



## Morphophysiological responses of *Crambe abyssinica* Hochst. lineages submitted to water deficit during flowering

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### Abstract

Water deficit (WD) at the start of the flowering stage can negatively affect the productivity of plants. The aim was to investigate the morphophysiological strategies of two crambe lineages (FMS CR 1326 and 1307) submitted to WD during the flowering stage and their connection with the progeny's germination. Plants were submitted to WD at the start of flowering for 12 d and then were irrigated again (water resumption, WR). As a control, plants were cultivated with uninterrupted daily irrigation. Under WD, reductions were observed in the stomatal conductance, the number of xylem vessels, and the mass of grains. Positive K- and L-bands occurred, indicating lower stability and efficiency in the use of energy under WD. In the WR period, plants presented photochemical recovery. WD induced less vigorous seeds. FMS CR 1307 had the highest capacity to maintain its photochemical performance, due to alterations in water conductivity, resulting in greater seed production and vigor.

**Keywords:** chlorophyll *a* fluorescence; grain yield; plant anatomy; progeny; water stress.

### Introduction

*Crambe abyssinica* Hochst. (crambe) is an oilseed plant belonging to the Brassicaceae family, with promising

traits for industrial use due to the high contents of oil (30–35%) and erucic acid (63–64%) (Wang *et al.* 2000, L alas *et al.* 2012), meaning it has huge potential, especially for the production of biodiesel (Oliveira *et al.*

### Highlights

- Lineage FMS CR 1326 had lower photochemical adjustment under WD
- FMS CR 1307 had the best photochemical performance due to alterations in water conductivity
- FMS CR 1307 had greater progeny seed production and vigor

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**Abbreviations:** ABS/RC – absorption flux per RC; Chl – chlorophyll; CI – continuously irrigated plants; DI<sub>0</sub>/RC – dissipated energy flux per RC; ET<sub>0</sub>/RC – electron transport flux per RC; F<sub>0</sub> – minimal fluorescence yield of the dark-adapted state; F<sub>1</sub> – fluorescence intensity at 30 ms; F<sub>2</sub> – fluorescence intensity at 2 ms; F<sub>K</sub> – fluorescence intensity at 0.3 ms; F<sub>m</sub> – maximal fluorescence yield of the dark-adapted state; F<sub>v</sub>/F<sub>0</sub> – ratio of photochemical to nonphotochemical quantum efficiencies (PSII potential activity); g<sub>s</sub> – stomatal conductance; GSI – germination speed index; M<sub>0</sub> – number of closed and/or inactive reaction centers; OEC – oxygen-evolving complex; PI<sub>(ABS)</sub> – performance index based on absorption; PI<sub>(TOTAL)</sub> – overall performance index, which measures the performance up until the final electron acceptors of PSI; RC – reaction center; RC/ABS – total number of active reaction centers per absorption; S1CI – control plants – progeny of continuously irrigated plants; S1WD – progeny of plants submitted to WD for 12 d; S<sub>m</sub> – assumed proportional to the number of reduction and oxidation of one Q<sub>A</sub><sup>-</sup> molecule during the fast OJIP transient, and therefore related to the number of electron carriers per electron transport chain; TR<sub>0</sub>/RC – trapping flux per RC; WD – water deficit; WR – water resumption; %G – germination percentage; φD<sub>0</sub> – quantum yield of energy dissipation; φE<sub>0</sub> – quantum yield of electron transport; φP<sub>0</sub> – maximum quantum yield of primary photochemistry; ψE<sub>0</sub> – probability that a trapped exciton moves an electron into the electron transport chain beyond Q<sub>A</sub><sup>-</sup>.

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2015). At present, biofuels are considered a viable alternative to diversify the energy mix of countries (Costa *et al.* 2019a). Many studies have been conducted to increase the production of crambe in the world (Costa *et al.* 2019b, Li *et al.* 2019, Kwiatkowski *et al.* 2020). In Brazil, the Midwest is the main region where crambe is grown; productivity varies from 1,000 to 1,500 kg ha<sup>-1</sup> (Batista *et al.* 2018). New lineages have already been tested to expand the growing areas to improve productivity in Brazil, such as FMS CR 1307. This lineage is considered to be a pilot candidate and the FMS CR 1326 is also being tested by Fundação Mato Grosso do Sul (Fundação MS) (Oliveira *et al.* 2015).

Water deficit (WD) is the main environmental factor that limits the growth of plants throughout the world (Zhang *et al.* 2018a). Under WD, plants present reduced cell turgescence and stomatal conductance, diminishing the transpiration rates, which in turn impairs the assimilation of CO<sub>2</sub> and causes damage to the reaction centers of PSII, reducing its efficiency (Mehta *et al.* 2010). The consequent reduction of photosynthesis lowers plant productivity (Velázquez-Márquez *et al.* 2015). Furthermore, when this water deficiency occurs during the flowering phenological stage, senescence of the flowers occurs (abortion), reducing the number and/or size of grains (Mathobo *et al.* 2017). The lower yield means a lower value and stunted or deformed grain can even prevent the sale of certain crops. Therefore, identification and selection of genotypes with morphophysiological attributes that show drought-tolerance responses during certain plant development stages, such as the flowering stage, can be promising in the maintenance of crops' production (Santos *et al.* 2012, Anjum *et al.* 2017). Boiago *et al.* (2018) observed a decrease in the uptake of potassium (K) and sodium (Na) of crambe plants (cultivar Brillhante) under WD at the flowering stage, resulting in lesser growth of the plants.

