS/RC, DI_0 /RC, TR_0 /RC and ET_0 /RC (electron transport), even with 7 days after drainage. In addition, a slightly decrease in ψ_{E_0} (efficiency/probability for electron transport) and ϕ_{E_0} (the quantum yield for electron transport) was observed. Further, a decrease occurred in the parameters δRo and ϕRo , besides a strong reduction in PI_{total} and PI_{ABS} with 7 dayd of recovery (Fig. 3d).

When Chl *a* fluorescence data were normalized between the steps O (50 μ s) and I (30 ms) and presented as relative variable fluorescence (W_{OI}), we observed a slight increase in the curve that serves to distinguish the sequence of events from exciton trapping by PSII up to plastoquinone (PQ) reduction upon flooding (Fig. 4a) and recovery conditions (Fig. 4b), compared to its respective control treatments. On the other hand, the sequence of events from the PSI-driven electron transfer to the end electron acceptors on the PSI acceptor side, starting at PQH_2 (plastoquinol) (W_{IP} and $W_{OI} > 1$ in the insert), did not change compared to the control plants during flooding (Fig. 4c), while decreased in all recovery times (Fig. 4d).

To evaluate the difference kinetics of Chl *a* fluorescence, normalization and corresponding subtraction were applied against treated and control plants

between the steps O ($50 \mu\text{s}$) and K ($300 \mu\text{s}$) (ΔW_{OK}), which revealed the presence of L-band and at the steps O ($50 \mu\text{s}$) and J (2 ms) (ΔW_{OJ}), the K-band. The presence of L-band (Fig. 4e) during flooding and recovery times, especially 7 days after the return of the root system to normoxia conditions (Fig. 4f), indicated a low energy connection and non-efficient consumption of the excitation energy and low stability of the system. The presence of positive K-band in plants subjected to flooding (Fig. 4g) and its increase

Fig. 4 Chl *a* fluorescence transients (OJIP) of dark-adapted leaves of common bean plants subjected to flooding (a, c, e and g) and recovery (b, d, f and h) conditions. Relative variable fluorescence between the steps O and I ($W_{OI} = 0$ to 1; a and b) on logarithmic time scale; Relative variable fluorescence between the steps I and P (W_{IP} ; c and d) and $W_{OI} (\geq 1)$ in the insert. Average kinetics between the steps O and K (ΔW_{OK} ; e and f); Average kinetics between the steps O and J (ΔW_{OJ} ; g and h). ms = milliseconds. ($n = 15$)

