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Differential response of photosystem II and I photochemistry in leaves of two *Crambe abyssinica* Hochst lineages submitted to water deficit

P.C.S. BRAGA$^+$, J.P.R. MARTINS, R. BONOMO, R.M. BORGES, J.V.G. SILVA, and A.R. FALQUETO

Plant Ecophysiology Laboratory, Federal University of Espírito Santo, Campus São Mateus, Espírito Santo State, Brazil

Abstract

Water stress is one of the most important abiotic factors affecting plants worldwide. This study compared the photochemical responses of two lineages of *Crambe abyssinica* (FMS CR 1307 and 1326) under water deficit (WD) conditions. The relative water content (RWC), stomatal conductance, chlorophyll (Chl) index, and Chl *a* fluorescence were evaluated. FMS CR 1326 showed greater reductions in RWC under WD. The FMS CR 1307 showed greater reductions in the connectivity of energy (L-band) and electron transfer from the oxygen-evolving complex (K-band), followed by a lower ability to maintain the reduction of plastoquinone. However, the increased size of the pool of the final electron acceptors in PSI for this lineage was observed. The FMS CR 1326 plant lineage showed an advantage over the FMS CR 1307 in terms of stability and efficiency of PSII under low water availability conditions.

Keywords: chlorophyll *a* fluorescence; JIP-test; K-band; OJIP curves; performance index; photochemical tolerance.

Introduction

Water deficit (WD) is one of the most important and complex factors that hinder plant development and crop productivity (Lang *et al*. 2018). With an increase in global temperature, changes in the world rainfall distribution patterns are expected to occur, favoring more drought periods in many regions (Lau and Kim 2015). Furthermore, the increase in air temperature has caused a decrease in soil capacity to retain moisture, resulting in the acceleration of WD conditions (Sikder *et al.* 2015). Consequently, farmers have increased irrigation practices to sustain the high productivity of plants. However, the high cost and inadequate engineering of the irrigation systems associated with limited water resources have restricted its use (Çakir 2004).

Plants have developed many strategies to maintain growth when under limited or irregular water availability (Hummel *et al.* 2010). Drought tolerance is defined as the capacity of plants to grow and produce satisfactorily under limited water availability or periodic deficiency of rainwater (Ashley 1993). Nevertheless, WD negatively affects growth and other physiological processes, even in drought-tolerant plants (Covre *et al.* 2016, Wang *et al.* 2017). These effects are the result of osmotic stress, which reduces cell turgescence, stomatal conductance, and transpiration rates, which negatively affect CO_2 assimilation and cause damages to the reaction centers of PSII (Mehta *et al.* 2010). Consequently, those effects can confer low PSII efficiency, which may lead to reduced plant productivity (Velázquez-Márquez *et al.* 2015).

Chl *a* fluorescence is a noninvasive method that can reveal the functional state or efficiency of the photosynthetic apparatus (Borawska-Jarmułowicz *et al.* 2020). As such, it has been widely used in experiments for photochemical identification of species/genotypes of plant tolerance/susceptibility to abiotic stresses (Mehta *et al.* 2010, Falqueto *et al.* 2017, Kalaji *et al.* 2018). Under WD, Chl *a* fluorescence analyses have shown positive values for L- and K-bands in two cultivars of *Passiflora edulis* L.,

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$^+$Corresponding author; phone: (+55) 27 3312 1683, e-mail: priscila.csbraga@gmail.com

Abbreviations: ABS/RC – absorption flux (of antenna Chl) per RC; Chl – chlorophyll; OEC – oxygen-evolving complex; DI_0/RC – dissipation flux per RC; DM – dry mass; ET_0/RC – electron transport flux (further than Q_A^-) per RC; F_0 – minimum fluorescence, when all PSII RCs are open; FM – fresh mass; F_m – maximum fluorescence, when all PSII RCs are closed; g_s – stomatal conductance; P_{680} – primary electron donor of PSII; P_{700} – primary electron donor of PSI; PI_{total} – performance index; PQ – plastoquinone; PQH_2 – plastohydroquinone; RC – reaction centre; RWC – leaf relative water content; TM – turgid mass; TR_0/RC – trapping flux per RC; V_{IP} – variable fluorescence between steps I (30 ms) and P (300 ms); V_{OI} – variable fluorescence between steps O (2 ms) and I (30 ms); V_{OJ} – variable fluorescence between steps O (0.02 ms) and J (2 ms); V_{OK} – variable fluorescence between steps O (0.02 ms) and K (0.3 ms); V_{OP} – variable fluorescence between steps O (0.02 ms) and P (300 ms); WD – water deficit; ΔV_{IP} – amplitude of the relative variable fluorescence of the I-to-P-rise; φ_{P0} – maximum quantum yield of primary PSII photochemistry.