Many regions in Brazil and the rest of the world are subject to low or no rainfall during certain periods of the year. This shortage of water resources requires irrigation techniques to maintain the potential productivity of crambe. However, no studies are investigating the responses of the two mentioned crambe lineages (FMS CR 1326 and 1307) to WD. According to Vasconcelos *et al.* (2018) and Araújo *et al.* (2013), identifying species/genotypes with the ability to adapt to water restriction is a valuable strategy to increase productivity. Thus, this work aimed to investigate the morphophysiological strategies of these two crambe lineages under WD in the flowering stage and the association with the production and germination of progeny.

## Materials and methods

**Plant material and experimental design:** The experiment was conducted in greenhouse conditions. Seeds of *Crambe abyssinica* (lineages FMS CR 1326 and 1307) were obtained from Fundação Mato Grosso do Sul (Fundação MS), located in the municipality of Maracaju, Mato Grosso do Sul. The seeds were previously disinfected with 70%

ethanol for 2 min, 1% (v/v) sodium hypochlorite for 20 min, and the fungicide *Ridomil*® for 10 min, followed by triple washing in distilled autoclaved water. The seeds were then sown to germinate in pots with a capacity of 14.3 L (15 seeds per pot) filled with soil (75.5% sand, 17.2% clay, and 5.2% silt). The soil had previously been analyzed chemically for correction of nutrients and pH, according to the recommendations of Oliveira *et al.* (1991). Thirty grams of simple superphosphate were added every 30 d during the experiment. Besides this, insecticide (*Evidence*®) and fungicide (*Ridomil*®) were applied according to the manual for the crop. Twenty days after sowing, the plantlets were thinned, leaving only one plant per pot.

The pots were watered daily to keep the soil at about 80% of field capacity ( $\approx 0.20 \text{ m}^3 \text{ m}^{-3}$ ) until the start of the flowering period when they were divided into two groups: (1) continually irrigated plants (CI) and (2) plants submitted to water deficit (WD). The WD treatment began when more than one-half (50% + 1) of the plants were undergoing inflorescence. For the FMS CR 1326 and FMS CR 1307 lineages, this period started 53 and 42 d after sowing, respectively. The WD was imposed by suspending the irrigation for 12 d (determined in preliminary studies) after the start of flowering. After this period, the irrigation was resumed daily (water resumption, WR) to maintain the soil moisture at about 80% of field capacity until the end of the experiment (end of the crop cycle, or 110 d after sowing).

The water levels in the soil [ $\text{m}^3 \text{ m}^{-3}$ ] were monitored with a soil water sensor (*ProCheck version 4, Dispositivos Decagon*). All the physiological measurements were carried out between 05:00 and 10:00 h with completely expanded leaves (third or fourth leaf from the apex).

### Stomatal conductance and chlorophyll *a* fluorescence:

The stomatal conductance ( $g_s$ ) and chlorophyll (Chl) *a* fluorescence transient were measured in nine plants ( $n = 9$ ) from each treatment during the periods of WD (0, 3, 6, 9, and 12 d of WD) and successive WR (3 and 12 d of WR). The  $g_s$  was measured on the abaxial leaf surface using a leaf porometer (*SC-1, Dispositivos Decagon*). The  $g_s$  was expressed in [ $\text{mmol}(\text{H}_2\text{O}) \text{ m}^{-2} \text{ s}^{-1}$ ]. The Chl *a* fluorescence transient was measured with a portable fluorometer (*Handy-PEA, Hansatech, UK*). The leaves were previously adapted to the dark for 20 min using leaf clips (*Hanstech*) to assure complete oxidation of the photosynthetic system. The light intensity on the leaves,  $3,000 \mu\text{mol}(\text{photon}) \text{ m}^{-2} \text{ s}^{-1}$ , was sufficient to generate the maximum fluorescence for all the samples. The transient fluorescence was recorded from 10  $\mu\text{s}$  to 1 s. The intensity of fluorescence at 20  $\mu\text{s}$  ( $F_0$ ), 100  $\mu\text{s}$ , 300  $\mu\text{s}$ , 2 ms ( $F_1$ ), and 30 ms ( $F_1$ ), and maximum fluorescence ( $F_m$ ) were recorded and analyzed by the JIP-test (Strasser *et al.* 2004, Stirbet and Govindjee 2011, Wang *et al.* 2016).

**Leaf and petiole anatomy:** After the periods of WD (12 d) and subsequent WR (12 d), the number of xylem vessels of leaves and petioles for each treatment was determined in seven plants ( $n = 7$ ). The samples were collected

randomly, fixed in FAA (formaldehyde, acetic acid, and ethanol at 50%, 0.5:0.5:9, v/v) for 72 h, followed by storage in 50% ethanol (Johansen 1940). Cross-sections were obtained with a double edge razor blade in the middle region of the third fully expanded leaf from the apex. The sections were observed and the images were captured with a *Leica DM 1000* light microscope coupled to a *Leica ICC50 HD* digital camera (Wetzlar, Germany). The *UTHSCSA-Imagetool*® software was used to calculate the anatomical measurements revealed by the photomicrographs.

**Plant growth and grain yield:** On the last day of the experimental period (110 d after sowing), the root dry mass and shoot dry mass were analyzed with 16 plants from each treatment ( $n = 16$ ). These two masses were determined in grams after the plants had been stored in paper bags for drying in a forced-air oven for 72 h at 60°C.

The hundred-grain mass and grain yield per plant were evaluated after the harvest and drying of the grains. The hundred-grain mass was determined by counting and weighing eight samples ( $n = 8$ ) of 100 grains from each treatment. The production of grains per plant was ascertained by weighing the grains produced by each plant of each treatment on a precision scale. The hundred-grain mass and grain yield were calculated with correction for 13% moisture.

