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Genetic parameters of morpho-agronomic and physiological traits of crambe genotypes under drought conditions[1]

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ABSTRACT - This work aims at evaluating and discriminating crambe genotypes regarding tolerance to water deficit by means of morpho-agronomic and physiological traits. The trial was carried out in a greenhouse under a randomized complete block design with four blocks, in a 2 x 8 factorial scheme, being two environments (with and without stress) and eight genotypes (FMS CR 1101, FMS CR 1106, FMS CR 1203, FMS CR 1305, FMS CR 1307, FMS CR 1312 and FMS CR 1326 and cultivar FMS Brilhante). The genotypes were evaluated according to physiological and morpho-agronomic traits. The significance of the mean squares was tested by the F test ($P < 0.05$) and the genetic parameters were estimated as ratio b between the genetic (CVg) and experimental (CVe) coefficients of variation, broad-sense heritability at genotypic mean level ($\hat{h}_m^2$), intraclass correlation (r) and selective accuracy (Ac). In general, water deficit did not cause severe physiological limitations to the plants. Plant height (PH), total dry mass (TDM) and grain yield (GY) were the traits that stood out for presenting the highest estimates of ratio b, $\hat{h}_m^2$, CVg and r, revealing the possibility of genetic gains with the selection. Nevertheless, after evaluating the performance of genotypes in terms of physiological and morpho-agronomic traits, it was concluded that cultivar FMS Brilhante and inbred lines FMS CR 1326 and FMS CR 1106 had the most favorable traits for the selection, therefore, presenting potential for water deficit tolerance.

Key words: Agroenergy. *Crambe abyssinca* Hostch. Gas exchanges. Plant breeding. Water deficit.

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INTRODUCTION

The impacts of global climate changes resulting from the increase of the concentration of greenhouse gases will subject crops to a variety of environmental stress. Drought tends to expand its frequency and intensity, causing a great impact on economy, society and the environment (NAUMANN *et al.*, 2018). In Brazil, water deficit, which used to be restricted to arid and semi-arid regions, today is extended to a great part of the Brazilian territory, directly affecting the production chain and sustainability in agriculture.

Water deficit is characterized by the insufficient availability of water in the soil to fulfill the requirements of plants at a certain time, jeopardizing several of their vital functions (TARDIEU; SIMONNEAU; MULLER, 2018). Among the few existing options to mitigate the drought effects on crop production, the development of tolerant cultivars has turned out to be the most effective and promising alternative. A plant which is tolerant to water deficit is the one that keeps its production during gradual and moderate water deficit in the soil (TARDIEU; SIMONNEAU; MULLER, 2018). Crambe (*Crambe abyssinca* Hostch) is one example of a species of agricultural interest which presents this tolerance (MARTINS *et al.*, 2017; MOURA *et al.*, 2018).

Crambe stands out for the high content of oil in its seeds (30-35%) with a high content of erucic acid (63-64%) and other valuable chemical compounds (LALAS *et al.*, 2012), which make the culture an important raw material for the industry of biodiesel, synthetic rubber, biodegradable plastic, cosmetics, lubricants, hydraulic fluid for agricultural applications, among others (FANIGLIULO *et al.*, 2021; GÄLLSTEDT *et al.*, 2017; LOVATTO *et al.*, 2017). Favorable agronomic traits, such as low nutritional need, rusticity, short cycle and wide adaptability are other factors that contribute to the success of the species (BASSEGIO *et al.*, 2016; ZANETTI *et al.*, 2016).

Recent research revealed tolerance of cultivar FMS Brilhante to water deficit (MARTINS *et al.*, 2017, MOURA *et al.*, 2018). With grain yield between 1000 and 1500 kg ha^{-1}, the cultivar presents wide variability for agronomic traits of interest, which has generated genetic gains with the selection of superior plants within the cultivar itself (LARA-FIOREZE *et al.*, 2016; PITOL; BROCH; ROSCOE, 2010). Crambe breeding in Brazil culminated in the development of inbred lines derived from cultivar FMS Brilhante, aiming at an increase of grain yield and oil quality (PITOL; BROCH; ROSCOE, 2010). The obtainment of plants tolerant to abiotic stress has also become the focus of attention of current breeding programmes. The development of cultivars tolerant to low water availability is the most efficient option for the increase of productivity, allowing the expansion of the culture to other promising regions all over the world. (MARTINS *et al.*, 2017).

The identification and characterization of tolerant genotypes, as well as the study of their behavior in conditions of water deficit, will make it easier, in the future, to select genotypes with a good combination of superior agronomic traits, cumulatively contributing to better yields under dry conditions (TARDIEU; SIMONNEAU; MULLER, 2018). In this sense, the phenotypic selection based on morpho-physiological traits, also including production and its components, is an important tool in the discrimination of genotypes and in the evaluation of their performance. This way, this work aims at evaluating and discriminating crambe genotypes regarding tolerance to water deficit by means of morpho-agronomic and physiological traits.

MATERIAL AND METHODS

Plant Material and Experimental Design

The trial was carried out from August to November 2018, in a greenhouse in Experimental Field “Diogo Alves de Melo” (7.703.630 N, 720.570 E, 648 m altitude) and in the Laboratory of Oilseed Breeding, both belonging to the Department of Agronomy of the Federal University of Viçosa (UFV), in Viçosa, Minas Gerais, Brazil. The randomized experimental block design was used, with four blocks in factorial scheme 2 x 8, being two environments (with and without stress) and eight genotypes (Inbred lines FMS CR 1101, FMS CR 1106, FMS CR 1203, FMS CR 1305, FMS CR 1307, FMS CR 1312 and FMS CR 1326 and cultivar FMS Brilhante) given by the Fundação Mato Grosso do Sul.

The seeds used were treated with fungicide Derosal® (Carbedazin + Thiram) and then they were sown in plastic vessels with a capacity of 12 liters of soil. Five seeds were put in each vessel and, after emergence, the seedlings were pruned, leaving only one per vessel. All the other culture traits and fertilization were carried out according to the recommendations for the culture (PITOL; BROCH; ROSCOE, 2010).

Plant Growth Conditions

The management of the plants was carried out normally up to the flowering stage (40-60 days after germination). From this stage onwards, the plants of the “stress” condition were induced to water deficit for 15 days. At the end of flowering, and after the 15 days, the plants started to be managed at field capacity again. The maximum average and minimum temperatures (Figure 1) were taken daily, with the help of a digital thermometer, located inside the greenhouse.

Water deficit was induced according to the methodology proposed by Nascimento *et al.* (2021). In order to adopt this methodology it was necessary to perform a physical analysis of the soil (Table 1) for the determination of its texture and for further preparation of the water retention curve (Figure 2), built through a pressure chamber.

Based on the equation of the curve ($\theta = 23.501T^{-0,056}$, Figure 1), it was possible to estimate soil moisture at field capacity and in the desired stress situation, as well as the critic moisture in which the plant significantly reduces its productivity. Conceptually, it is believed that sandy soils are at field capacity at the tension of 10 kPa (CASSEL; NIELSEN, 1986). For the simulation of water stress, 900 kPa tension was applied, such a value that is higher than the critical moisture, able to cause stress, and lower at the permanent wilting point of the soil (1500 kPa).