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FB200 and FB300 (Gomes *et al.* 2012), two cultivars of *Zea mays* L., SD609 and ZD958 (Zhang *et al.* 2015), two genotypes of *Crambe abyssinica* Hochst, FMS Brilhante and FMS CR 1101 (Martins *et al.* 2017), and two clones of *Hevea brasiliensis* L., RRIM600 and FX3864 (Falqueto *et al.* 2017), and these results have revealed a decrease in energy connectivity between PSII units and impaired performance of the oxygen-evolving complex (OEC). Thus, it is possible to differentiate the tolerance of species/cultivars/genotypes to drought using this method.

Crambe (*Crambe abyssinica* Hochst, Brassicaceae) is an annual plant that is native to East Africa, particularly Ethiopia (formerly called Abyssinia, from where its name is derived), and was first domesticated in the Mediterranean region. It is now cultivated in various tropical and subtropical regions. The grains are produced only for industrial purposes, aiming at the extraction of valuable oil and other substances (*e.g.*, erucic acid) that have various applications, such as the manufacture of plastics, lubricants, and biodiesel (Carlsson *et al.* 2007). As it does not compete with oilseeds used for human nutrition and occupies arable lands in the winter (as a second crop), crambe has become an innovative crop in the Brazilian agribusiness sector, particularly presenting potential supply of insulating vegetable oil (Oliveira *et al.* 2015).

According to Pitol *et al.* (2012), new crambe lineages have been tested to expand the crop and increase yields in Brazil. Also, according to Oliveira *et al.* (2015), the FMS CR 1307 crambe lineage is one of those being considered as a pilot candidate, while the FMS CR 1326 lineage is still under analysis by the Mato Grosso do Sul Foundation for Research and Diffusion of Agricultural Technologies (Fundação MS). Both lineages have yields greater than the cultivar FMS Brilhante, the only registered genotype (approximately 1,368; 1,357; and 1,254 kg ha^{-1}, respectively) in conditions with adequate water supply.

Considering that water resources are limited and that agriculture consumes large amounts of water, demands for water resources can be mitigated by the selection of species/genotypes of plants that are tolerant to WD (Meng and You 2020). Therefore, the objective of this study was to evaluate the effects of WD on the PSII and PSI photochemistry in leaves of two crambe lineages (FMS CR 1307 and 1326) during the vegetative phase, identifying the action site of stress on the photosynthetic apparatus.

Materials and methods

Plant growth conditions and treatments: Seeds of *Crambe abyssinica* Hochst, lineages FMS CR 1307 and FMS CR 1326, were previously disinfected with ethanol 70% for 2 min, 1% sodium hypochlorite [v/v] for 20 min, and *Ridomil*® fungicide for 10 min, followed by multiple cleanings with autoclaved distilled water and placed to germinate in pots of 5.5 L (15 seeds per pot) filled with soil (75.5% sand, 17.2% clay, and 5.2% silt). Previously, chemical analysis of soil was performed to adjust nutrient concentrations and pH, following the recommendations of Oliveira *et al.* (1991). Thirty grams of single superphosphate were added per pot at three separate intervals during the experiment. Besides, insecticides (*Evidence*®) and fungicides (*Ridomil*®) were applied according to the culture manual. After 20 d, the seedlings were thinned to one per pot.

All plants (both lineages) were watered daily to maintain the soil humidity at approximately 80% of field capacity, *e.g.*, 0.20 m^3 m^{-3}. At the age of 50 d, the plants were split into two groups: for each lineage, the first group of plants was maintained with daily irrigation (used as control); and the others were subjected to progressive drought by withholding watering. When the humidity level reached 30% (around 0.06 m^3 m^{-3}) and the stomatal conductance (g_s) reached values below 10 mmol(H_2O) m^{-2} s^{-1} (Martins *et al.* 2017), the analysis was performed at 7 d without irrigation and plants presented visual symptoms of predawn wilting. All sampling and measurements were conducted between 5 and 9 h using the third or fourth fully expanded leaf from the apex.

Leaf relative water content (RWC), stomatal conductance (g_s), and Chl index were measured in 3, 9, and 11 plants per treatment, respectively; the plants were randomly selected. For RWC measurements, five leaf discs (0.78 cm^2) were extracted, and they were immediately weighted together for obtaining the fresh mass (FM). Then, the turgid mass (TM) was obtained after the hydration of the leaf discs through submersion in distilled water for 24 h in Petri dishes. The dry mass (DM) was obtained after drying the leaf discs over 72 h at 65°C (Martins *et al.* 2017). The RWC was calculated according to the following expression, and the results expressed in percentage [%]: RWC = [(FM – DM)/(TM – DM)] × 100 (Barrs and Weatherley 1962). The g_s and Chl index were measured using a leaf porometer (*SC-1*, *Decagon Devices*, USA) and a *ClorofiLOG (CFL 1030*, *Falker*, Porto Alegre, Brazil), respectively (Arunyanark *et al.* 2008, Maes *et al.* 2009). The g_s was measured on the leaf abaxial surface.