**Germination of progeny:** At the end of the experimental period (110 d after sowing), dried seeds were sown for germination and were described as S1CI seeds [control plants – progeny of continuously irrigated plants (CI)], and S1WD (progeny of plants submitted to WD for 12 d). To determine the germination percentage (%G) and germination speed index (GSI), the crambe seeds were previously sterilized with a 70% ethanol solution for 2 min, 1% (v/v) sodium hypochlorite for 20 min, and the fungicide *Ridomil*® for 10 min, followed by triple washing with autoclaved distilled water. The seeds were then placed in *Gerbox*® plastic boxes (11 × 11 × 3 cm) on filter paper moistened with 10 ml of autoclaved distilled water and placed to germinate in a growth room at 25°C and photoperiod of 16-h light and 8-h dark. The germination test was carried out with 40 seeds per sample and 5 *Gerbox*® plastic boxes ( $n = 5$ ). The germination percentage (%G) was determined according to the criterion of radicle emission, and the germination speed index (GSI) was measured during 7 d according to Braga *et al.* (2019), calculated as described by Maguire (1962) using the formulas:  $\%G = (\sum ni/N) \times 100$  and  $GSI = (G1/N1) + (G2/N2) + \dots (Gn/n)$ , where: %G – germination percentage (with radicle emission considered to be germination);  $\sum ni$  – total number of seeds germinated;  $N$  – number of seeds placed to germinate; GSI – germination speed index; G1, G2, Gn – number of seeds germinated on the first, second, and last day; N1, N2, and Nn – number of days since the first, second and last day of sowing.

**Statistical analysis:** The experimental design was completely randomized in a 2 × 2 factorial scheme, with two

irrigation regimes [continuously irrigated plants (CI) – 80% of field capacity – approximately 0.20 m<sup>3</sup> m<sup>-3</sup> of soil moisture, and plants submitted to water deficit (WD – 12 d irrigation suspension)] and two lineages of crambe (FMS CR 1326 and 1307). The data were submitted to analysis of variance (*ANOVA*) and the means were compared by the *Tukey's* test ( $p < 0.05$ ). The statistical analysis was applied to monitoring time and water regime, as well as genotypes and water regime. All the analyses were carried out with the *Sisvar*® program (Ferreira 2011).

## Results

**Stomatal conductance ( $g_s$ ) and soil moisture:** After 12 d of WD, symptoms (wilted leaves) were observed in all the plants. The WD at the start of the flowering period influenced the soil moisture and  $g_s$  of crambe plants (Fig. 1). The soil moisture and  $g_s$  diminished during the 12 d of WD. The plants of both lineages showed partial recuperation of  $g_s$  after the resumption of irrigation.

**Leaf anatomy:** At 12 d of WR, the number of leaf xylem vessels was significantly greater in the plants of the FMS CR 1326 lineage compared to the FMS CR 1307

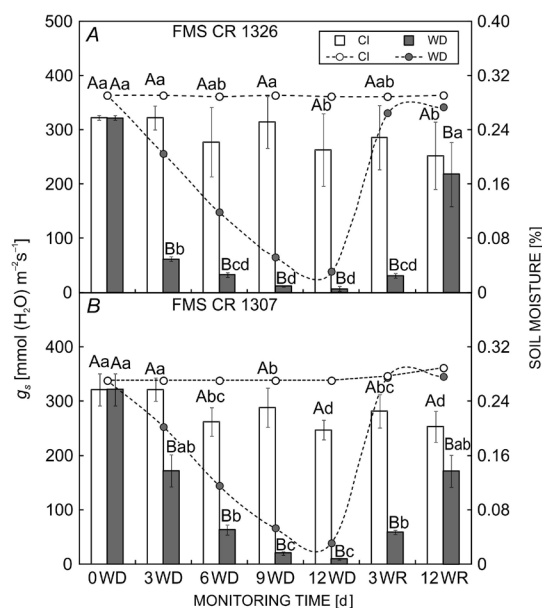


Fig. 1. Stomatal conductance,  $g_s$  (columns – primary ordinate scale on the left) in relation to soil moisture (rows – secondary ordinate scale on the left) of two lineages of crambe, FMS CR 1326 (A) and FMS CR 1307 (B) submitted to water deficit (0, 3<sup>rd</sup>, 6<sup>th</sup>, 9<sup>th</sup>, and 12<sup>th</sup> d of WD) followed by resumed irrigation (3<sup>rd</sup> and 12<sup>th</sup> d of WR) for recuperation. For the  $g_s$ , means  $\pm$  SD ( $n = 9$ ) followed by the same letter, uppercase for water regimes (comparing the CI and WD in each monitoring day) and lowercase for monitoring days (comparing the monitoring days in each water regime), do not differ significantly by the *Tukey's* test ( $p < 0.05$ ). CI – continuously irrigated plants; WD – water deficit; WR – water resumption.

lineage after the WD and resumption of irrigation (Figs. 2, 3A). Furthermore, the lineages also responded differently regarding the number of xylem vessels per vascular bundle in the petiole (Fig. 3B). After WD and WR, the reduction was observed in the number of xylem vessels per vascular bundle in the petiole in the FMS CR 1326 lineage and an increase in the FMS CR 1307 lineage.