After installing the trial, a system was designed, consisting of water mass (M_{water}), mass of dry soil (M_{ds}), vessel mass (M_{vessel}) and plant mass (M_{plant}), and the equation of the mass of the system was defined as: $Msys = Mvessel + Mds + Mwater + Mplant \cdots (1)$ (NASCIMENTO *et al.*, 2021). Plant mass was determined from the measurement of the fresh matter of plants in different phenological stages. In order to do so, additional plants were added to the plots, grown in parallel with the evaluated plants. An electronic spreadsheet was organized, where the weight of the system and its respective treatments were monitored and kept as the weight to be reached every day by means of irrigation management. During the period of stress imposition, a representative sample of the vessels per block was weighed at 10 am and 4 pm, and, from the difference of mass corresponding to each treatment, the water blade was applied to balance the masses of the system.

Physiological Traits

The following evaluations were performed after the period of water deficit imposition (15 days after flowering):

Gas Exchanges and Chlorophyll Fluorescence

After the flowering stage of the plants, 15 days after the stress application, the parameters of gas exchanges and chlorophyll α fluorescence were read simultaneously, with the aid of portable IRGA (LI-6400XT, Li-Cor Inc., Lincoln, NE) equipped with a fluorescence chamber (LI-6400). The net CO_2 assimilation rate (*A*), stomatal conduntance (g_s), transpiratory rate (*E*), internal CO_2 concentration in the leaf (C_i), the ratio between internal and external CO_2 concentration (C_i/C_a) and efficiency of water use (*A/E*) were measured in the fully expanded leaf of the plants, with a saturating light of 1200 µmoL $m^{-2}s^{-1}$, 400 µmoL CO_2 moL^{-1} and 25 °C environment temperature. The readings were performed between 8 am and 10 am.

Figure 1 - Values of maximum, minimum and average temperatures, taken daily and presented by five-day intervals, during the experimental period

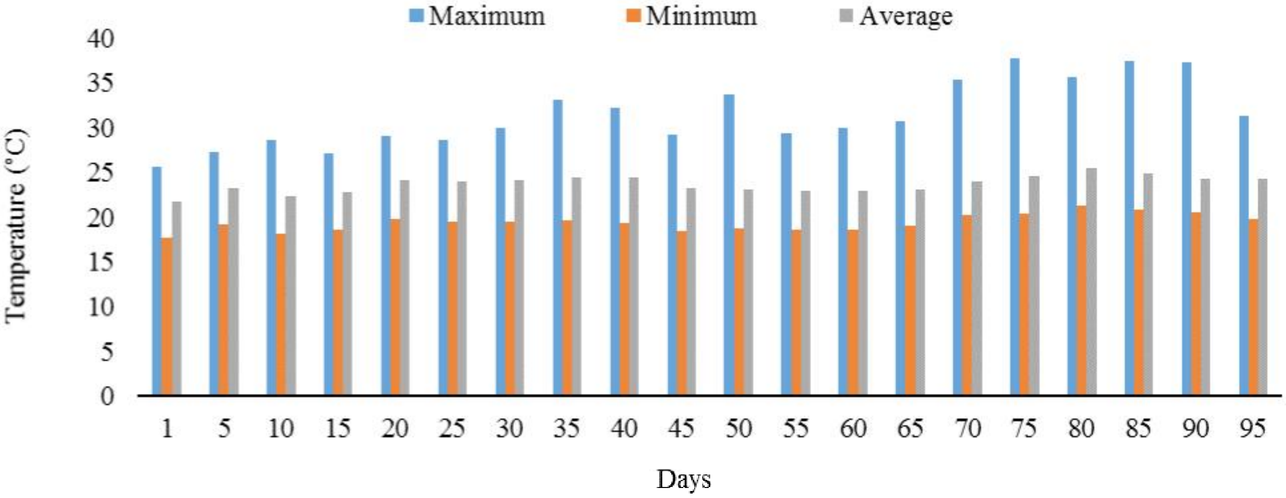
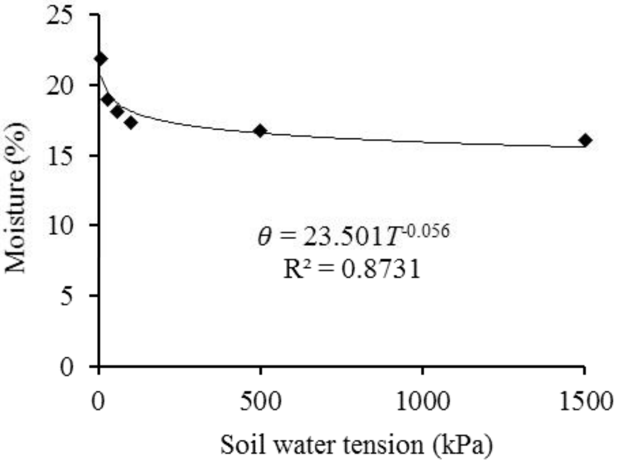


Table 1 - Physical traits of the soil used to fill in the vessels

Clay	Silt	Sand	Texture Classification	Type of soil
--------------------- % --------------------				
20	8	72	Sandy-Loam	1 - Sandy

Source: Viçosa Laboratory of Soil Analysis Ltda, Viçosa, MG

Figure 2 - Soil water retention curve, where θ represents moisture in percentage, and T represents soil water tension



The leaves, previously adapted to darkness (4 am and 5 am), were illuminated with a modulated red light of low intensity (0,03 µmoL $m^{-2}s^{-1}$) to obtain minimum fluorescence (F_0). Next, saturating pulses of white light of 8000 µmoL $m^{-2}s^{-1}$ were applied for 0,8 seconds to ensure the emission of maximum fluorescence (F_m). Having these values in hand, the potential quantum yield of photosystem II was calculated $(F_v / F_m) = (F_m - F_0 / F_m)$ (MAXWELL; JOHNSON, 2000).

Leaf Water Potential (Ψw)

Leaf water potential (Ψw) was taken on the fifteenth day after stress exposure with a Scholander portable pressure chamber. Evaluations were performed using the fifth fully expanded leaf during the night (3 am to 4 am).

Chlorophyll Content

The Chlorophyll readings were determined, indirectly, using clorofiLOG® CFL1030 ®, a portable chlorophyll meter, at the end of the stress imposition period. Three leaves of each plant were evaluated for further obtainment of average data for analysis.

Morpho-agronomic Traits

The following evaluations were carried out at the end of the crop cycle (one hundred days after planting): plant height (PH, in cm); height of the first productive branch (HFPB, in cm); stem diameter (SD, in mm); number of branches per plant (NB); total dry mass of the plant (TDM, in g); grain yield (GY, in g); and weight of 100 seeds (W 100, in g).