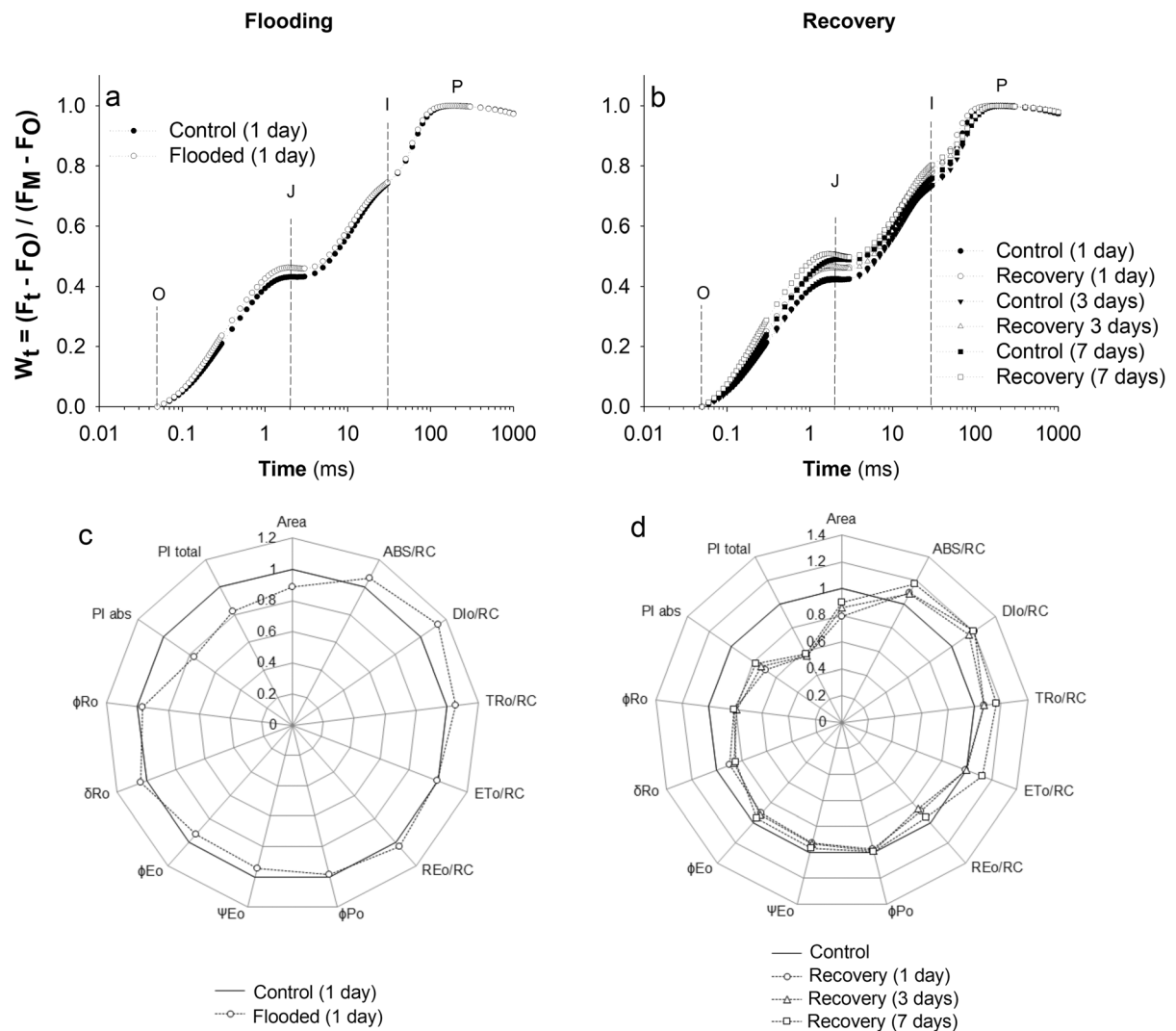
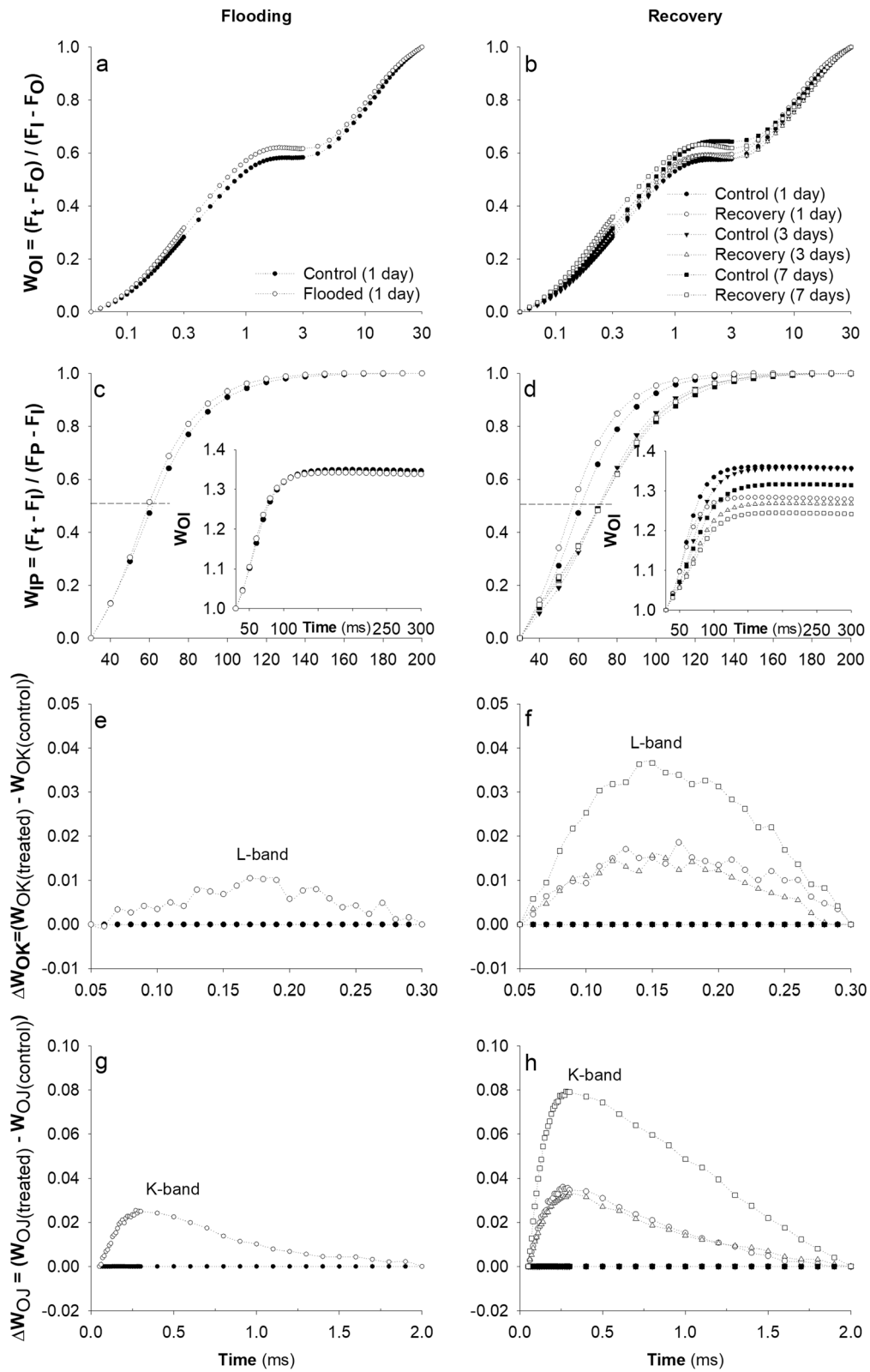