Chl *a* fluorescence (OJIP transients and JIP-test): Fluorescence signals were registered using a plant efficiency analyzer (*Handy-PEA*, *Hansatech*, King's Lynn, Northfolk, UK), as described by Strasser and Strasser (1995). The measurements were collected from five plants per treatment in leaves that were previously adapted to the dark for 20 min using leaf clips (*Hansatech*, UK). The fast fluorescence kinetics (F_0 to F_P) was recorded from 10 μs to 1 s after the application of saturated light flash with intensity reaching 3,000 μmol(photon) m^{-2} s^{-1}. The fluorescence intensity at 0.02 ms (considered as F_0), 0.15 ms (F_L), 0.3 ms (F_K), 2 ms (F_J), 30 ms (F_I), and 300 ms (F_m) was collected and used to obtain the parameters from JIP-test (Strasser *et al.* 2004, Stirbet and Govindjee 2011). OJIP curves were normalized according to the method of Yusuf *et al.* (2010).

Statistical analysis: The experiment was performed following a completely randomized design in a factorial scheme, considering two water treatments [daily irrigated (control) and nonirrigated plants (WD)] and two crambe lineages (FMS CR 1307 and 1326). All statistical analysis was conducted using the statistical software *SISVAR* version *5.6*. Significant differences between the treatments were evaluated using analysis of variance (*ANOVA*) and *Tukey*'s test ($p \leq 0.05$).

Results

RWC, g_s, and Chl index: Significant differences of RWC were observed as a function of WD and crambe lineages (Fig. 1). The RWC values obtained for plants irrigated daily (control) were 65.7 and 62.7% for the lineages FMS CR 1307 and 1326, respectively. After water deficit conditions, reduced values of RWC were observed in both crambe lineages compared to the control plants. Lower RWC values were obtained for the FMS CR 1326 (25.9%) in comparison to the lineage FMS CR 1307 (34.9%).

Reductions in g_s were also observed when crambe lineages were submitted to WD [152.3 to 7.7 mmol(H_2O) m^{-2} s^{-1}]. No significant differences in g_s were observed between the crambe lineages (Fig. 2*A*). The Chl index *a* and *b* increased significantly when the plants were submitted to WD (Chl *a* from 36.9 to 40.05 and Chl *b* from 15.8 to 23.7), but no significant difference was observed between crambe lineages (Fig. 2*B*).

OJIP Chl *a* fluorescence and normalizations: To evaluate the polyphasic behavior of OJIP curves for the photochemical events reflected in the O–J, J–I, and, I–P phases, the Chl *a* fluorescence data were double normalized between F_0 and F_m as $V_{OP} = (F_t - F_0)/(F_m - F_0)$ and showed the difference kinetic, ΔV_{OP} [$\Delta V_{OP} = V_{OP(treatment)} - V_{OP(control)}$] (Strasser *et al.* 2007). Under WD, the positive amplitudes of ΔV_{OP} were obtained for both crambe lineages. However, the higher positive amplitude (≈ 0.08) was obtained for the FMS CR 1307 lineage when compared to FMS CR 1326 lineage ($\Delta V_{OP} \approx 0.06$) (Fig. 3*A*).

The relative fluorescence between the O- and K-steps (obtained at 0.02 and 0.3 ms, respectively), reported as $V_{OK} = (F_t - F_0)/(F_K - F_0)$, and between O- and J-steps (obtained at 0.02 and 2 ms, respectively), reported as $V_{OJ} = (F_t - F_0)/(F_J - F_0)$, were normalized and presented as the kinetics difference $\Delta V_{OK} = V_{OK(treatment)} - V_{OK(control)}$ and $\Delta V_{OJ} = V_{OJ(treatment)2} - V_{OJ(control)}$, respectively. The kinetic difference ΔV_{OK} and ΔV_{OJ} made the L- and K-bands visible, respectively. In this study, both crambe lineages presented an L-band (obtained at 0.15 ms) and K-band (obtained at 0.3 ms) with positive amplitudes after submission to WD (Fig. 3*B*,*C*). The larger K-band values were obtained from the FMS CR 1307 crambe lineage (0.13), followed by the FMS CR 1326 lineage (0.06). Similarly, a higher positive amplitude for the L-band was observed in the FMS CR 1307 crambe lineage when compared to FMS CR 1326 (0.19 and 0.12, respectively).