Statistical Procedure

The data obtained were subjected to the univariate analysis of variance to evaluate the existence of genetic variability among the genotypes, according to the following statistical model:

$$Y_{ijk} = m + b_k + g_i + \alpha_j + g\alpha_{ij} + e_{ijk} \qquad (2)$$

Where Y_{ijk} is the value observed for a given trait, related to the i-th genotype, in the j-th environment and in the k-th block; m is the general mean of the trial; b_k is the effect associated with the k-th block; g_i is the effect associated with the i-th genotype; α_j is the effect associated with the j-th environment; $g\alpha_{ij}$ is the effect associated with the genotype x environment interaction and e_{ijk} is the effect associated with the experimental error associated with the i-th genotype, j-th environment of the k-th block.

In this model, factor environment (with and without stress) was considered fixed and the effects of genotypes, blocks and experimental error were considered random. The significances of the mean squares were tested by the F test ($p < 0,05$). For the morpho-agronomic traits, the following genetic parameters were estimated: genetic variance($\hat{\sigma}_g^2$); variance of interaction genotype x environment ($\hat{\sigma}_{g\alpha}^2$); environmental variance ($\hat{\sigma}_e^2$); broad-sense heritability at plot mean level ($\hat{h}_m^2$); intraclass correlation (r); coefficient of genetic variation (CV_g); ratio b (CVg/CVe) and selective accuracy (A_c). All the analyses of the experimental data were performed on the Genes software (CRUZ, 2013).

RESULTS AND DISCUSSION

Physiological Traits

Leaf Water Potential (Ψw)

The water deficit applied in the soil induced significant reduction in the water potential of the crambe leaves, if compared to the environments (Figure 3); however, no significant variance was observed for genotypes and for interaction genotype x environment (F = 0.52239; p = 100.0^{ns}).

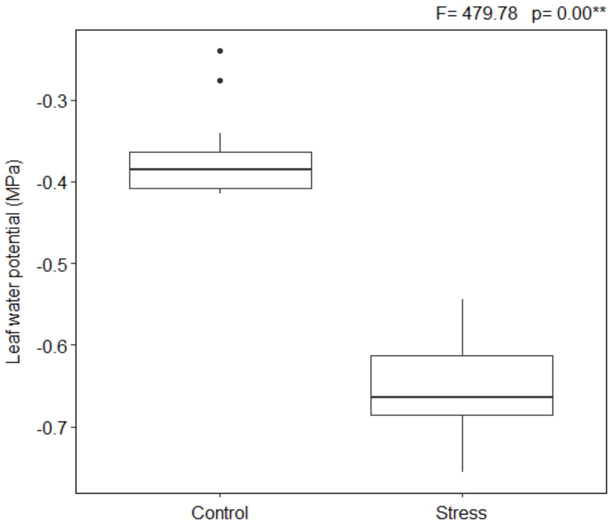
According to Choat *et al.* (2012), the occurrence of very low Ψ_{am} lead the water transport system to a stress situation, since there is a continuous tendency to increase the soil-plant-atmosphere water resistance due to problems in the xylem and in the extra-axillary tissues. If a more severe water limitation was applied, the transport of water by the plants could be completely interrupted (CHOAT *et al.*, 2012). The mean potential verified in the stressed plants (-0,63 MPa) agrees with the results of Batista *et al.* (2018), when working with controlled irrigation at the level of 50% of the water retention capacity in the soil with the crambe culture, verified mean potentials varying from -0,4 to -0,6 Mpa, which also did not cause the death of the plants.

Gas Exchanges and Chlorophyll Fluorescence

The results of the analyses of variance (Table 2) pointed out significant effects by the F test, at 5% significance, for genotypes, in variables *A*, *E*, C_i/C_a and *A/E* and, for environments, in variables F_v/F_m, C_i, C_i/C_a and *A/E*. The only trait which did not present a significant effect for any of the sources of variation being studied was g_s. The genotype x environment interaction was not significant for any variables analyzed (Table 32, highlighting independence between these two factors. Thus, it can be said that the differences among genotypes were essentially the same in the two environments studied (with and without stress).

For variable F_v/F_m, the only difference observed was between environments (Table 2), which indicates the efficiency of the stress applied, revealing the occurrence of damage in the photosynthetic apparatus of the plants (F_v/F_m < 0,8), even in the absence of significant variation among the genotypes (Table 3). F_v/F_m is considered to be a parameter which indicates stress in plants and it is understood as an estimator of the maximum efficiency of the photochemical activity of photosystem II (PSII), when all its reaction centers are open. In non-stressed plants, value F_v/F_m is about 0.8; values under that are interpreted as an indicator of plants under stress (MAXWELL; JOHNSON, 2000). In *A*, unlike F_v/F_m, a significant variation was observed only among genotypes, thus indicating that damage to the crambe photosynthetic machinery was not enough to decrease the CO_2 assimilation rate of the plants. The highest values of net photosynthesis were registered in cultivar FMS Brilhante (1) and in inbred lines FMS CR 1326 (6) and FMS CR 1106 (8) (Table 3).

Figure 3 - Boxplot with the means for leaf water potential (Ψw**)** regarding eight crambe genotypes grown under different water regimes (with and without stress)



** Significant, by the F test, at 1% level of probability

The readings performed allowed to observe a general mean concentration of 221 μmol mol^{-1} of internal CO_2 in the leaves (C_i), showing a significant variation only between environments (Table 2). The opposite was verified in variable *E*, which showed significant differences only among genotypes (Table 2); the highest values were also observed in cultivar FMS Brilhante (1) and in inbred lines FMS CR 1326 (6) and FMS CR 1106 (8) (Table 3).

Reductions in *A*, g_s and *E* are usually expected in plants sensitive to drought, which was not observed in this study, since none of these variables showed a significant variation between environments (Table 2). As there was no limitation or closing of stomata (Table 3), the CO_2 flow towards the sites of chloroplast carboxylation was not reduced (Table 3) and, therefore, did not affect the net photosynthesis rate or make transpiration difficult, since the stomata remained open, behaving as the channel where most part of the evapotranspiration of the plants occurs.

