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Early screening for cold tolerance in sugarcane breeding

Abstract – The objective of this work was to evaluate the efficiency of early screening in the selection of sugarcane (*Saccharum* spp.) families for cold tolerance in a controlled environment. Fourteen sugarcane families were evaluated for leaf damage and chlorophyll content, in a controlled environment and in the field, after cold stress. The variance components and genotypic values of the families were estimated via REML/BLUP, as well as the genetic correlations between variables and environments. The heritabilities in the narrow sense and for family averages showed values above 0.23 and 0.76, respectively, and a genetic coefficient of variation above 13%, which confirmis the existence of variability and enables selection in both environments. The genetic correlations ranged from 0.59 to 0.88 and were significant for all combinations of variables and environments. The average coincidence index for the families between environments was 60% for the selected group and 85% for the unselected group. The early screening in the first stages of selection is efficient in breeding for cold tolerance in sugarcane families.

Index terms: *Saccharum*, abiotic stress, freezing temperature, REML/BLUP, selection strategy, speed breeding.

"Screening" precoce quanto à tolerância ao frio no melhoramento de cana-de-açúcar

Resumo – O objetivo deste trabalho foi avaliar a eficiência do *screening* precoce na seleção de famílias de cana-de-açúcar (*Saccharum* spp.) quanto à tolerância ao frio em ambiente controlado. Avaliaram-se 14 famílias de cana-de-açúcar quanto ao dano foliar e ao teor de clorofila, em ambiente controlado e em campo, após estresse por frio. Estimaram-se os componentes de variância e os valores genotípicos das famílias via REML/BLUP, bem como as correlações genéticas entre variáveis e ambientes. As herdabilidades no sentido restrito e para médias de famílias apresentaram valores superiores a 0,23 e 0,76, respectivamente, com coeficiente de variação genético superior a 13%, o que confirma existência de variabilidade e torna possível a seleção em ambos os ambientes. As correlações genéticas variaram de 0,59 a 0,88 e foram significativas para todas as combinações de variáveis e ambientes. O índice de coincidência médio para as famílias entre ambientes foi de 60% para o grupo selecionado e de 85% para o grupo não selecionado. O "screening" precoce, nas primeiras fases de seleção, é eficiente no melhoramento genético para tolerância ao frio em famílias de cana-de-açúcar.

Termos para indexação: *Saccharum*, estresse abiótico, baixas temperaturas, REML/BLUP, estratégia de seleção, melhoramento acelerado.

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Introduction

Low temperatures and the formation of frosts (°C) cause irreversible damage to plant tissues and are considered as a severe stress that limits the management practices and productivity (Edmé & Glaz, 2013). Sugarcane cultivation is exposed to cold damage in more than 25% of producing countries (Hale et al., 2016). Temperatures ranging from 0.0 to -2.2°C cause the death of terminal buds and the apical meristem, from -2.8 to -3.9°C cause damage to the lateral buds (Edmé & Glaz, 2013; Hale et al., 2016), and those from -2.5 to -5.0ºC cause the death of leaf tissues (Brinholi, 1972). Cold stress causes the decrease of chlorophyll contents and, consequently, lower photosynthetic rates, with more accentuated reductions in sensitive genotypes (Zhang et al., 2015).

The use of tolerant genotypes is the most effective measure to minimize the negative effects caused by cold stress (Edmé & Glaz, 2013; Verissimo et al., 2020). However, due to the complex genetic nature and semiperennial cycle of sugarcane, the genetic improvement of this crop requires long selection cycles and experimental tests, which is an onerous process with high demand for labor and cost (Barbosa et al., 2012; Verissimo et al., 2020). In this sense, the selection of sugarcane families under cold stress is an important tool to obtain genotypes with a greater tolerance to low temperatures (Verissimo et al., 2018, 2020; Kar et al., 2019).

Experimental conduction and selection methodologies in a controlled environment have substantially advanced in recent years, aiming to accelerate the selection phases and obtain a greater control of environmental conditions (Watson et al., 2018). For this purpose, the controlled conditions should simulate natural conditions, considering the acclimatization period, the stress intensity and extent, which are the main factors influencing the tolerance to cold (Kazemi-Shahandashti & Maali-Amiri, 2018).