To evaluate the O–I phase that occurred between 0.02 and 30 ms, the normalizations [$V_{OI} = (F_t - F_0)/(F_I - F_0)$] and the kinetic differences [$\Delta V_{OI} = V_{OI(treatment)} - V_{OI(control)}$] were utilized, allowing observation of the O–I phase in $V_{OI} \leq 1$ (Strasser *et al.* 2004). In this study, the crambe lineages showed positive amplitudes in the O–I phase when submitted to water deficit. A greater incline was observed for $V_{OI} \leq 1$ in the FMS CR 1307 crambe lineage (Fig. 3*D*). Also, the I–P phase was evaluated as $V_{OI} \geq 1$ and V_{IP}. $V_{OI} \geq 1$ was plotted from 30 to 300 ms when the fluorescence data were normalized between the O–I phase (Fig. 4*A*). In the control group plants, higher values were obtained for $V_{OI} \geq 1$ in the FMS CR 1326 lineage (1.25) when compared to the FMS CR 1307 lineage (1.23). However, WD resulted in reduced $V_{OI} \geq 1$ values in both crambe lineages evaluated. The FMS CR 1326 lineage showed higher $V_{OI} \geq 1$ values (1.4) when compared to the FMS CR 1307 crambe lineage (1.36).

The normalization $V_{IP} = (F_t - F_I)/(F_m - F_I)$ and the kinetic difference $\Delta V_{IP} = V_{IP(treatment)} - V_{IP(control)}$ values were plotted between 30 and 300 ms (Fig. 4*B*,*C*). According to

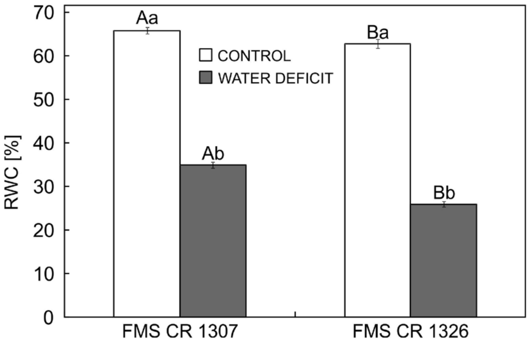

Fig. 1. Leaf relative water content (RWC) of two *Crambe abyssinica* lineages submitted to water deficit. Means (± SD), *n* = 3, followed by *the same letter* (*uppercase* for each water regime – comparing the two crambe lineages; and *lowercase* for each lineage – comparing the two water regimes) do not differ significantly according to the *Tukey*'s test, $p \leq 0.001$.

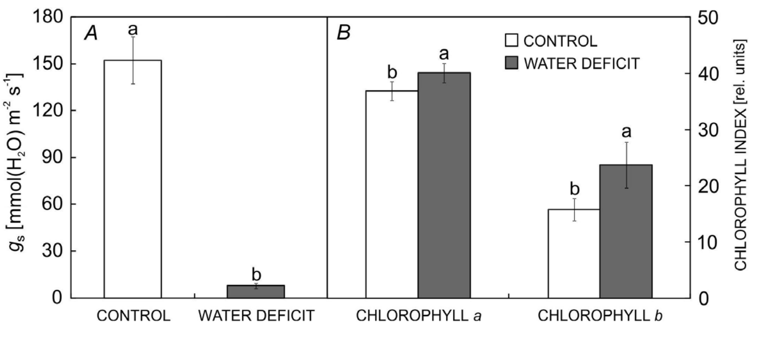

Fig. 2. Stomatal conductance (g_s) (*A*) and chlorophyll *a* and *b* index (*B*) of two lineages of *Crambe abyssinica* submitted to water deficit. Means (± SD) followed by *the same letter* do not differ significantly according to the *Tukey*'s test, $p \leq 0.001$. Data are the means of nine and eleven replicates, respectively.