Table 2 - Summary of the analysis of variance for potential quantum yield of photosystem II (F_v/F_m), CO_2 assimilation rate (*A*, μmol m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}), internal CO_2 concentration in the leaf (C_i, μmol mol^{-1}), transpiration rate (*E*, mmol m^{-2} s^{-1}), ratio between internal and external CO_2 concentration (C_i/C_a) and efficiency of water use (*A/E*, μmolCO_2 (mmol H_2O)$^{-1}$) regarding eight crambe genotypes grown under different water regimes (with and without stress)

SV	Mean Squares							
	gl	Fv/Fm	*A*	*gs*	*Ci*	*E*	*Ci/Ca*	*A/E*
Block	3	0.0021	111.4050	0.0343	17018.1913	11.2219	0.0637	0.8260
Genotype (G)	7	0.0020	75.7990*	0.0182	3494.2827	5.0140*	0.0244*	5.6884*
Environment (E)	1	0.0372*	13.0215	0.0001	13588.8854*	0.6452	0.0506*	10.7529*
G x E	7	0.0015	21.8580	0.0088	3265.2277	1.2658	0.0076	0.5168
Error	45	0.0020	21.597	0.0097	092.1298	2.1727	0.0119	2.2962
Mean		0.81	9.01	0.12	221.87	2.66	0.62	3.50
CV (%)		5.65	51.56	80.42	25.06	55.44	17.63	43.26

* Significant, by the F test, at 5% level of probability

Table 3 - Means for potential quantum yield of photosystem II (F_v/F_m), CO_2 assimilation rate (A, µmol m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}), leaf internal CO_2 concentration (C_i, µmol mol^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}), rate between internal and external CO_2 concentration (C_i/C_a) and efficiency of water use (A/E, µmolCO_2 (mmol $H_2O)^{-1}$) regarding eight crambe genotypes grown under different water regimes (with and without stress)

Environments	Genotypes								
	1	2	3	4	5	6	7	8	Mean
					Fv/Fm				
Control	0.82	0.82	0.84	0.85	0.81	0.84	0.86	0.82	0.83 a
Stress	0.81	0.78	0.79	0.79	0.75	0.81	0.75	0.78	0.78 b
					A				
Control	11.80	4.05	4.94	7.91	8.03	11.94	9.09	16.00	9.16
Stress	10.49	11.92	3.08	5.14	7.64	12.84	8.30	14.00	9.16
					gs				
Control	0.16	0.13	0.06	0.07	0.08	0.20	0.10	0.20	0.12
Stress	0.13	0.05	0.04	0.23	0.09	0.17	0.12	0.21	0.13
					Ci				
Control	173.6	224	206	222.7	208.5	227	169	236	208.32 b
Stress	274.3	187	248	246.6	229.8	288	199	251	240.40 a
					E				
Control	3.46	2.72	1.52	1.49	2.07	3.52	2.33	3.60	2.59
Stress	3.73	1.37	1.55	3.14	2.50	4.08	2.55	3.50	2.80
					Ci/Ca				
Control	0.59	0.57	0.57	0.66	0.53	0.63	0.52	0.70	0.59 b
Stress	0.71	0.54	0.62	0.69	0.61	0.74	0.59	0.70	0.65 a
					A/E				
Control	3.40	4.38	3.30	3.73	3.92	3.32	3.47	6.30	3.97 a
Stress	2.69	3.12	1.88	2.37	3.11	2.90	3.12	5.30	3.05 b

Means followed by the same letters do not differ from each other by the F test at 5% level of probability (n = 4). Genotypes: 1 (FMS Brilhante), 2 (FMS CR 1307), 3 (FMS CR 1312), 4 (FMS CR 1203), 5 (FMS CR 1305), 6 (FMS CR 1326), 7 (FMS CR 1101) and 8 (FMS CR 1106)

Internal CO_2 concentration in the leaves (C_i) was significantly different between environments (Table 2), even if there were no significant differences in the assimilation pattern (A) and there was no occurrence of stomatal closure (Table 2). This can be justified by the fact that CO_2 assimilation does not exclusively depend on the level of stomatal opening, but also on the resistance to diffusion, on CO_2 demand in the mesophile and on the enzymatic activity (INMAN-BAMBER; SMITH, 2005). The increase of internal CO_2 concentration in the plants subjected to water deficit (Table 3) is an indicative that there was no restriction in CO_2 acquisition by the crop, but that its fixation was jeopardized when it reached the mesophile cells (INMAN-BAMBER; SMITH, 2005).

Variables C_i/C_a and A/E were the only ones that showed significance for both genotypes and environments (Table 2). With regard to the C_i/C_a ratio, inbreed line FMS CR 1203 stood out, in addition to cultivar FMS Brilhante and inbred lines FMS CR 1326 and FMS CR 1106. Through this ratio, the efficiency or inefficiency in the reactions of carbon fixation can be observed. According to Guerra, Costa and Tavares (2017), values closer to 1.0, as the ones detected in the plants subjected to water deficit in this study, indicate less efficiency of the process, and this can be an indicative that the activity of some enzymes involved in CO_2 fixation has been affected. The selection of genotypes that present a low C_i/C_a ratio constitutes a valuable strategy in plant breeding aiming at greater efficiency in water use.

Inbred line FMS CR 1106 showed itself as the most efficient in relation to water use (Table 3), presenting greater values for this ratio than those observed for commercial cultivar FMS Brilhante. The ratio between the CO_2 net assimilation rate and the water transpiration rate allows to estimate the instantaneous efficiency in water use (*A/E*) by the plants. It was observed that the plants in the environment control were more efficient in water use, as they registered the greatest mean value of *A/E (*Table 3*)*, indicating that a greater amount of CO_2 was absorbed to the detriment of less water loss.

Chlorophyll Content

The contents of chlorophyll *a* and *b* showed significant differences between genotypes and environments (Table 4). However, no difference was observed for interaction genotype x environment, for both variables. The analyses of the chlorophyll meter were carried out with considerable experimental precision, since the coefficient of variation was 5.72% for the contents of chlorophyll *a*, and 15.36% for chlorophyll *b*. A significant reduction was observed in both variables with regard to the control, when subjected to water deficit (Table 5). For content of chlorophyll *a*, a reduction of 10,13% was verified regarding the control, while a reduction of 27,65% was observed for chlorophyll *b*.

Silva *et al*. (2014) reported the association of water deficit to the decrease in the chlorophyll contents of the plants, justifying, this way, the importance of the analysis of these pigments as an evaluation tool of the integrity of the internal apparatus of the cell during the photosynthesis, constituting a good physiological indicator for the selection of tolerant genotypes. The capacity to reduce chlorophyll contents in the plant tissues, under water restriction, especially occurs from the production of reactive oxygen species (ROSs) in thylakoids (CUI *et al*., 2022), which destroy these pigments; this can justify the significant reduction observed in the contents of both chlorophylls in both treatments being studied.

From the chlorophyll content, the genotype capacity to tolerate water deficit can be inferred and those that keep greater contents of these pigments in stress conditions are expected to be more tolerant, due to the direct relationship between chlorophylls, photosynthesis and productivity (O'NEILL; SHANAHAN; SCHEPERS, 2006). Less content of chlorophyll *a* was observed in inbred line FMS CR 1307 under stress (Table 5), which is possibly related to a greater sensitivity to drought. As for the content of chlorophyll *b*, a similar behavior was observed in inbred lines FMS CR 1307, 1326, 1101 and 1106.