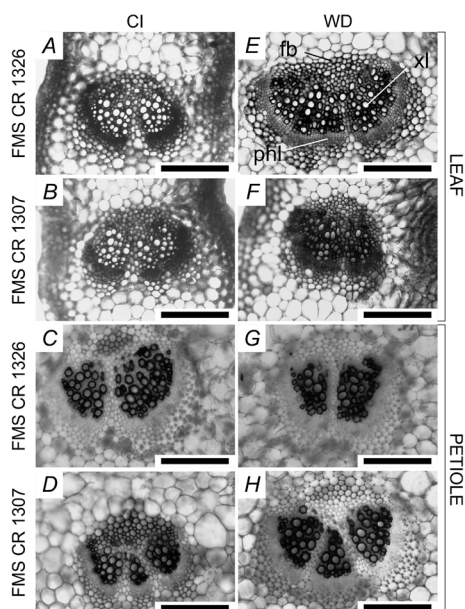


Fig. 2. Cross-sections of crambe plants (leaves and petioles) of two lineages previously submitted to 12 d of WD, followed by 12 d of WR. Bar = 50  $\mu$ m. xl – xylem; phl – phloem; fb – fibers. CI – continuously irrigated plants; WD – water deficit; WR – water resumption.

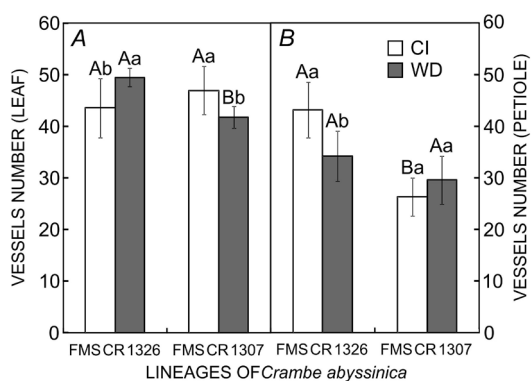


Fig. 3. Number of xylem vessels per vascular bundle in leaves (A) and petioles (B) of two lineages of crambe (FMS CR 1326 and FMS CR 1307) submitted to water deficit followed by water resumption. Means  $\pm$  SD ( $n = 7$ ) followed by the same letter, uppercase for lineages (comparing FMS CR 1326 and FMS CR 1307 in each water regime) and lowercase for water regimes (comparing CI and WD in each lineage), do not differ significantly by the Tukey's test, ( $p < 0.05$ ). CI – continuously irrigated plants; WD – water deficit.

**Chl *a* fluorescence transient – OJIP and JIP-test:** The WD also affected the photosynthetic apparatus (Figs. 4, 5). The variable fluorescence related to the O (20  $\mu$ s) and J (2 ms) steps and between the O (20  $\mu$ s) and K (300  $\mu$ s) steps were normalized and are reported as kinetic differences, making the K- and L-bands visible, respectively. The bands peaked at around 0.27 and 0.15 ms, respectively. The two crambe lineages presented positive K- and L-bands. In the FMS CR 1326 lineage, we observed increases in K- and L-bands as the WD became more intense (Fig. 4A,D). For the FMS CR 1307 lineage, positive K- and L-bands were only observed on 9 and 12 d of WD, respectively, and negative bands were observed on 3 and 6 d of WD, respectively, and also during 3 and 12 d of WR, respectively (Fig. 4B,E). The FMS CR 1307 lineage presented lower values in the K- and L-bands in comparison to the FMS CR 1326 lineage during the periods of WD and successive WR (Fig. 4C,F). The water restriction and resumption influenced the parameters of the JIP-test. All the data were normalized to day zero of WD (0 WD) and varied in function of the water regimes and days under water deficit/recuperation, as depicted in Fig. 5. Significant increases of  $M_0$  and reductions of  $F_v/F_0$  and  $S_m$  were observed for both lineages during WD and WR. Nevertheless, the FMS CR 1307 lineage presented lower values than that of the FMS CR 1326.

Concerning the parameters of the specific energy fluxes (per active RC of PSII), increases in the values of absorption (ABS/RC), trapping ( $TR_0/RC$ ), transport ( $ET_0/RC$ ), and dissipation ( $DI_0/RC$ ) per RC and decreases of RC/ABS were observed for the FMS CR 1326 lineage during the entire experimental period (Fig. 5A). However, for FMS CR 1307, these increases were observed only on the 12 d of WD (Fig. 5B). The FMS CR 1307 lineage presented reductions of 19.6, 16.5, 14.1, and 66.2%, respectively, for specific energy fluxes parameters on the 12 WD in comparison with FMS CR 1326. Reductions of the values of  $\phi E_0$  and  $\psi E_0$  and increases of  $\phi D_0$  were observed for the FMS CR 1326 plants exposed to WD (Fig. 5A). In contrast, for the FMS CR 1307 lineage, these increases were only observed on the 12 WD (Fig. 5B).

The performance indexes [ $PI_{(ABS)}$  and  $PI_{(TOTAL)}$ ] declined for the two lineages under WD (Fig. 5). However, the plants showed progressive recuperation of  $PI_{(ABS)}$  and  $PI_{(TOTAL)}$  after WR. The FMS CR 1307 plants presented the largest increases in the values of  $PI_{(ABS)}$  and  $PI_{(TOTAL)}$  in the period of recovery after WD (Fig. 5B).