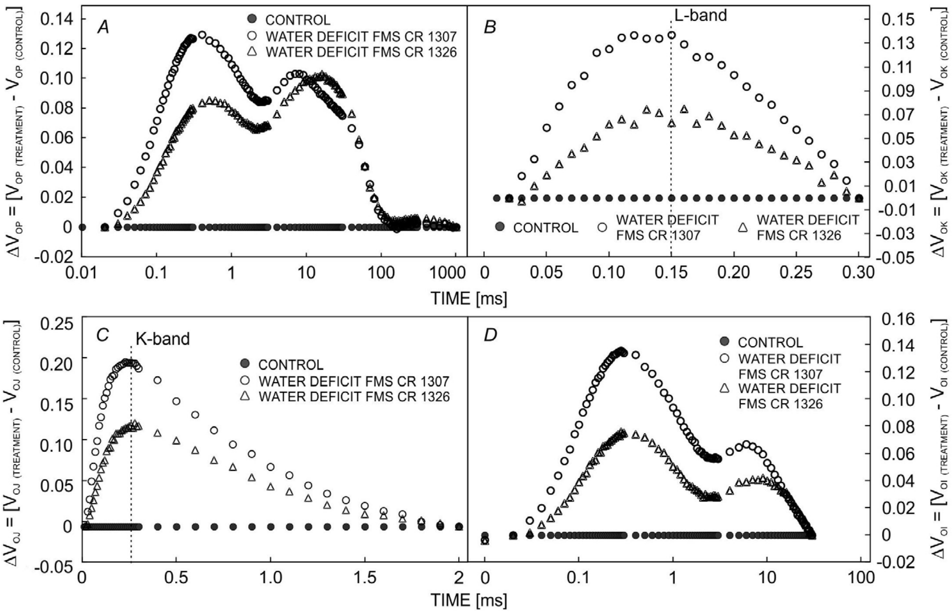

Fig. 3. Kinetic difference of V_{OP} (*A*), V_{OK} (*B*), V_{OJ} (*C*), and V_{OI} (*D*) obtained through the double normalization of the O–P phase [$\Delta V_{OP} = V_{OP(treatment)} - V_{OP(control)}$], O-K phase [$\Delta V_{OK} = V_{OK(treatment)} - V_{OK(control)}$], and O–I phase [$\Delta V_{OI} = V_{OI(treatment)} - V_{OI(control)}$] respectively, in two lineages of crambe FMS CR 1307 and FMS CR 1326 submitted to water deficit. Data represent means of five replicates.

the Michaelis-Menten equation, where $V_{IP} = 0.5$ (Yusuf *et al.* 2010), the V_{IP} data showed higher velocity constants for the FMS CR 1326 compared to the FMS CR 1307 (0.80 and 0.54, respectively).

This result was better elucidated by analyzing the kinetic difference ΔV_{IP} (Fig. 4*C*), as it showed that the negative amplitude band was higher in the FMS CR 1326 lineage (–0.08).

JIP-test: The specific energy fluxes varied significantly in WD and crambe lineages (Fig. 5). However, for the φ_{P0} and PI_{total}, this variation occurred independently from WD treatment (Fig. 6). The specific energy flux values (ABS/RC, TR_0/RC, and DI_0/RC) increased significantly in plants exposed to WD. For ABS/RC, TR_0/RC, and DI_0/RC, the FMS CR 1307 and FMS CR 1326 crambe lineages presented 3.26, 2.42, and 0.84 and 2.74, 2.15, and 0.58, respectively. For ET_0/RC, no significant difference was observed between treatments and crambe lineages (Fig. 5). Also, WD reduced the maximum quantum yield of primary photochemistry of PSII (φ_{P0}) and the performance index (PI_{total}) compared to control plants (from 0.86 to 0.76 and from 35.33 to 9.75, respectively), independent of crambe lineage evaluated (Fig. 6).

Discussion

The ability of plants to tolerate water deficit conditions can be attributed to several mechanisms, including the efficiency in maintaining a high RWC and stomatal closure (Oukarroum *et al.* 2007), by reducing water loss through transpiration (Lawson and Blatt 2014). The two crambe lineages evaluated in this study showed different RWC values. Under well-irrigated conditions (control), the plants of the FMS CR 1326 lineage showed RWC values 4.5% lower than that of the FMS CR 1307 lineage. Under WD, the plants of the FMS CR 1326 lineage

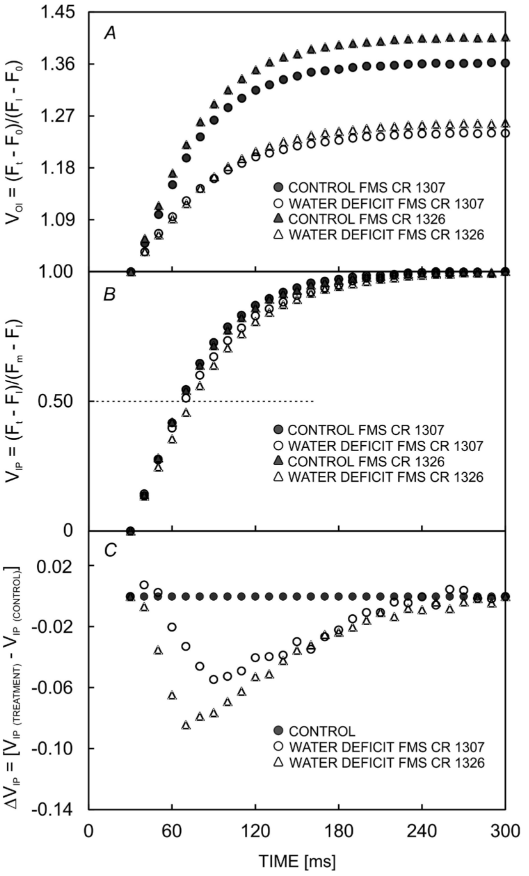

Fig. 4. Transient chlorophyll fluorescence in the O–I phase ($V_{OI} = F_t - F_0)/(F_I - F_0)$ (*A*) and I–P phase ($V_{IP} = F_t - F_I)/(F_m - F_I)$ (*B*) and kinetic difference V_{IP} [$\Delta V_{IP} = V_{IP(treatment)} - V_{IP(control)}$] (*C*), respectively, in two lineages of crambe FMS CR 1307 and FMS CR 1326 submitted to the water deficit. Data are the means of five replicates.