Table 4 - Summary of the analysis of variance for the contents of chlorophyll *a* and *b* regarding eight crambe genotypes grown under different water regimes (with and without stress)

		Mean Squares	
SV	gl	Chlorophyll *a*	Chlorophyll *b*
Block	3	13.0039	8.6313
Genotype (G)	7	9.2802*	12.2383*
Environment (E)	1	170.8744*	218.7493*
G x E	7	5.8952	3.4847
Error	45	3.3544	3.4609
Mean		32.0198	12.1131
CV (%)		5.72	15.36

* Significant, by the F test, at 5% level of probability

Table 5 - Means for contents of chlorophyll *a* and *b* regarding eight crambe genotypes grown under different water regimes (environments with and without stress)

	Genotype								
Environments	1	2	3	4	5	6	7	8	Mean
					Chlorophyll *a*				
Control	33.22	32.56	33.67	33.57	34.10	33.27	35.46	33.08	33.61 a
Stress	31.67	26.56	31.58	31.06	30.86	31.99	29.36	29.53	30.33 b
					Chlorophyll *b*				
Control	14.25	12.11	15.45	15.52	15.19	11.77	14.63	12.29	13.90 a
Stress	11.50	8.34	11.57	10.22	10.76	11.05	8.81	8.75	10.12 b

Means followed by the same letters do not differ from each other by the F test at 5% level of probability (n = 4). Genotypes: 1 (FMS Brilhante), 2 (FMS CR 1307), 3 (FMS CR 1312), 4 (FMS CR 1203), 5 (FMS CR 1305), 6 (FMS CR 1326), 7 (FMS CR 1101) and 8 (FMS CR 1106)

As a whole, the water deficit applied did not cause severe physiological limitations to the plants. Inbred lines FMS CR 1326 and FMS CR 1106 were the ones that obtained performance close to the one of cultivar FMS Brilhante, presenting the highest values of net photosynthesis and transpiration (Figure 4), indicating that, even under stress, these patterns were not affected, which suggests that they have greater potential for water deficit tolerance. Inbred line FMS CR 1106 (8) also presented the greatest efficiency in water use (Figure 4), if compared to the other genotypes, which confirms its potential tolerance. A high content of chlorophyll *a* and C_i/C_a ratio lower than 1.0 were also verified in the inbred lines and cultivar cited above, which justifies the values of net photosynthesis found (Figure 4). Inbred line FMS CR 1307 (2) was the only one to present a low content of chlorophyll *a*, also associated with a low content of chlorophyll *b*, which raises doubts about its tolerance.

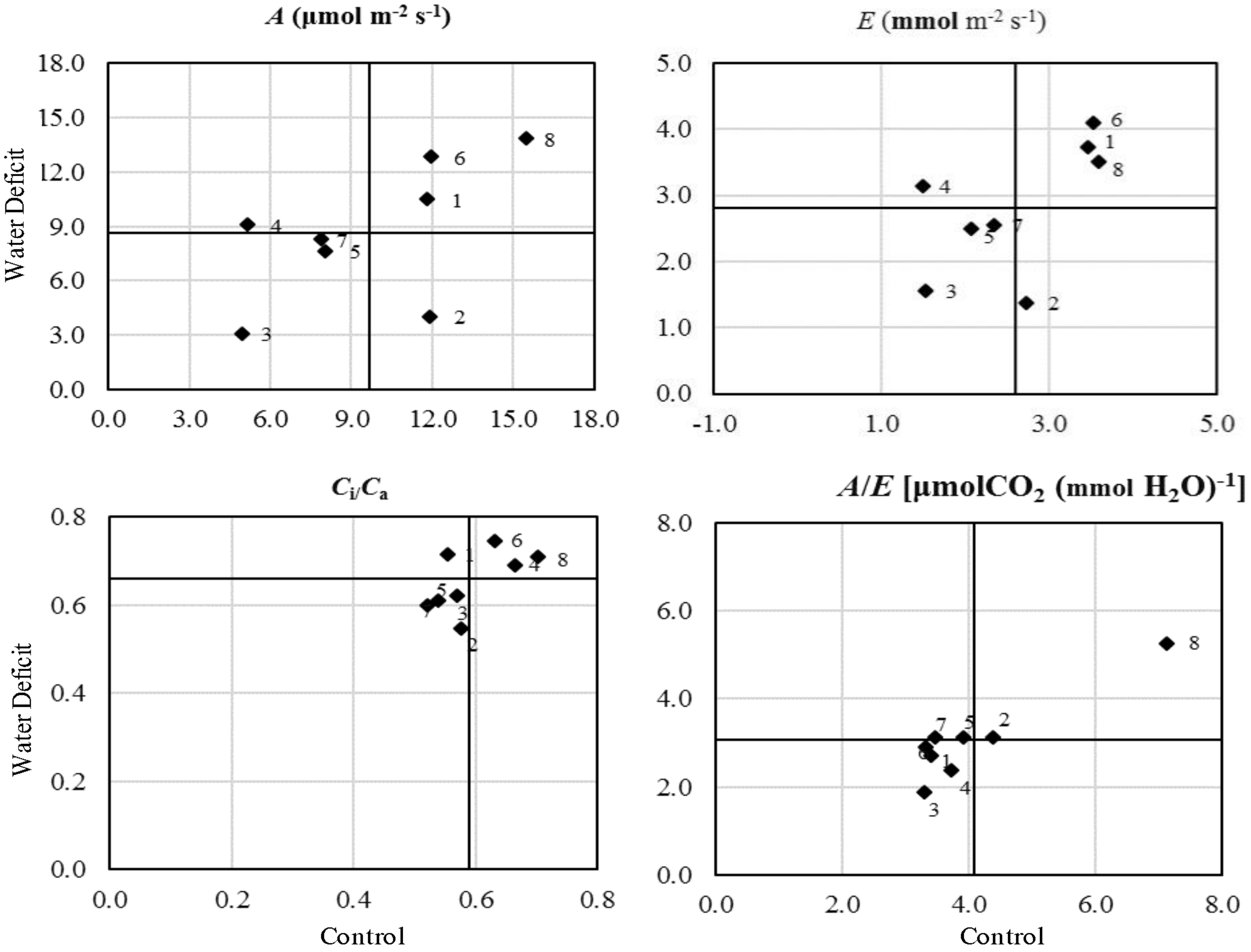
Morpho-agronomic traits

Analysis of Variance

Through the results of the analysis of variance, significant effects ($p < 0,05$) were verified among genotypes for six (PH, SD, NB, TDM, GY, W100) of the seven variables studied (Table 6), indicating the existence of genetic variability. Variable PH was the only one to present significant differences for the environments (Table 6).

Cultivar FMS Brilhante remained in the group of genotypes with superior means in almost all variables (Table 7), and the only exception was verified in variable TDM. An even better behavior was observed in inbred line FMS CR 1307 (2), which remained in the group of superior means in all variables analyzed. Inbred lines FMS CR 1326 and FMS CR 1106 presented a similar performance as the one verified in the physiological

Figure 4 - Behavior of the CO_2 assimilation rate (*A*, μmol m^{-2} s^{-1}), transpiration rate (*E*, mmol m^{-2} s^{-1}), ratio between internal and external CO_2 concentration (C_i/C_a) and efficiency of water use (*A/E*, μmolCO_2 (mmol H_2O)$^{-1}$) regarding eight crambe genotypes grown under different water regimes (environments with and without stress). Genotypes: 1 (FMS Brilhante), 2 (FMS CR 1307), 3 (FMS CR 1312), 4 (FMS CR 1203), 5 (FMS CR 1305), 6 (FMS CR 1326), 7 (FMS CR 1101) and 8 (FMS CR 1106)



variables, being close to cultivar FMS Brilhante in some traits, such as, SD, NB and GY (Table 7). As for variable PH, a significant average reduction of 6,84 cm was observed with the stress imposition (Table 7).