Fig. 3 Chl *a* fluorescence transients (OJIP) of dark-adapted leaves of common bean plants subjected to flooding (a and c) and recovery (b and d) conditions. Relative variable fluorescence between the steps O and P (W_t ; a and b) on logarithmic

time; Photosynthetic parameters deduced by JIP-test analysis of fluorescence transients normalized using as reference the control (c and d). ms = milliseconds. ($n = 15$)



in the recovery period (Fig. 4h), indicated changes in PSII antenna size.

3.2 Gas exchange upon recovery after short-term root flooding

Net assimilation rate (A) declined in about two-fold upon 1 d of root flooding, compared to control plants (Fig. 5a) and did not recover within 7 days of re-oxygenation (58% lower; Fig. 5b). Besides, g_s (Fig. 5c) and E (Fig. 5g) decreased (72 and 67%, respectively) and remained lower upon 7 days of recovery (Fig. 5d, h), in comparison to control plants. On the other hand, C_i decreased only with 7 days of recovery (Fig. 5f).

3.3 Oxidative damage and enzyme activity

Oxidative damage to lipid (lipid peroxidation) was higher only upon 7 days of recovery (Fig. 6b). On the other hand, $O_2^{\cdot-}$ generation rate was twofold higher (Fig. 6c) and H_2O_2 content increased 37% (Fig. 6e) with 1 day of flooding. Moreover, ROS did not decrease to the levels of control plants even 7 days after recovery, with both $O_2^{\cdot-}$ generation rate kept

twofold (Fig. 6d) and H_2O_2 in about 40% (Fig. 6f) higher.