Due to the seasonality of occurrence, duration, and intensity of abiotic stresses under field conditions, the simulation of this stress in a controlled environment is important for the standardization and validation of methodologies (Yu et al., 2004; Parra-Lodono et al., 2018). Some tests of cold stress simulation have been carried out for sugarcane, however, these studies are limited to a small number of genotypes (Friesen et al., 2014; Huang et al., 2015; Tang et al., 2015; Wang et al., 2016; Li et al., 2018; Kar et al., 2019). In this sense, the objective of this work was to evaluate the efficiency of early screening in the selection of sugarcane families for cold tolerance.

Materials and methods

The populations used in this work were determined based on previous results observed by Verissimo et al. (2020), and on information generated for sugarcane genetic breeding programs from the Rede Interuniverstitária para o Desenvovimento Sucroenergético (Ridesa) (Barbosa et al., 2012). Botanical seed from 14 families of sugarcane full-sib families of the RB18 series from Ridesa were used. These families were obtained from hybridizations carried out at the Serra do Ouro experimental station (in Murici, Alagoas state, Brazil) which belongs to the Universidade Federal de Alagoas (Table 1).

After processing, seed were sent to the experimental station of Embrapa Clima Temperado, in the municipality of Pelotas (31°39'39"S, 52°27'28"W, at 50 m altitude), in the state of Rio Grande do Sul, Brazil, where controlled and field experiments were performed. The regional climate, according to the Köppen-Geiger's classification, is Cfa, humid subtropical, with no defined dry seasons, and with hot summers. The soil is classified as Argissolo Vermelho-Amarelo, according to the Brazilian Soil Classification System (Santos et al., 2018), wich corresponds to Typic Rhodudult soil according to the International Soil Taxonomy (Cunha & Silveira, 1996). The seed total of each family was divided into two aliquots of 1.0 g each, thus forming the populations in the different evaluated environments.

For the experiment in field conditions, sowing was carried out in the last 10-day-period of September 2018, in polystyrene trays filled with commercial substrate (Turfa Fértil, Criciúma, SC, Brazil). Seedlings were obtained in a greenhouse subjected to controlled temperature at 28°C and automated sprinkler irrigation. At 10 days after the emergence, the seedlings were individualized into polyethylene tubes filled with substrate. After 75 days of driving in a controlled environment, the plants were subjected to acclimatization in a natural environment for 40 days, under a shaded screen (50%). After this period, leaves

were pruned and the plants were transplanted to the field, in the last 10-day-period of January 2019.

The experimental area was in fallow and it was previously prepared with chemical desiccation, plowing, and harrowing. The soil correction was performed four months before preparation, according to following chemical analysis: 5.8 for pH (water); 42.7 mg dm^{-3} P; 83 mg dm^{-3} K; 0.1 $cmol_c$ dm^{-3} Al; 3.7 $cmol_c$ dm^{-3} Ca; 2.2 $cmol_c$ dm^{-3} Mg; 3.1 $cmol_c$ dm^{-3} H+Al; 1.6% Al saturation; 9.2 $cmol_c$ dm^{-3} cation exchange capacity at pH 7.0; 67.0% base saturation; and 17.9 g dm^{-3} organic matter. The planting lines were made with a furrower at 0.3 m soil depth, and 1.4 m spacing between rows. Base fertilization in the furrow was applied with 600 kg ha^{-1} N-P_2O_5-K_2O, at 10-20-20 ratio, and covered with 0.2 m of soil.

The experiment implementation was carried out in a randomized block design with six replicates; the plots consisted of 4.5 m lines, with manual transplantation of nine plants per plot, spaced at 0.5 m apart. For the monitoring of weather conditions, measurements of minimum, average, maximum temperature (°C), and relative humidity (%) were performed using a meteorological data logger LogBox-THT_LCD (Novus, Centennial, CO, USA) installed in the center of the experiment, at the height of the plant canopy, and data were attained every 10 min. Precipitation data were collected at the Embrapa Clima Temperado automatic meteorological station, at 3 km from the experimental area.