Table 6 - Summary of the analysis of variance for plant height (PH, cm), height of the first productive branch (HFPB, cm), stem diameter (SD, mm), number of branches (NB), total dry mass of the plant (TDM, g), grain yield (GY, g) and weight of one hundred seeds (W100, g), regarding eight crambe genotypes under different water regimes (environments with and without stress)

SV	gl	Mean Squares						
		PH	HFPB	SD	NB	TDM	GY	W100
Block	3	80.60	1.59	1.33	1.12	8.18	0.07	0.0029
Genotype (G)	7	535.60*	1.06	2.31*	17.87*	128.90*	4.45*	0.0072*
Environment (E)	1	921.90*	0.71	0.30	0.03	1.17	0.41	0.0004
G x E	7	144.90*	1.49*	2.59*	6.15	42.76*	0.63	0.0075*
Error	45	63.50	0.66	0.94	4.58	14.64	0.36	0.0017
Mean	-	75.08	5.65	8.76	16.94	13.38	2.35	0.50
CV (%)	-	10.61	14.42	11.08	12.63	28.60	25.71	8.16

* Significant, by the F test, at 5% level of probability

Table 7 - Means for plant height (PH), height of the first productive branch (HFPB), stem diameter (SD), number of branches (NB), total dry mass of the plant (TDM), grain yield (GY) and weight of one hundred seeds (W100), regarding eight crambe genotypes grown under different water regimes (environments with and without stress)

	Genotypes								
Environments	1	2	3	4	5	6	7	8	Mean
					PH (cm)				
Control	81.73	91.87	81.86	71.32	84.53	78.37	75.01	66.66	78.9 a
Stress	78.87	80.37	65.00	63.50	87.75	56.82	71.78	66.25	71.2 b
					HFPB (cm)				
Control	6.00	5.80	5.81	5.62	5.75	5.00	5.37	5.00	5.54 a
Stress	5.44	6.00	4.33	5.75	6.75	6.00	6.18	5.59	5.76 a
					SD (mm)				
Control	9.34	9.44	8.99	6.66	9.06	9.52	8.72	8.89	8.83 a
Stress	9.21	9.24	7.38	8.97	8.41	8.81	8.63	8.88	8.69 a
					NB				
Control	20.25	18.00	17.50	13.50	17.25	18.00	16.50	14.33	16.92 a
Stress	16.87	17.33	17.00	15.50	18.25	18.83	15.75	16.13	16.96 a
					TDM (g)				
Control	13.70	19.40	16.76	9.34	11.01	10.43	18.66	6.66	13.24 a
Stress	15.02	17.98	7.08	9.85	17.20	13.83	18.98	8.19	13.52 a
					GY (g)				
Control	3.09	3.19	2.22	2.19	2.06	3.17	2.49	0.95	2.42 a
Stress	2.61	3.10	1.93	2.09	2.03	2.16	3.48	0.71	2.26 a
					W100 (g)				
Control	0.51	0.54	0.50	0.51	0.49	0.42	0.54	0.47	0.50 a
Stress	0.50	0.50	0.52	0.53	0.49	0.55	0.53	0.41	0.50 a

Means followed by the same letters do not differ from each other by the F test at 5% level of probability (n = 4). Genotypes: 1 (FMS Brilhante), 2 (FMS CR 1307), 3 (FMS CR 1312), 4 (FMS CR 1203), 5 (FMS CR 1305), 6 (FMS CR 1326), 7 (FMS CR 1101) and 8 (FMS CR 1106)

In general, the means of the morpho-agronomic traits evaluated showed themselves lower to those described in the literature. This can be explained by period the trial was carried out, in which the phenological stages of great importance in plant development coincided with the exposure to high temperatures. The mean values of variables PH and HFPB, which are important traits when considering mechanical harvesting, were 75,08 and 5,65 cm, respectively. PH is in the range of growth variation (from 60 to 100 cm) of crambe plants (PITOL; BROCH; ROSCOE, 2010). As soybean machinery is used in the crambe harvest, the ideal would be PH varying from 10-12 cm, a desired height for the first pod of soybean, which minimizes losses in the harvest of the culture.

The mean observed for SD (8.76 mm) was lower than the one verified by Oliveira *et al.* (2018), when working with the same genotypes (11.05 mm). However, similar values were observed for variable NB, being 16.94 in this study, and 16.89 in the work cited above. With regard to TDM and GY, mean values of 13.38 and 2.35 g were found. Higher TDM values reveal greater efficiency in the conversion of the energy generated by the plant.

Variables PH, HFPB, SD, TDM and W100 showed significance ($p < 0,05$) for the interaction between genotypes and environments (Table 6), which means that the environment influenced the expression of genotypes differentially. For these variables, a factor was unfolded within another one (Table 8) in order to decompose its simple effects. Both water regimes, environments to which the plants were subjected, significantly influenced the behavior of inbred lines: FMS CR 1307 for variable PH; FMS CR 1312 for PH, HFPB, SD, TDM; FMS CR 1203 for SD; FMS CR 1305 for HFPB and TDM; FMS CR 1326 for PH, HFPB and W100 and FMS CR 1106 for W100. For traits NB and GY, which did not present significance for the interaction, the factors were studied in an isolated way (Table 6).

Estimates of genetic parameters

After the confirmation of the existence of genetic variability among the genotypes being studied, the genetic parameters were estimated (Table 9). The estimated $\hat{\sigma}_g^2$ reaffirmed the variability among genotypes, since for all the variables this estimate was different from zero.

Variables PH, HFPB, SD, NB and W100 presented higher estimates of $\hat{\sigma}_e^2$ than the estimates of $\hat{\sigma}_g^2$ The greatest value of $\hat{\sigma}_g^2$ was verified for variable PH (59.02), which shows that among the evaluated variables, PH has greater variability, and its highly promising for carrying out the selection.

Greater estimates for *CVg* (10.23; 28.24 and 30.45), *CVg/CVe* (0.96; 0.99 and 1.18), $\hat{h}_m^2$ (0.88; 0.89 and 0.92) and *r* (0.48; 0.49 and 0.58) were verified simultaneously in variables PH, TDM and GY, respectively. Accuracy (A_c), which demonstrates the degree of confidence of the results in the genetic evaluation of the trait, varied from 0.61 (HFPB) to 0.96 (GY).