**Plant growth and grain yield:** The water regimes and lineages influenced the growth traits and grain yield of the crambe plants, but this influence was independent [the factors (lineages and water regimes) did not have significant interaction]. Reductions of root and shoot dry mass were observed in the plants exposed to WD, irrespective of lineage (45.4 and 30.5%, respectively, compared to the CI plants). The hundred-grain matter and production of grains per plant exposed to WD also declined, independently of crambe lineages. When the

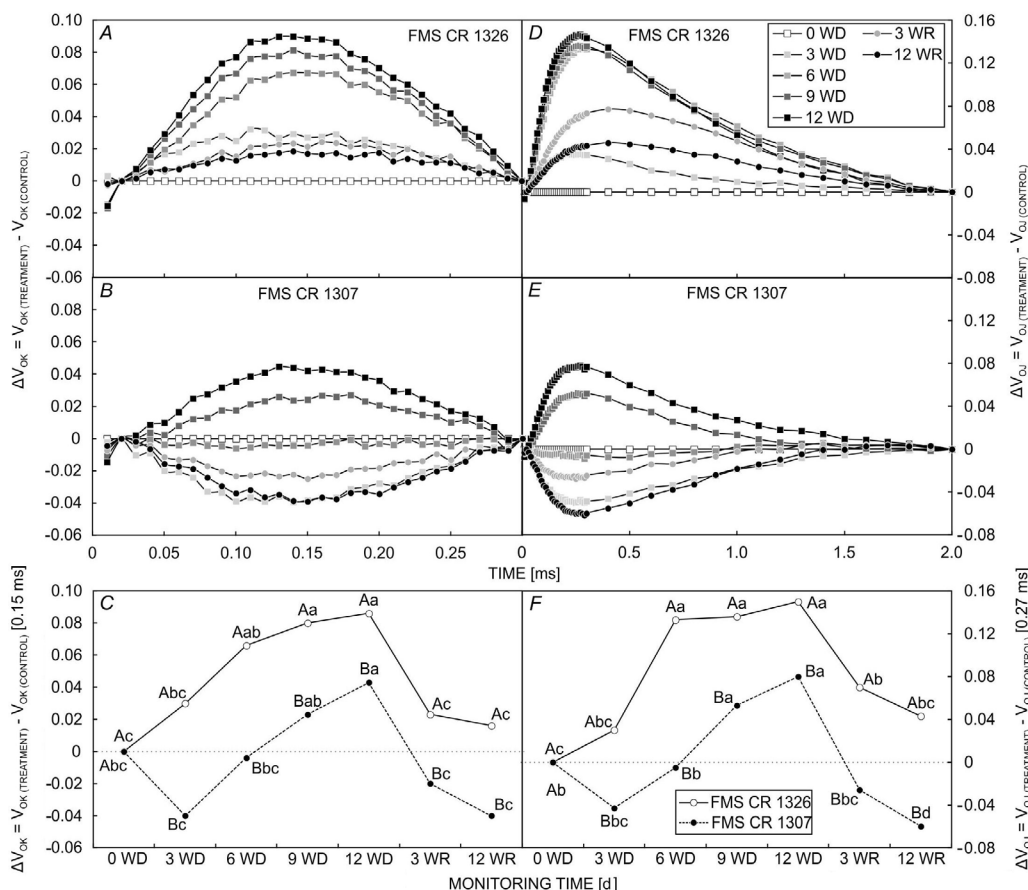


Fig. 4. Kinetic difference of  $V_{OJ}$  (A,B,E) and  $V_{OK}$  (C,D,F) obtained by double normalization of the O–J steps [ $\Delta V_{OJ} = V_{OJ(treatment)} - V_{OJ(control)}$ ] and O–K steps [ $\Delta V_{OK} = V_{OK(treatment)} - V_{OK(control)}$ ], respectively, in two lineages of crabe (FMS CR 1326 and FMS CR 1307) submitted to water deficit (0, 3<sup>rd</sup>, 6<sup>th</sup>, 9<sup>th</sup>, and 12<sup>th</sup> d of WD) followed by water resumption (3<sup>rd</sup> and 12<sup>th</sup> d of WR). Means ( $n = 9$ ) with the same letters in the row, uppercase for lineages (comparing the FMS CR 1326 and FMS CR 1307 in each monitoring day) and lowercase for monitoring days (comparing the monitoring days in each lineage), do not differ significantly by the Tukey's test ( $p < 0.05$ ). WD – water deficit; WR – water resumption.

hundred-grain matter was compared between the two lineages, FMS CR 1307 presented higher grain yield values than that of the FMS CR 1306 lineage (Table 1).

**Germination of the progeny seeds:** The WD in the flowering stage, as well as the lineages, influenced the GSI of the S1CI and S1WD of seeds (Fig. 6A). The germination percentage of the S1CI and S1WD seeds was only influenced by the water regime (Fig. 6B). Reductions in the GSI of the S1WD seeds of the FMS CR 1326 lineage were observed, as well as reductions of the germination percentage of the S1WD seeds (about 17.2%; Fig. 6).

## Discussion

In this study, we investigated how WD influences the morphophysiology of two lineages of *C. abyssinica* at the start of the flowering stage. The plants of lineages FMS CR 1307 and FMS CR 1326 presented distinct responses during the WD and after WR. These differences were

related to alterations of the anatomy and physiology, which were fundamental to maintain survival and also influenced effective grain production.

The monitoring of the control plants' transpiration of both lineages allowed noting a trend for the decrease of  $g_s$  in the leaves during the reproductive stages (flowering and grain development). This was probably related to the greater allocation of water to the flowers than would normally be required. Indeed, water is an essential resource and its scarcity limits the development of shoots, maintenance of physiology and longevity of flowers, which in turn directly influences the attraction of pollinators (Zhang *et al.* 2017a, Roddy *et al.* 2019). WD at the start of the flowering stage hampers many physiological and metabolic processes, affecting the development and grain yield of plants (Zhang *et al.* 2017b). Since crabe plants are pollinated by insects (Simioni *et al.* 2015), maintenance of good hydration of the reproductive organs is a critical aspect for attraction of pollinators, besides influencing the floral longevity and effective formation of grains (zygotic embryos).