GO activity did not vary during the course-time of flooding and recovery treatments (Fig. 6g, h). The enzymes SOD and APX did not increase with 1 day of flooding (Fig. 6i, m), while increased $\sim 70\%$ higher (Fig. 6j, n) during the recovery time in comparison to control plants. CAT only increased upon 3 days of recovery (Fig. 6k, l). GPOD was the most induced enzyme upon 1 day of flooding (56% higher; Fig. 6o) and reaching up to fourfold higher in comparison to control plants with 7 days of recovery (Fig. 6p).

4 Discussion

Common bean is a flooding sensitive species, as demonstrated by the previous result of our group (Posso et al. 2018). Four days of root flooding induced strong effects on the photosynthetic process by showing different strategies of some genotypes to counteract the negative effect of oxygen level deprivation in the root system. In addition, we demonstrated that even short-term of root flooding affect severely the recovery process of photosynthesis and the ability of plants to

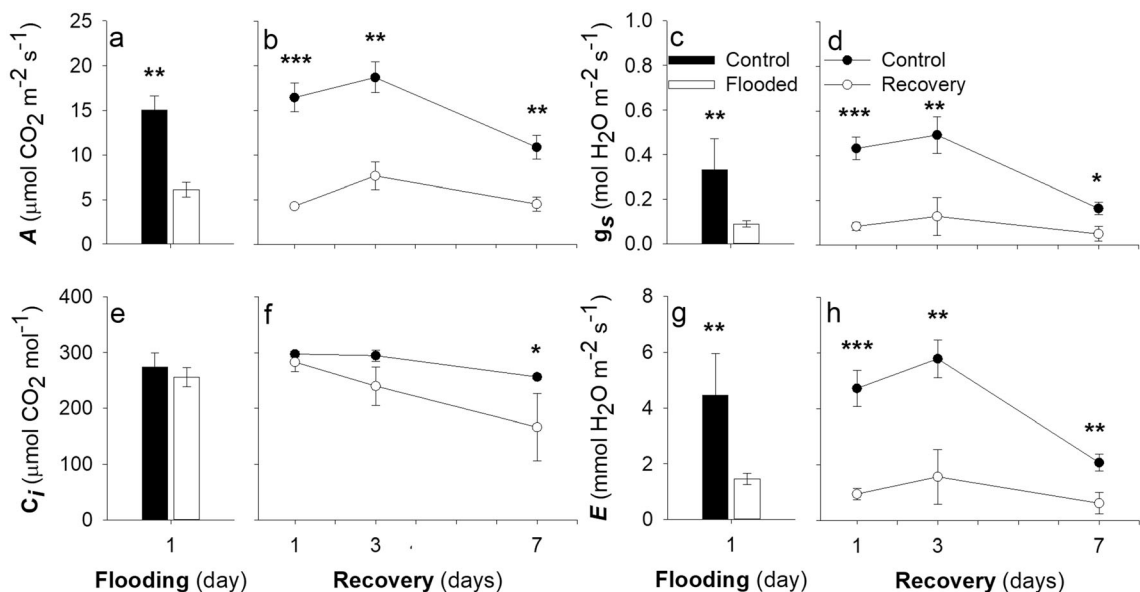


Fig. 5 Gas Exchange in leaves of common bean plants subjected to flooding (a, e, c and g) and recovery (b, d, f and h) conditions. Values represent the mean \pm standard deviation (SD). Asterisk indicates a significant difference by the t-test (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.0001$; $n = 4$) between

control and stressful condition (flooding or recovery), in each period, independently. Net assimilation rate (A ; a and b); stomatal conductance (g_s ; c and d); Intercellular CO_2 concentration (C_i ; e and f); Transpiration rate (E ; g and h)