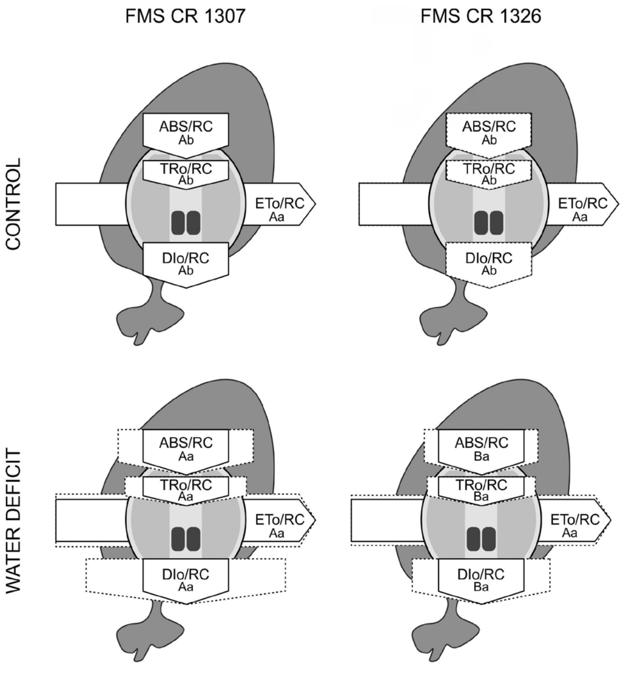

Fig. 5. Models of energy flux per reaction centre (RC) of two lineages of *Crambe abyssinica* (FMS CR 1307 and 1326 control) under two water regimes. The response of each of the parameters can be observed by *the dotted lines* compared to the control. Relative values inside the figures followed by *the same letter* (*uppercase* for each water regime – comparing the two crambe lineages; and *lowercase* for each lineage – comparing the two water regimes) do not differ significantly according to the *Tukey*'s test, $p \leq 0.05$. Data are the means of five replicates. ABS/RC – absorption flux per RC; ET_0/RC – electron transport flux (further than Q_A^-) per RC; DI_0/RC – dissipated energy flux per RC (at t = 0); TR_0/RC – trapping flux (leading to Q_A reduction) per RC.

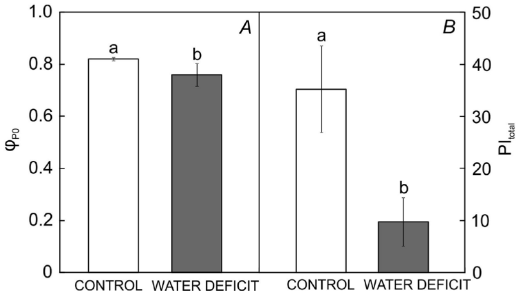

Fig. 6. Maximum quantum yield of primary photochemistry of PSII (φ_{P0}) (*A*) and photosynthetic performance index (PI_{total}) (*B*) of two *Crambe abyssinica* lineages submitted to water deficit. Means (± SD) followed by *the same letter* do not differ significantly according to the *Tukey*'s test, $p \leq 0.001$. Data represent the mean values of five replicates.

showed RWC of approximately 25.7% while FMS CR 1307 lineage had 34.9%. This difference in RWC between the lineages can be related to the different capacities for conducting water through the xylem in response to a specific hydraulic signal (Sinclair *et al.* 2008). This trait may have been acquired *via* natural selection since the two crambe lineages under the current study originated from natural crosses between different genotypes of the cultivar FMS Brilhante. According to Lara-Fioreze *et al.* (2013), the FMS Brilhante crambe cultivar is considered to be tolerant to WD. In this study, the higher RWC values observed in lineage FMS CR 1307 under water deficit and control conditions are associated with its greater ability to maintain a high water content in the leaf tissue, compared to FMS CR 1326 plants.