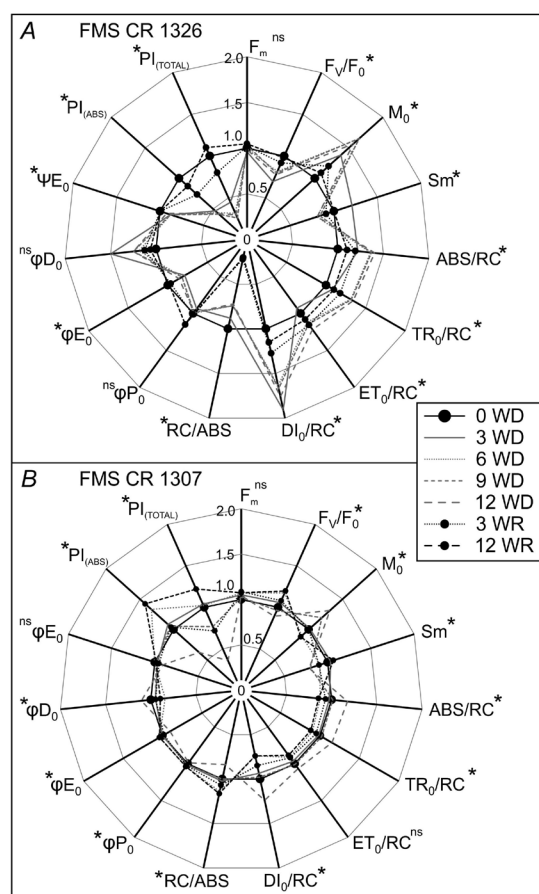


Fig. 5. JIP-test parameters of two lineages of crambe, FMS CR 1326 (A) and FMS CR 1307 (B), submitted to water deficit (0, 3<sup>rd</sup>, 6<sup>th</sup>, 9<sup>th</sup>, and 12<sup>th</sup> d of WD) followed by water resumption (3<sup>rd</sup> and 12<sup>th</sup> d of WR). Means ( $n = 9$ ) followed by asterisks (\*) are significantly different in relation to monitoring days under WD and WR according to the Tukey's test, (\*  $-p < 0.05$ ; ns – not significant). ABS/RC – absorption flux per RC; DI<sub>0</sub>/RC – dissipated energy flux per RC; ET<sub>0</sub>/RC – electron transport flux per RC; F<sub>m</sub> – maximal fluorescence yield of the dark-adapted state; F<sub>v</sub>/F<sub>0</sub> – ratio of photochemical to nonphotochemical quantum efficiencies (PSII potential activity); M<sub>0</sub> – number of closed and/or inactive reaction centers; PI<sub>(ABS)</sub> – performance index based on absorption; PI<sub>(TOTAL)</sub> – overall performance index, which measures the performance up until the final electron acceptors of PSII; RC/ABS – total number of active reaction center per absorption; Sm – assumed proportional to the number of reduction and oxidation of one Q<sub>A</sub><sup>-</sup> molecule during the fast OJIP transient, and therefore related to the number of electron carriers per electron transport chain; TR<sub>0</sub>/RC – trapping flux per RC; WD – water deficit; WR – water resumption; φD<sub>0</sub> – quantum yield of energy dissipation; φE<sub>0</sub> – quantum yield of electron transport; φP<sub>0</sub> – maximum quantum yield of primary photochemistry; ψE<sub>0</sub> – probability that a trapped exciton moves an electron into the electron transport chain beyond Q<sub>A</sub><sup>-</sup>.

The reductions of  $g_s$  observed as at 3 WD allow inferring that closing of the stomatal pores (ostioles) occurred. Plants close their stomata to limit water loss and reduce the negative effects of WD. This physiological

response can be mediated by abscisic acid (ABA) (Saradadevi *et al.* 2017, Kuromori *et al.* 2018). The biosynthesis of this hormone is related to the regulation of the expression of genes responsive to water stress in the roots (Rasheed *et al.* 2016). In this study, the reduction of the soil moisture was more drastic on the 12 WD, which induced a sharper decline of the transpiration via stomatal conductance. These modulations of transpiration and water conductivity are keys for drought tolerance because they decelerate the loss of water to the atmosphere (Zhang *et al.* 2018a).

During WD, the water conductivity can be compromised due to the loss of functionality of the xylem vessels by an embolism (Tombesi *et al.* 2015). The vulnerability of leaves induced by embolism under stress conditions provoked by drought can lead to leaf mortality (Cardoso *et al.* 2020). Therefore, phenotypic plasticity of the anatomical traits involved in water conductivity is fundamental for plants to adjust to water stress. In this study, the two crambe lineages presented different responses to the water stress conditions imposed. The plants of the FMS CR 1326 lineage had a higher number of xylem vessels per vascular bundle in the new leaves formed after the WD period and subsequent WR. This can be interpreted as a compensatory response to losses of water conductivity by embolism. In contrast, the FMS CR 1307 plants had reduced water conductivity due to a smaller number of vessel elements. This makes sense because water conductivity is modulated by the diameter and/or the number of vessels in the xylem (Tulik *et al.* 2010, Zhang *et al.* 2018b). A decrease in water conductivity due to alterations in the number or diameter of xylem vessels can contribute to reduce the transpiration through the stomata and increase the drought tolerance, as reported by Zhang *et al.* (2018b) and El-Sayed *et al.* (2019). Indeed, the adjustments of water conductivity (confirmed by the values of  $g_s$ ) observed in the FMS CR 1307 plants were efficient and permitted a faster recovery of these plants after 3 WR (demonstrated by the analyses of Chl *a* fluorescence).