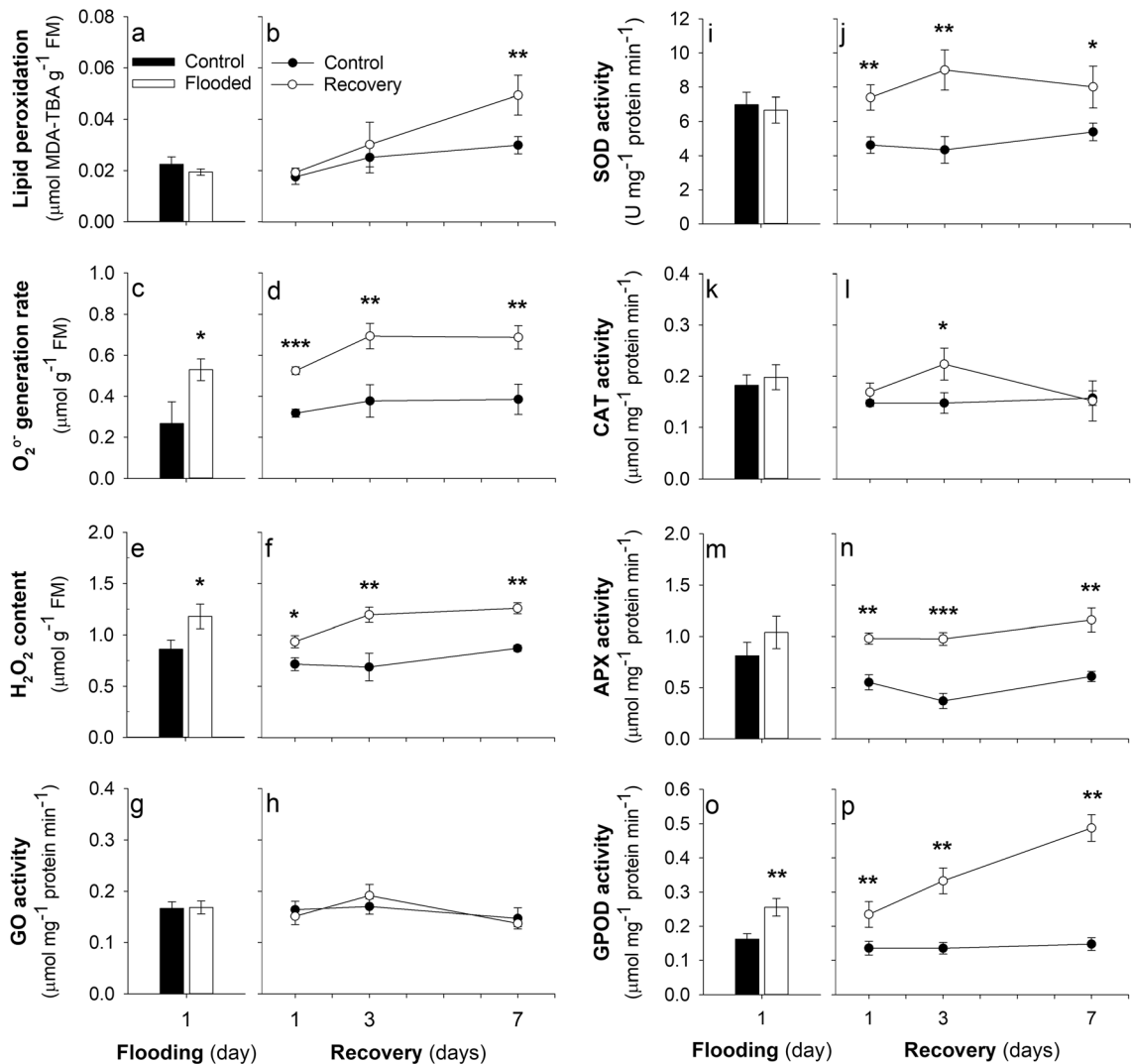


Fig. 6 Oxidative damages and enzyme activity in leaves of common bean plants subjected to flooding (**a, c, e, g, i, k, m** and **o**) and recovery (**b, d, f, h, j, l, n** and **p**) conditions. Asterisk indicates a significant difference by the t-test ($*p \leq 0.05$, $**p \leq 0.01$ and $***p < 0.0001$; $n = 4$) between control and stressful condition (flooding or recovery), in each period,

independently. Superoxide ($O_2^{\cdot-}$) generation rate (**c** and **d**); Hydrogen peroxide (H_2O_2) content (**e** and **f**); Glycolate oxidase (GO) activity (**g** and **h**); Superoxide dismutase (SOD) activity (**i** and **j**); Catalase (CAT) activity (**k** and **l**); Ascorbate peroxidase (APX) activity (**m** and **n**); Guaiacol peroxidase (GPOD) activity (**o** and **p**)