Table 8 - Unfolding of factors genotypes and environments for plant height (PH), height of the first productive branch (HFPB), stem diameter (SD), total dry mass of the plant (TDM), and weight of one hundred seeds (W100), in eight crambe genotypes grown under different water regimes (with and without stress)

SV		Mean Squares					
		gl	PH	HFPB	SD	TDM	W100
Genotype/Environment		14	340.27*	1.27*	2.41*	85.82*	0.0074*
Genotypes	Control	7	253.79*	0.57	3.39*	86.47	0.0067*
	Stress	7	426.75*	1.96*	1.43	85.17*	0.0081*
Environment/Genotype		8	242.05*	1.39*	2.23*	37.55*	0.0067*
Environments	FMS Brilhante	1	16.34	0.63	0.33	3.48	0.0004
	FMS CR 1307	1	264.50*	0.080	0.0790	4.0054	0.0035
	FMS CR 1312	1	569.53*	4.37*	5.15*	187.73*	0.0010
	FMS CR 1203	1	112.50	0.031	10.71*	0.5200	0.0005
	FMS CR 1305	1	21.13	2.00*	0.86	76.73*	0.0000
	FMS CR 1326	1	930.96*	2.00*	1.04	23.12	0.038*
	FMS CR 1101	1	21.13	1.32	0.02	0.19	0.0008
	FMS CR 1106	1	0.35	0.71	0.00	4.66	0.0096*

*Significant by the F test, at 5% level of probability

Table 9 - Estimates of genetic parameters for plant height (PH), height of the first productive branch (HFPB), stem diameter (SD), number of branches (NB), total dry mass of the plant (TDM), grain yield (GY) and weight of one hundred seeds (W100), in eight crambe genotypes grown under different water regimes (environments with and without stress)

Estimates	PH	HFPB	SD	NB	TDM	GY	W100
$\hat{\sigma}_g^2$	59.02	0.05	0.17	1.66	14.28	0.51	0.0007
$\hat{\sigma}_{g\alpha}^2$	10.18	0.10	0.20	0.20	3.51	0.03	0.0007
$\hat{\sigma}_e^2$	63.46	0.66	0.94	4.58	14.14	0.36	0.0017
$\hat{h}_m^2$	0.88	0.37	0.59	0.74	0.89	0.92	0.77
r	0.48	0.07	0.15	0.27	0.49	0.58	0.29
CV_g	10.23	3.93	4.72	7.61	28.24	30.45	5.20
b CV_g/CV_e	0.96	0.27	0.43	0.60	0.99	1.18	0.64
A_c	0.94	0.61	0.77	0.86	0.94	0.96	0.87

$\sigma^2_{g:}$ mean genotypic variance; $\sigma^2_{gxa:}$ mean genotypic variance of genotype x environment interaction; $\sigma^2_{e:}$ mean environmental variance; $h^2_{m:}$ heritability coefficient at genotypic mean level; *r:* intraclass correlation; $CV_{g:}$ coefficient of genotypic variation; $CV_g/CV_{e:}$ ratio between the coefficient of genotypic variation and the coefficient of environmental variation; $A_{c:}$ accuracy of genotype selection

The existence of genetic variability is an indispensable factor for the obtainment of genetic gains, and, therefore, it is a fundamental requirement for the success of a breeding program. Thus, the results found suggest that the evaluated genotypes present potential for crambe breeding, since the presence of genetic variability among genotypes was verified in six out of the seven variables being studied.

According to Cruz, Regazzi and Carneiro (2012), genetic variance ($\hat{\sigma}_g^2$) and heritability ($\hat{h}_m^2$) are the most important parameter estimates to confirm variability. $\hat{\sigma}_g^2$ is related to the number of favorable alleles existing for each variable, allowing, this way, the obtainment of information about the variables being selected. $\hat{h}_m^2$, in turn, quantifies how much of the genetic variation can be transmitted to the next generation after the selection (ALLARD, 1999), expressing the ratio of the genotypic variance by the phenotypical variance. The $\hat{h}_m^2$ magnitudes in all the variables of this study are considered high ($\hat{h}_m^2$ > 0,50), according to what was proposed by Resende (2002), reflecting in less environmental influence and greater discriminatory power of the traits. Vasconcelos *et al.* (2020), when evaluating crambe progenies in different places and years, also reported high broad-sense heritability for the production of grains (82%), indicating the possibility of a direct selection of these traits.

The estimated values for ratio b (CVg/CVe) for PH, TDM and GY indicate a favorable condition for the selection, since, according to Vencovsky and Barriga (1992), values close to or greater than 1.00 obtained for maize progenies favor the selection, pointing out that genetic variation outweighs environmental variation, such a fact that was observed for TDM and GY. Such estimates corroborate the ones found by Vasconcelos *et al.* (2020), who also observed high b values for variables PH and GY. Following this tendency, variables PH, TDM and GY were also the same ones which presented higher $\hat{h}_m^2$ values (0.88; 0.89 and 0.92, respectively) and *CVg* (10.23; 28.24 and 30.45, respectively), which indicates the possibility of a direct selection on these variables. The same behavior was verified with regard to the *r* estimates (0.48; 0.49; 0.58, respectively), observed in the three variables. According to Laviola *et al.* (2014), the smaller the *r*, the greater the influence of interaction genotype x environment in the population. This way, it was observed that these variables are less influenced by the interaction. Variable GY, strongly influenced by the environment, revealed the greatest estimates of $\hat{h}_m^2$ and *CVg*, which shows that genetic variance was great for this variable and that gains can be obtained with the selection of more productive superior plants.

Accuracy (A_c) is associated with the precision of selection, reflecting the quality of the information and procedures used in the prediction of genetic values. The greater the estimate of A_c the greater the confidence in the evaluation and in the predicted genetic value. In this study, high values (A_c > 0,70) were observed for all the variables, revealing the good quality of the trial (RESENDE, 2002).

The eight genotypes studied presented potential for crambe breeding, confirmed by the existence of genetic variability among them. According to the estimate of the genetic parameters, it was found that variables PH, TDM and GY stood out because they showed the highest values of ratio b, $\hat{h}_m^2$, CV_g and *r* (Table 9). This reveals that it is possible to obtain genetic gains by selecting superior plants for each one of them, reaffirmed by the high values of selective accuracy. From these variables, differences regarding the imposed environments were verified only for PH (Table 6), indicating that, for GY and TDM, the selected genotypes tend to present an alleged tolerance to water deficit as well, because they did not differ statistically with the stress imposition.

Genotypes FMS Brilhante, FMS CR 1307, FMS CR 1326, FMS CR 1101 and FMS CR 1106 presented the greatest means of grain yield (Table 7). When selecting variable PH, it is noticed that the greatest means are associated with cultivar FMS Brilhante and inbred lines FMS CR 1307 and FMS CR 1326. However, as all genotypes conform to the height variation expected for the culture, inbred lines FMS CR 1101 and FMS CR 1106 can be included in this list, since they present values close to the ones cited above with regard to grain yield. Inbred lines FMS CR 1307 and FMS CR 1326 also stood out due to the highest means of dry mass (Table 7), which reveals good efficiency in energy conversion by the plants.