Water deficit regimes also influence the performance of the photosynthetic apparatus. In conditions of water restriction, the analysis of Chl *a* fluorescence consistently shows positive values for K- and L-bands (Falqueto *et al.* 2017, Martins *et al.* 2017, ). Positive values of K- and L-bands indicate physiological disturbances in response to stress (Meng *et al.* 2016). The positive amplitudes of the K-band of both lineages under WD indicated partial inactivation of the oxygen-evolving complex (OEC) (Rosa *et al.* 2018). This behavior was confirmed in the present study, by the reduction of F<sub>v</sub>/F<sub>0</sub> in the FMS CR 1326 plants on all WD and WR periods. This result suggests reduced efficiency on the donor side of PSII, *i.e.*, inactivation of the OEC (Kalaji *et al.* 2016). The FMS CR 1307 plants were more tolerant and only showed reductions in the F<sub>v</sub>/F<sub>0</sub> values at 9 WD and 12 WD.

The positive amplitudes of the L-band of both lineages can be interpreted as inhibition of the donation of electrons to YZ (Tyr161 of the protein D1) and disorganization of PSII (Huang *et al.* 2017). The rising

Table 1. Plant growth and grain yield of crambe as a function of lineages (FMS CR 1326 and FMS CR 1307) or water regimes (CI and WD). For water regime or lineages of crambe, means  $\pm$  SD (growth traits:  $n = 16$ ; grain yield:  $n = 8$ ) followed by the same letter in the column do not differ significantly according to the Tukey's test ( $p < 0.05$ ). CI – continuously irrigated plants; WD – water deficit.

Water regime	Root dry mass [g per plant]	Shoot dry mass [g per plant]	100-grain mass [g]	Production of grains per plant [g per plant]
CI	0.011 $\pm$ 0.005 <sup>a</sup>	0.085 $\pm$ 0.018 <sup>a</sup>	2.43 $\pm$ 0.50 <sup>a</sup>	61.40 $\pm$ 5.94 <sup>a</sup>
WD	0.006 $\pm$ 0.003 <sup>b</sup>	0.059 $\pm$ 0.014 <sup>b</sup>	1.76 $\pm$ 0.41 <sup>b</sup>	35.70 $\pm$ 8.01 <sup>b</sup>
Lineages of crambe	Root dry mass [g per plant]	Shoot dry mass [g per plant]	100-grain mass [g]	Production of grains per plant [g per plant]
FMS CR 1326	0.011 $\pm$ 0.005 <sup>a</sup>	0.081 $\pm$ 0.014 <sup>a</sup>	1.72 $\pm$ 0.36 <sup>b</sup>	47.75 $\pm$ 16.91 <sup>a</sup>
FMS CR 1307	0.006 $\pm$ 0.003 <sup>b</sup>	0.063 $\pm$ 0.022 <sup>b</sup>	2.47 $\pm$ 0.48 <sup>a</sup>	49.46 $\pm$ 20.40 <sup>a</sup>

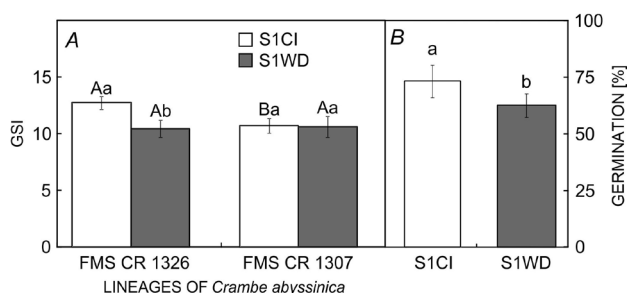


Fig. 6. Germination speed index (GSI) and germination rate [%] of the progeny of crambe plants previously submitted to water deficit followed by water resumption. (A) GSI of the progeny of two lineages of crambe (FMS CR 1326 and FMS CR 1307) submitted to water deficit followed by water resumption and (B) germination rate of the progeny of crambe plants, irrespective of the lineage (only influenced by the previous water regime treatments). Means  $\pm$  SD ( $n = 8$ ) followed by the same letter, uppercase for lineages (comparing the FMS CR 1326 and FMS CR 1307 in each water regime) and lowercase for water regime (comparing the S1CI and S1WD), do not differ significantly according to the Tukey's test ( $p < 0.05$ ). S1CI – seeds of progeny of continuously irrigated plants; S1WD – seeds of progeny of plants submitted to water deficit.

increase of the positive amplitudes in the L-band of the FMS CR 1326 plants suggests that each day of WD aggravates the damages to energy connectivity or grouping among the PSII units. For the FMS CR 1307 plants, the photochemical damages began on the 9 WD. Between the two lineages, FMS CR 1307 was better able to maintain the balance between the electrons at the donor and acceptor sites of PSII when submitted to moderate WD (6 WD), but not intense WD (9 WD and 12 WD). In turn, the FMS CR 1326 plants were unable to maintain the equilibrium between electrons even at the start of the water deficit (3 WD).