cope with the oxidative burst in leaves upon the re-entrance of oxygen on the root system, mainly due to the capacity of soil drainage during the recovery time. Since life on Earth evolved after the oxygen amount increased in the atmosphere due to photosynthesis (Johnson 2016), a decrease in oxygen level (hypoxia), impairs ATP production and activate fermentative pathway that consumes the cell stock sugars and metabolic contents to maintain low energy production in the root cells (Kreuzwieser and Rennenberg 2014;

van Dongen and Licausi 2015). However, the re-oxygenation and re-establishment of metabolic functions after a flooding condition can be limited due to the low capacity of the soil to rapid recovery the flow of oxygen levels to the root of plants. The slow decline in soil moisture recovery after drainage extends the negative effects of flooding, in addition to the direct effects of the re-oxygenation on root cells that lead to cellular damages (Blokhina et al. 2003; Herzog et al. 2016), even when soil moisture reaches control levels.

Oxygen deprivation on root cells declines ATP production which limits water absorption and transport to the shoot (Tournaire-Roux et al. 2003; Herrera 2013; Bansal and Srivastava 2015). The deficit of water transported to shoot cells may limit the stomatal conductance of common bean plants subjected to root flooding conditions, which limits the transpiration rate and CO₂ intake through stomata, consequently, leading to a decline in net assimilation rate. In addition, continuous light exposure force plants to deal with energy absorption through photosynthetic pigments by changing the dynamic dissipation of photosynthetic energy and increasing Chl *a* fluorescence emission as evidenced in common bean plants subjected to 1 day of root flooding. The decline in water transport by plants under root flooding has been associated with the acidification of the cytosol (Aroca et al. 2012), which inhibits the activity of aquaporins (limiting water absorption) (Landi et al. 2017). Moreover, the extent negative effects limits the recovery of gas exchange even when soil moisture reaches the levels of control plants (7 days of recovery), which can be a result of the low capacity of the soil to rapid decline the moisture over the time, maintaining plants under waterlogging conditions for an extended period, increasing cellular damages.

Besides limitation in CO₂ assimilation by plants, lower Chl indexes can be a result of Chl degradation and low turnover rates along the recovery time (up to 7 days of recovery), due to a reduction in mineral nutrient uptake and transport through xylem sap to the shoot, mainly nitrogen forms (Lanza et al. 2014; Garcia et al. 2020). Decreases in photosynthetic pigments led to a reduction in energy absorption and an imbalance in electron transport chain (ETC), which was prolonged over 7 days of the recovery period. This explains the increase in Chl *a* fluorescence emission during the flooding and recovery treatment as indicated by the JIP-test parameters.

During the recovery process of plants, the prolonged time of moisture in soil induced plants to increase ABS/RC, which means an increase in the functional size of the antenna system or inactivation of RC fraction by being transformed to non-Q_A-reducing centers (Yusuf et al. 2010), an answer reported by plants under stressful conditions even when photosynthetic pigments decrease (Posso et al. 2018). The increase in ABS/RC reflects an attempt of plants to increase the specific absorption flow of the continuous

illumination to maintain ETC working, which increases TR₀/RC and further decrease RE₀/RC, δ_{Ro} and φ_{Ro} leading to a decreases in reducing energy power (Yusuf et al. 2010). On the other hand, as most of the absorbed energy is not used in photochemical reactions and, therefore, dissipated as heat (DI₀/RC) and fluorescence. This non-photochemical mechanism of dissipation is important for plants to deal with the excess of energy and might be a response of increased TR₀/RC, and an increase in the sequence of events from exciton trapping by PSII up to plastoquinone (PQ) reduction (OI-phase; W_{OI} from 0 to 1). Although electrons may be trapped, the electron transport flux (ET₀/RC) was not impaired and did not limit the flow of electrons further than Q_A⁻ through the ETC upon re-establishment of normoxic conditions.