After the occurrence of frost events, in the last ten-day-period of July 2019, the visual evaluation of leaf damage (LD) was carried out, and the proportion of the damaged leaf area by freezing was classified by a single evaluator, as follows: class 1 (highly tolerant) for plants with all-green leaves; class 2 (tolerant), plants with up to 30% dry leaves; class 3 (medium tolerant), plants with up 60% dry leaves; class 4 (sensitive), plants with up 90% dry leaves; class 5 (highly sensitive, plants with 100% dry leaves (Härter et al., 2021). The evaluation for chlorophyll contents (CHL, SPAD Index) was carried out in the first ten days of August, and the measurement was taken between 9:30 h and 11:30 h, in the median portion of the leaf +3 of the main culm of the clump, using a portable chlorophyllometer SPAD-502 Plus (Konica Minolta, Tokyo, Japan).

The experiment in controlled environment was carried out in the last ten-day-period of April 2019, in polystyrene trays filled with commercial substrate, as previously described. The development period of the seedlings, ranging from individualization to 70 days, was conducted in a greenhouse subjected to controlled temperature and automated sprinkler irrigation.

After the growth phase, the populations were placed in an uncontrolled environment for 20 days, with maximum, average, and minimum temperatures at 5.0, 12.5, and 23.0°C, respectively. These environmental conditions are important for plant acclimatization, as they induce the natural cold tolerance mechanisms, avoiding the "thermal shock" effect during the stress simulation (Zub et al., 2012). Temperature data in the greenhouse and in the uncontrolled environment were recorded every 10 min, in a meteorological data logger (Novus, LogBox-THT_LCD) installed on the canopy of the plants. The temperature inside the refrigeration chamber was monitored, using three type K thermocouple sensors made of chromium and aluminum, positioned in the plant canopy. These sensors were coupled to a CR1000 datalogger with an interface to the PC200W software (Campbell Scientific, North Logan, UT, USA), configured for data acquisition at 15-second intervals. The temperature information recorded in the different environments is presented (Figure 1).

Table 1. Parents used to obtain biparental crosses of sugarcane used to assess cold tolerance.

ID[1]	♀	♂	ID	♀	♂
01	RB996962	RB016916	08	RB965902	RB946903
02	RB016916	RB006970	09	RB946903	RB006970
03	RB975952	RB966229	10	RB966220	RB946903
04	RB867515	RB92579	11	RB006970	RB965902
05	RB996963	RB92579	12	BJ7504	RB835089
06	BJ7504	RB106802	13	RB835089	RB006655
07	SP70-1143	RB036088	14	RB006655	RB835089

[1]ID, identification of families; ♀, female parent; ♂, male parent.

After the acclimatization period, a cold stress simulation was carried out in a refrigeration chamber Reefer container 40 feet High Cube (Thermo King, MN, USA), with internal measurements of 2.29 m (width) x 2.50 m (height) x 11.55 m (length), totaling 66.1 m^3, with internal polyurethane insulation, stainless steel internal lining, and cooling system with 15 CV (380 V) three-phase motor, and an operating temperature range between +25.0°C and -25.0°C.

A subchamber was built inside the refrigeration chamber, with a wooden structure measuring 1.0 m (width) x 2.0 m (height) x 10.0 m (length), and covered with 200 µm plastic film. This structure aims to promote a gradual exchange of temperature between the refrigeration chamber and the internal environment of the subchamber.

On 08/21/2019 (day 91), the populations were transferred to the cold chamber at 0:00 h; the plants were put inside the subchamber, in a randomized block design with six replicates, with a plot consisting of nine plants. Before starting the temperature reduction, the canopy surface was humidified with a manual sprayer, to simulate the formation of dew, and the subchamber was closed. From 0:45 hours onwards, a gradual temperature reduction of the refrigeration chamber was started. The tests were conducted in the absence of light, at a cooling rate of 2.0°C per hour, which was manually controlled on the refrigeration chamber display, until reaching -2.5°C at 6:00 h. This temperature was maintained for 30 min, followed by a gradual increase of temperature on the same scale. The intensity and duration of stress was determined based on the meteorological information recorded in the field experiment. The relative humidity of the air was maintained at 85% ±5 during the stress simulation. After the chamber reached the external environment temperature, the plants were placed back to the uncontrolled environment.