$M_0$  is associated with the number of closed and/or inactive reaction centers (Einali and Shariati 2015) and the electron transport between quinone A ( $Q_A$ ) and quinone B ( $Q_B$ ). An increase of this parameter's values can indicate inhibition on the acceptor side of PSII (Wang *et al.* 2016). Therefore, the reduction of the values of  $M_0$

of the FMS CR 1307 plants indicates total recovery in the rehydration period, besides an increase of primary photochemical activity. The  $F_v/F_0$  ratio is considered the most sensitive parameter of the electron transport chain, since it is proportional to the activity of the OEC, *i.e.*, of the donor side of PSII. An increase of this parameter indicates an improved electron transport (Ghassemi-Golezani and Lotfi 2015). The reductions of  $F_v/F_0$  and  $S_m$  observed for the FMS CR 1326 plants indicate greater impairment of the electron transfer system caused by WD.

The increases of ABS/RC values and reductions of RC/ABS in the FMS CR 1326 lineage also indicate reduced stability of the RC, *i.e.*, a reduction in the connection with the light-harvesting antenna complexes (Chen *et al.* 2014, Li *et al.* 2020). For both lineages, an increase of ABS/RC resulted in increases of  $TR_0/RC$  and  $DI_0/RC$ . According to Souza *et al.* (2019), increases in the values of  $TR_0/RC$  proportional to increases of ABS/RC indicate impairment of OEC due to suppressed repair of the damaged PSII (Takahashi and Murata 2008). This response was confirmed by the increase of the positive values of the K-band and reductions of  $F_v/F_0$ , which were more pronounced in the FMS CR 1326 plants. Besides this, the reductions of  $\phi E_0$  and  $\psi E_0$  and increases of  $\phi D_0$  observed in the FMS CR 1326 plants submitted to WD also indicate that the energy absorbed was not trapped and used to reduce the  $Q_A$ , which increased the loss of energy by dissipation ( $\phi D_0$ ) (Meng *et al.* 2016, Martins *et al.* 2019). In rice plants (*Oryza sativa* L. of the Liangyoupeijiu genotype), response to WD was also related to increases in the energy dissipation flux ( $DI_0/RC$ ), as well as higher quantum yield for energy dissipation ( $\phi D_0$ ) and decrease of RC/ABS, indicating lower energy use during the WD period (Wang *et al.* 2017).

The performance indexes [ $PI_{(ABS)}$  and  $PI_{(TOTAL)}$ ] are considered the most sensitive parameters of the JIP-test. According to Yusuf *et al.* (2010), reductions of the PI values denote a decrease in the energy conservation potential of the photosynthetic apparatus. The reductions of  $PI_{(ABS)}$  and  $PI_{(TOTAL)}$  in the two crambe lineages analyzed showed reduced efficiency of the energy fluxes of the electron transport chain under WD. In general, the FMS CR 1326 lineage suffered greater impairment of the photosynthetic apparatus under WD, *i.e.*, the worse

performance of the photosynthetic apparatus, while the FMS CR 1307 plants presented increases in these parameters after rehydration (WR), revealing the ability to maintain efficient energy transfer and capture of photosystem PSII, indicating greater recuperation and photochemical tolerance to WD. This better performance of the photosynthetic apparatus was related to the alterations of the water conductivity and consequent more efficient use of water in adverse conditions.

Water shortage often stunts the vegetative growth of plants (Covre *et al.* 2016), as observed in this study. The reduction of the accumulation of dry matter of the crambe plants under stress was associated with the worse performance of the photosystems. This response may be associated with hormonal balance. According to Masondo *et al.* (2019), the increased concentration of endogenous ABA activates various response mechanisms to drought stress, impairing the performance of the photosynthetic apparatus. Differences in the accumulation of dry biomass and embryonic grain development in the plants submitted to temporary water deprivation were observed. The FMS CR 1326 plants presented greater accumulation of dry mass (vegetative growth), but the effective grain production was partially impaired since many grains were unfilled due to the inability to form zygotic embryos. Hence, there was a preponderance of energy drain (embryos) over energy source (mother plant). On the other hand, the FMS CR 1307 plants accumulated less dry biomass but had greater hundred-grain mass. This means that most of the grains were filled with zygotic embryos. The development of viable seeds (with all components such as cotyledons, embryo, seed coat, and endosperm) demands considerable energy; hence the preponderance of energy drains to source. The energy synthesized from sucrose in green (photosynthetically active) tissues is carried through the phloem to support the growth and maturation of the grains (seeds) (Aguirre *et al.* 2018). We suggest that the lower accumulation of dry biomass by the FMS CR 1307 plants occurred due to the energy demand for the development of embryos. This greater energy demand did not occur in the FMS CR 1326 plants in the final reproduction stages because of the lower number of zygotic embryos per plant (hundred-grain mass).

WD can also cause consistent impacts on the successive processes of plants (Shi *et al.* 2018), such as seed development of progeny. Exposure to environmental factors of the mother plant can have subsequent effects on the seeds of the progeny (Postma and Agren 2015, Chen *et al.* 2020). The alterations in the GSI of the seeds generated by the FMS CR 1326 plants might be associated with the endogenous content of ABA in the seeds. Likewise, the lower germination rate of the seeds (less vigor) produced by the mother plants previously exposed to WD might be involved with the accumulation of ABA in the seed endosperm. This hormone plays a central role in seed dormancy and germination (Wasilewska *et al.* 2008).

**Conclusions:** The two crambe lineages evaluated showed distinct responses to WD at the start of the flowering

stage. The FMS CR 1326 plants had less ability for photochemical adjustment and lower effective grain yield when submitted to water restriction. The water stress produced a residual effect on the progeny, resulting in less vigorous seeds. The FMS CR 1307 plants had a greater ability to maintain the performance of the photosynthetic apparatus under WD due to alterations in the water conductivity and thus more efficient use of water. The FMS CR 1307 lineage is thus more tolerant to water stress during the flowering stage and has a greater effective grain yield.

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