CONCLUSIONS

1. After a joint evaluation of the performance of the genotypes with regard to physiological and morpho-agronomic traits, it is concluded that cultivar FMS Brilhante and inbred lines FMS CR 1326 and FMS CR 1106 bring together most part of the traits favorable for the selection, presenting, this way, potential for water deficit tolerance;
2. In order to confirm this tolerance and evaluate the performance of the other genotypes again, the replication of the trial at other times of the year and at differentiated stress levels is suggested.

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REFERENCES

ALLARD, R. W. **Principies of plant breeding**. 2. ed. New York: John Wiley & Sons, 1999. 254 p.

BASSEGIO, D. *et al.* Oilseed crop crambe as a source of renewable energy in Brazil. **Renewable and Sustainable Energy Reviews**, v. 66, p. 311-321, 2016.

BATISTA, P. F. *et al.* Nitric oxide mitigates the effect of water deficit in *Crambe abyssinica.* **Plant Physiology and Biochemistry**, v. 129, p. 310-322, 2018.

CASSEL, D. K.; NIELSEN, D. R. Field capacity and available water capacity. *In:* KLUTE, A. (ed.). **Methods of soil analysis**. Part 1: Physical and mineralogical methods. 2. ed. Madison: American Society of Agronomy, 1986. cap. 5, p. 901- 926.

CHOAT, B. *et al.* Global convergence in the vulnerability of forests to drought. **Nature**, v. 491, p. 4-8, 2012.

CRUZ, C. D. Genes: Software para análise de dados em estatística experimental e em genética quantitativa. **Acta Scientiarum - Agronomy**, v. 35, n. 3, p. 271-276, 2013.

CRUZ, C. D.; REGAZZI, A. J.; CARNEIRO, P. C. **Métodos biométricos aplicados ao melhoramento genético**. 4. ed. Viçosa, MG: UFV, 2012. 414 p.

CUI, L. *et al.* Tomato methionine sulfoxide reductase B2 functions in drought tolerance by promoting ROS scavenging and chlorophyll accumulation through interaction with Catalase 2 and RBCS3B. **Plant Science**, v. 318, p. 111206, 2022.

FANIGLIULO, R. *et al.* Semi-refined *Crambe abyssinica* (Hochst. EX RE Fr.) oil as a biobased hydraulic fluid for agricultural applications. **Biomass Conversion and Biorefinery**, p. 1-13, 2021.

GÄLLSTEDT, M. *et al.* Film extrusion of *Crambe abyssinica*/ wheat gluten blends. **Journal of Visualized Experiments**, v. 119, p. 10-15, 2017.

GUERRA, A. M. M. N.; COSTA, A. C. M.; TAVARES, P. R. F. Atividade fotossintética e produtividade de alface cultivada sob sombreamento. **Revista Agropecuária Técnica**, v. 7467, p. 125-132, 2017.

INMAN-BAMBER, N. G.; SMITH, D. M. Water relations in sugarcane and response to water deficits. **Field Crops Research**, v. 92, p. 185-202, 2005.

LALAS, S. *et al.* Full characterisation of *Crambe abyssinica* Hochst. seed oil. **Journal of the American Oil Chemists Society**, v. 89, n. 12, p. 2253-2258, 2012.

LARA-FIOREZE, A. C. da C. *et al*. Genetic variation and gain in progenies of crambe. **Crop Breeding and Applied Biotechnology**, v. 16, n. 2, p. 132-140, 2016.

LAVIOLA, B. G. *et al.* Desempenho agronômico e ganho genético pela seleção de pinhão manso em três regiões do Brasil. **Pesquisa Agropecuária Brasileira**, v. 1, p. 356-363, 2014.

LOVATTO, N. de M. *et al.* Crambe (*Crambe abyssinica*) and sunflower (*Helianthus annuus*) protein concentrates: production methods and nutritional properties for use in fish feed. **Anais da Academia Brasileira de Ciencias**, v. 89, n. 3, p. 2495-2504, 2017.

MARTINS, R. F. A. *et al.* Physiological responses to intense water deficit in two genotypes of crambe (*Crambe abyssinica* Hochst). **Australian Journal of Crop Science**, v. 11, n. 7, p. 821-827, 2017.

MAXWELL, K.; JOHNSON, G. N. Chlorophyll fluorescence: a practical guide. **Journal of Experimental Botany**, v. 51, n. 345, p. 659-668, 2000.

MOURA, L. M. de F. *et al.* Drought tolerance in potential oilseed plants for biofuel production. **Australian Journal of Crop Science**, v. 12, n. 2, p. 289-298, 2018.

NASCIMENTO, H. R. *et al*. A new methodological approach for simulating water deficit in soybean genotypes. **Journal of Agronomy and Crop Science**, v. 207, n. 6, p. 946-955, 2021.

NAUMANN, G. *et al*. Global changes in drought conditions under different levels of warming. **Geophysical Research Letters**, v. 45, n. 7, p. 3285-3296, 2018.

OLIVEIRA, R. L. *et al*. Divergence and estimates of genetic parameters in *Crambe abyssinica*: an oilseed plant for industrial uses. **Revista Ceres**, v. 1, p. 500-506, 2018.

O'NEILL, P. M.; SHANAHAN, J. F.; SCHEPERS, J. S. Use of chlorophyll fluorescence assessments to differentiate corn hybrid response to variable water conditions. **Crop Science**, v. 46, n. 2, p. 681-687, 2006.

PITOL, C.; BROCH, D. L.; ROSCOE, E. R. **Tecnologia e produção**: Crambe. Maracaju: Fundação Mato Grosso do Sul, 2010. 60 p.

RESENDE, M. D. V. **Genética biométrica e estatística no melhoramento de plantas perenes**. Brasília, DF: Embrapa Informação Tecnológica; Colombo: Embrapa Florestas, 2002. 975 p.

SILVA, M. A. *et al*. Pigmentos fotossintéticos e índice spad como descritores de intensidade do estresse por deficiência hídrica em cana- de-açúcar. **Bioscience Journal**, v. 30, n. 1, p. 173-181, 2014.

TARDIEU, F.; SIMONNEAU, T.; MULLER, B. The physiological basis of drought tolerance in crop plants : a scenario-sependent probabilistic approach. **Annual Review of Plant Biology**, v. 69, p. 733-759, 2018.

VASCONCELOS, U. A. A. *et al.* Estimates of genetic parameters and path analysis of crambe: an important oil plant for biofuel production. **Acta Scientiarum-Agronomy**, v. 42, p. 1-7, 2020.

VENCOVSKY, R.; BARRIGA, P. **Genética biométrica no fitomelhoramento**. Ribeirão Preto: Sociedade Brasileira de Genética, 1992. 496 p.

ZANETTI, F. *et al*. Crambe abyssinica a non-food crop with potential for the Mediterranean climate: insights on productive performances and root growth. **Industrial Crops and Products**, v. 90, p. 152-160, 2016.