Five days after the stress simulation, the populations were transferred to a greenhouse, and the level of leaf damage (LD) was evaluated and classified as follows: class 1 (highly tolerant), for seedlings with absence

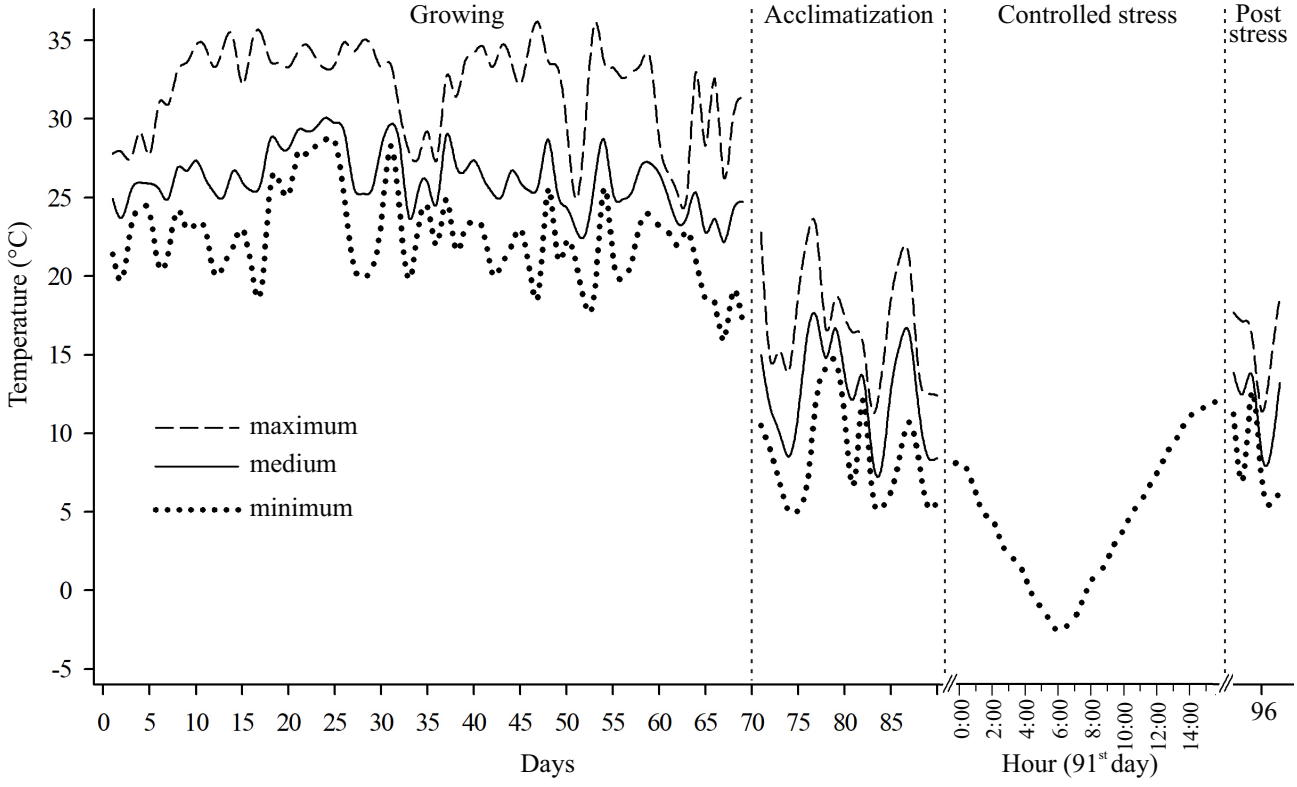

Figure 1. Minimum, medium, and maximum temperature for the periods of growth, acclimatization, stress, and post-stress in the early screening of sugarcane (*Saccharum* spp.) families for cold tolerance.

of symptoms of frost damage, with green and tender-looking leaves; class 2 (tolerant), seedlings with mild symptoms of leaf wilting; class 3 (medium tolerant), seedlings with intermediate symptoms of cell death and wilting present in all leaves, with onset of leaf discoloration, but no dry leaves; class 4 (sensitive), seedlings with intense symptoms of cell death, dry young leaves, and leaf curl; class 5 (highly sensitive), seedlings with severe symptoms of leaf freeze damage, with complete dehydration and curling of all leaves.