Despite the maintenance of electron flow, the maintenance of energy production did not occur as there was a decrease in RE₀/RC, δ_{Ro} and φ_{Ro}. Therefore, the reduction in energy production (reducing power), can be a direct consequence of a decline in gas exchange. The decline in g_s and *E* limit the diffusion of CO₂ from the air into the carboxylation sites of the Rubisco enzyme, leading to a reduction in CO₂ assimilation (Ahmed et al. 2002; Bansal and Srivastava 2015). As far as less CO₂ is assimilated into sugar, less ATP and NADPH may be recycled by the Calvin-Benson cycle to maintain energy production by the ETC, which leads to an over-reduction of ETC by increasing ABS/RC and TR₀/RC. Besides that, reduction in gas exchange upon 7 days of recovery in control plants can be a result of the fluctuation in the environment temperature, as reflected by a decrease of the temperature inside the greenhouse (Fig. 1).

Besides, there was a decline in specific energy fluxes per reaction center, such as RE₀/RC. This decline limits reducing energy power production which is supported by a decrease in quantum yield and efficiencies. The efficiency/probability with which an electron from the intersystem electron carries moves to reduce end electron acceptors at the PSI acceptor side (δ_{Ro}) was lower and limited the quantum yield for reduction of end electron acceptor at the PSI acceptor side (φ_{Ro}), although ET₀/RC remained functioning and, also a decline in PSI-driven electron transfer to the end electron acceptor on the PSI acceptor side starting at PQH₂ (IP-phase; W_{OI} > 1). In addition, the electron transport chain from the absorption of the photon to the reduction of the PSI

final acceptors, described by two performance indexes (PI_{total} and PI_{ABS}) showed a strong decrease, evidencing problems on the density of the reaction center, capture efficiency, and electron transport efficiency by limiting the well-functioning of the electron transport chain. Taken all together, this information supports the probability of lower NADPH and ATP production (Strasser et al. 2004; Yusuf et al. 2010).

The presence of positive L-band demonstrated lower energy connectivity of PSII, even after 7 days of recovery, which means low efficiency with which absorbed energy is transmitted between PSII units (Tsimilli-Michael and Strasser 2008), contributing to increasing in the dissipation of the energy absorbed via heat and fluorescence, as reported by Stirbet and Govindjee (2011) and Santos Jr. et al. (2015). In addition, the positive K-band showed an increase of the functional PSII antenna size, and/or inactivation of the OEC (Yan et al. 2013), which causes an imbalance in the energy flux in PSII and increases the probability of photooxidative damage due to the production of singlet oxygen ($^1O^2$) (Foyer et al. 2017), contributing to Chl degradation during the recovery time of plants.

It is noteworthy that CO_2 is not the only electron sink, though it is the most relevant. Photorespiration (C3 plants) is one of the most important alternative pathways that can drive the electron from ETC beyond net assimilation via Rubisco (Foyer and Noctor 2009) and commonly reported in plants under stress (Bräutigam and Gowik 2016), also in common bean plants under root flooding conditions through increase or maintenance of glycolate oxidase activity (Posso et al. 2018). Besides there is no increase in GO activity along the recovery period of plants, it also did not decrease which means that the maintenance of photorespiratory process through GO enzyme and low CO_2 assimilation represents an important way to alleviate, at least in part, the effects caused by flooding stress and even during the recovery time as an attempt to release ETC from over-reduction. The BRS Expedito genotype has been reported to maintain GO activity functioning at control levels while most other genotypes decrease its activity (Posso et al. 2018).