The lower g_s values verified in plants of crambe under WD suggest that there was the closure of the stomatal pore. Plants close stomata to limit water loss, and this is a response to hydraulic signals from changes in leaf turgor (Huber *et al.* 2019). Stomatal closure generally restricts stomatal diffusion of CO_2 and is coupled with a decrease in the mesophyll conductance of CO_2 (internal CO_2 diffusion) (Guha *et al.* 2013). Thus, the reduced g_s values of both crambe lineages may have hampered the supply of CO_2 to the carboxylation sites. Plants under moderate WD may present a reduced photosynthetic performance, mainly due to stomatal closure. However, if plants remain in such conditions (severe drought), the photosynthetic mechanism may also be affected by nonstomatal limitations, due to both reduced Rubisco content and activity and increased amounts of nonreducing Q_A PSII centers (Stirbet *et al.* 2018). In this work, plants showed clear signs of drought stress, which compromised the photosynthetic apparatus performance.

The crambe lineages subjected to WD showed increases in the Chl index (dimensionless). According to Arunyanark *et al.* (2008), the Chl index values are associated with the Chl density. Thus, the results obtained in this study indicate increases in the Chl density in the plants submitted to WD. In this condition, the leaf thickness can decrease, which is also associated with the RWC and cell volume reductions, and this results in more Chl per unit of area (Arunyanark *et al.* 2008). An increase in the Chl density under WD was already described by Puangbut *et al.* (2017) in artichoke genotypes (*Cynara scolymus* L.).

The signs of stress were evidenced by the kinetics of Chl *a* fluorescence, as well as the JIP-test parameters. The analysis of ΔV_{OP} corresponds to the primary photochemical events of photosynthesis on the acceptor side of PSII (Joliot and Joliot 2002). In this study, the two crambe lineages submitted to WD showed positive ΔV_{OP} amplitudes. The higher positive ΔV_{OP} amplitude observed in the FMS CR 1307 lineage under WD might have been caused by the lower efficiency in reoxidizing Q_A^- and the consequent reduction of electrons transport flux after Q_A (Zhang *et al.* 2015).

The energetic connectivity or grouping among the PSII units is verified by analyzing the L-band (Oukarroum *et al.* 2007, Tsimilli-Michael and Strasser 2008). Positive L-band values indicate low energy connectivity (Yusuf *et al.* 2010). In this study, the higher positive L-band amplitude observed in the FMS CR 1307 crambe lineage suggests less stability of the system and/or reduction of active reaction centers associated with PSII (Sharma *et al.* 2014). This result indicates that FMS CR 1326 maintained better utilization of excitation energy and greater stability of the system when exposed to WD (Oukarroum *et al.* 2007, Strasser *et al.* 2007). According to Redillas *et al.* (2011), increased connectivity can be considered a partially protective mechanism, directing more excitation energy to photochemistry. Therefore, the present results suggest that FMS CR 1326 lineage was more stable under WD for presenting a greater RWC maintenance. FMS CR 1326 lineage presented a 41.3% reduction and FMS CR 1307 a 53.1% reduction when they were compared to their control plants. Also, under WD, the RWC maintenance may be impaired by the osmotic action through the increase of proline content, which can indirectly affect the photosynthetic apparatus and stabilization of cell membranes (Rapacz *et al.* 2019).

The transfer of electrons from the oxygen-evolving complex (OEC) to the P_{680} reaction center refers to the K-band (Skotnica *et al.* 2000). Therefore, positive values indicate a reduction in the transfer of electrons to P_{680} (Jiang *et al.* 2008). The higher positive amplitude of the K-band observed in the FMS CR 1307 crambe lineage suggests the reduced transfer of electrons from the OEC to the P_{680} reaction center (Skotnica *et al.* 2000). De Ronde *et al.* (2004) suggested that positive K-band amplitudes indicate activation of some defense mechanisms, such as accumulation of glycine betaine, proline, and sugars.

In this study, the positive amplitude of the O–I phase ($V_{OI} \leq 1$) was observed in the two crambe lineages, noting a higher positive amplitude for the FMS CR 1307 lineage. This result is an indicator of low capacity to maintain the reduction of PQ since the O–I phase allows for evaluation of the sequence of photochemical events from energy capture starting in PSII to a reduction of PQ (Yusuf *et al.* 2010). All these results indicate that the FMS CR 1326 lineage is more efficient in reoxidizing Q_A^- and in exchanging energy between the PSII units. Furthermore, the low damage to the OEC (K-band), as well as the higher capacity to maintain the reduction of PQ described in FMS CR 1326, evidences higher photochemical tolerance to WD by FMS CR 1307.

The WD also resulted in decreased $V_{OI} \geq 1$. The I–P phase reflects the events related to the electron flow from PQH_2^- to the final PSI acceptors. Thus, the repeated reduction of plastocyanin and P_{700+} in PSI events can be evaluated (Chen *et al.* 2014). The lower $V_{OI} \geq 1$ values obtained in the FMS CR 1307 lineage indicates that less electron flux reached the final PSI acceptors. In this study, this apparent decline in the I–P phase occurred due to the drastic decrease of water content in the leaves, which could have reached the threshold of acclimation for drought (Guha *et al.* 2013).