The increase in TR_0/RC and the presence of positive L- and K-band increase the possibility of electron leakage from ETC, consequently leading to ROS production as evidenced by higher $O_2^{\cdot-}$ generation rate and H_2O_2 upon flooding and recovery treatment in common bean plants. The higher $O_2^{\cdot-}$

generation rate can be a result of an inefficient activation of SOD (Blokhina and Fagerstedt 2010a, b; Mittler 2017), and the Mehler reaction (another electron sink that reduces molecular oxygen on the acceptor side of PSI) (Foyer and Noctor 2009). Several authors have reported increased accumulation of ROS under hypoxic/anoxic conditions (for a review, see Blokhina et al. 2003). The higher production of ROS triggers oxidative damage to lipid membrane, as evidenced by an increase in lipid peroxidation in the common bean plants with 7 days of recovery, besides other membrane injuries, protein degradation, enzyme inactivation, DNA, RNA and nucleosides (Gill and Tuteja 2010; Ivanov et al. 2018).

The content of H_2O_2 is also a result of the detoxification of $O_2^{\cdot-}$ produced by the photosystems due to the activity of SOD (Gill and Tuteja 2010), and via GO activity (photorespiration), in peroxisome (Bräutigam and Gowik 2016). In addition to the direct production of H_2O_2 via a pool of plastoquinones (Ivanov et al. 2018). As chloroplast is one of the main sources of $O_2^{\cdot-}$ and H_2O_2 in plants, the electron flow to oxygen (ROS production) rather than $NADP^+$ can result in decreases in RE_0/RC , δ_{RO} and ϕ_{RO} , besides it, can be important to relieve reductive pressure within the ETC chain and balances ATP: NADPH ratios by allowing proton pumping without net reductant generation (Foyer et al. 2017). However, ROS production should be maintained at low levels (control levels), to work as signaling molecules (Mittler 2017) and to avoid oxidative damages, which was not evidenced in common bean plants as ROS production and lipid peroxidation increased along the recovery process, as above reported.

To avoid oxidative damage, an efficient antioxidative defense system composed of both enzymatic and non-enzymatic compounds is induced by root and shoot cells, to cope with high-level ROS production (Blokhina and Fagerstedt 2010a, b). The SOD activity increased and remained high during the recovery from flooding, probably to scavenge the high production of $O_2^{\cdot-}$ radicals into H_2O_2 via the one-electron steps of the dismutation reaction, by one of the three forms of SODs existing in plants containing different metal centers, such as manganese (Mn-SOD), iron (Fe-SOD), and copper-zinc (Cu/Zn-SOD), and constitutes the first defense line against $O_2^{\cdot-}$ production. Moreover, the increase in APX activity, an enzyme that scavenger H_2O_2 , may help to maintain its production

low in the cells (Gill and Tuteja 2010). The higher increase in APX and GPOD activity contribute to scavenger H_2O_2 . The increase in APX activity might be important to help the dissipation of reducing power (NADPH), once NADPH is a co-factor used by APX in the ascorbate–glutathione cycle (Blokhina and Fagerstedt 2010a, b; Mitler 2017). The increase of CAT only on the third day of recovery seems to be a balance in the activation of enzymes over the time since CAT was not reported as the main enzyme acting in ROS scavenging in BRS Expedito genotype upon flooding conditions (Posso et al. 2018).

Therefore, short-term root flooding impairs photosynthetic process recovery of common bean plants upon re-establishment of normoxic conditions by keeping long-time high soil moisture which prolongs the negative effects of flooding in common bean plants. The direct effects of flooding on roots influence the shoot by limiting stomatal conductance and transpiration rate, which decreases CO_2 intake and consequently impairs CO_2 assimilation during flooding and recovery of plants. The reduction in carboxylation reactions may decline energy power consumption leading to an impairment of ETC. To deal with the excess of light absorption and avoid over-reduction of ETC, plants induce changes in the dynamic dissipation of photosynthetic energy by increasing Chl *a* fluorescence and energy dissipation as heat. In addition, the impairment of ETC leads to electron leakage and ROS production which induces the antioxidant enzymatic system to cope with oxidative damages, mainly when plants return to normoxic conditions, indicating the high ability of common bean to induce/activate the enzymatic ROS scavenger system and keep it working along with the decline in soil moisture. Although plants can induce efficiently the antioxidant system, it is clear that the effects of flooding and the long-time of soil moisture during the recovery process allow high ROS production beyond the scavenger system capacity, resulting in an oxidative burst that leads to cell damage, even when soil moisture reaches control levels.

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