According to Yusuf *et al.* (2010), the inverse of the time to reach $V_{IP} = 0.5$ provides an estimate of the overall reduction rate of the final electron acceptors of PSI. In turn, ΔV_{IP} reflects the size of the final electron acceptors pool of PSI, *i.e.*, the efficiency of the electron transport through PSI to reduce the final acceptors beyond PSI (Salvatori *et al.* 2015, Souza *et al.* 2019). The present results indicate both lower efficiency of electrons transfer to the acceptor side of PSI (reduction rate) and smaller size of the PSI final electron acceptor pool in both crambe lineages studied under water deficit, noting a stronger effect in the FMS CR 1326 lineage.

Under the suppression of the linear electron transport in PSII, Wang *et al.* (2017) suggested that the cyclic electrons transport (CET) probably compensates for the loss of reduction capacity in PSI. The activation of CET is a common photoprotective response to several stress factors, which prevents the formation of reactive oxygen species (ROS) (Oukarroum *et al.* 2015). In this study, the two crambe lineages probably increased the CET to improve the metabolic energy (ATP) under WD, considering the suppression of electron transport in PSII. The results obtained in this study indicate a possible correlation between the higher electron flow suppression in PSII (evaluated through ΔV_{OP}, K-band, and L-band) and the increased cyclic electron flow (ΔV_{IP}). This provided greater protection to FMS CR 1307 since this crambe lineage showed a higher photochemical loss in PSII under WD. Campos *et al.* (2014) also suggested that the increase of ΔV_{IP} in *Agave salmiana* Otto ex Salm-Dyck grown under WD was probably due to the occurrence of CET, which contributed to increased ATP production under stress conditions. This is because not all electrons from reduced ferredoxin (Fd_{red}) are directed to the formation of NADPH by ferredoxin-NADP reductase. Instead, the electrons are transferred to O_2 through the Mehler reaction, resulting in ROS formation.

In this study, WD reduced the efficiency of photochemical events in both crambe lineages. The specific energy flows values increased in both lineages, noting a stronger effect in FMS CR 1307 (18.6, 12.5, and 44.8% for ABS/RC, TR_0/RC, and DI_0/RC, respectively) when compared to FMS CR 1326 crambe lineage (2.74 ABS/RC, 2.15 TR_0/RC, and 0.58 DI_0/RC). The increased ABS/RC values increased the trapping and dissipation energy flow per RC (TR_0/RC and DI_0/RC, respectively). These results indicate that some RCs were inactivated and the efficiency per RC was enhanced (Meng *et al.* 2016). The inactivation of RC (decrease of nonreduced Q_A or heat dissipation) can be indicative of susceptibility to photoinhibition, *i.e.*, the inactivation of RC is considered to be a downregulation mechanism to dissipate the excess of energy absorbed (Franić *et al.* 2018). Furthermore, increases of TR_0/RC can indicate impairment of the OEC, corroborated by positive K-band amplitudes, which showed higher amplitude in the FMS CR 1307 crambe lineage. The increase of ABS/RC and TR_0/RC did not result in increased transport flow (ET_0/RC) values, but rather, it caused an increase of DI_0/RC values, which implies a higher loss of excitation energy as heat (Kalaji *et al.* 2014).

The maximum quantum yield of the primary photochemistry of PSII (φ_{P0}) and PI_{total} were reduced in the crambe lineages submitted to WD. Reductions of φ_{P0} are usually related to the degradation of the polypeptide D1, which results in an increased number of inactive RCs associated with PSII (Yusuf *et al.* 2010). PI_{total} is considered one of the most sensitive parameters of the JIP-test. PI_{total} measures the performance up to the final electron acceptors of PSI (Tsimilli-Michael and Strasser 2008, Yusuf *et al.*

2010). This shows that primary photochemical reactions were affected by WD.

Conclusions: The water deficit had a significant effect on the photochemical activity of crambe during the vegetative phase, reducing photosynthetic apparatus performance. Under WD, there were compromises in the energy fluxes per RC and reduction in the use of energy of crambe lineages, but WD affected the FMS CR 1307 plants to a greater extent (higher values of ABS/RC, TR_0/RC, DI_0/RC, and higher positive amplitudes of L- and K-bands). Plants of the FMS CR 1307 lineage presented an increase in the pool size of final electron acceptors in PSI (ΔV_{IP}). The FMS CR 1326 lineage plants presented an advantage over FMS CR 1307 in terms of stability and efficiency of PSII under low water availability conditions.

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