Chlorophyll content (CHL, SPAD Index) was evaluated from 9:30 h to 11:30 h, in the middle portion of the leaf +1 in all plants, using a portable chlorophyllometer SPAD-502 Plus (Konica Minolta, Tokyo, Japan).

Descriptive analysis graphs were prepared with the SigmaPlot software, version 11.0. Leaf damage classes were associated with values, by the order of tolerance, as class 1 (highly tolerant) for the value 1, and successively up to class 5 (highly sensitive) with a value of 5.

The variance components and genetic parameters were estimated by the restricted maximum likelihood (REML), and the genotypic values of the progenies, by the best linear unbiased predictor (BLUP). Data were analyzed via mixed models with the Selegen REML/BLUP software (Resende, 2016). The statistical model 33 of this software was used, in association with the evaluation of full-sib families obtained under an unbalanced diallel crossing, unrelated parents, in a randomized block design, as $y = Xr + Za + Wp + Td + e$, in which: y is the data vector; r is the vector of replicate effects (assumed to be fixed), added to the overall average; a is the vector of the individual additive genetic effects (assumed to be random); p is the vector of the (random) plot effects; d is the vector of genetic dominance effects, associated with full-sib families (assumed to be random); and e is the vector of errors or residuals (random) with $e \sim N(0, R=I\sigma^2)$. Capital letters represent incidence matrices for each class of effects. Genetic parameters were estimated as described by Rodrigues et al. (2017).

To assess the association between characters within and between environments, the Spearman's correlation coefficients were estimated, using the predicted genotypic values of the families. The efficiency of the early screening was validated from the results of the field experiment, simulating four selection rates (15, 30, 50, and 70%). After defining the set of selected (selection) or discarded (no selection) families, at each selection rate, the coincidence index (%) between the groups "selection" and "no selection" was estimated, combining the intersection of the variables between the environments.

Results and Discussion

There were 13 days with negative temperatures, during the experimental assay in the field, with -2.9°C absolute minimum in July (Table 2). From May to August, 22 days were recorded with a minimum temperature between 0 and 5°C, which allowed of the occurrence of cold damage in sugarcane (Edmé & Glaz,

Table 2. Meteorological conditions of minimum, average, and maximum temperature, and rainfall, for the period from May to August 2019.

Month	Ten-day period	Temperature (°C)			Rain (mm)	Days	
		Minimum	Medium	Maximum		0 a 5°C	< 0°C
May	2	10.1	18.7	31.0	1.6	0	0
	3	3.0	15.2	24.7	40.0	1	0
June	1	2.2	13.4	29.5	1.2	2	0
	2	5.3	18.5	30.1	6.4	0	0
	3	-0.7	16.3	31.4	27.8	0	2
July	1	-2.9	7.5	23.7	0.8	3	7
	2	-0.6	12.3	28.8	6.8	3	1
	3	3.3	14.8	29.2	171.6	1	0
August	1	-1.0	12.5	29.7	89.4	3	2
	2	0.4	11.3	29.8	8.6	6	0
	3	-1.0	10.8	20.1	0.6	3	1

2013). Such environmental conditions were favorable for the assessment of cold tolerance in sugarcane families in the field, as well as the association of these results with the information obtained in the controlled environment.

A high variability between and within families was observed for leaf damage and chlorophyll content, in both evaluation environments (Figure 2). In the controlled environment, the families RB016916 x RB006970 and RB966962 x RB016916 showed 70% of the population with a low level of leaf damage (LD 1 and 2). In the field, the families RB835089 x RB006655, RB016916 x RB006970 and RB996963 x RB92579 showed a higher level of tolerance, and more than 65% individuals were classified as tolerant. Similar results for cold tolerance in sugarcane families were

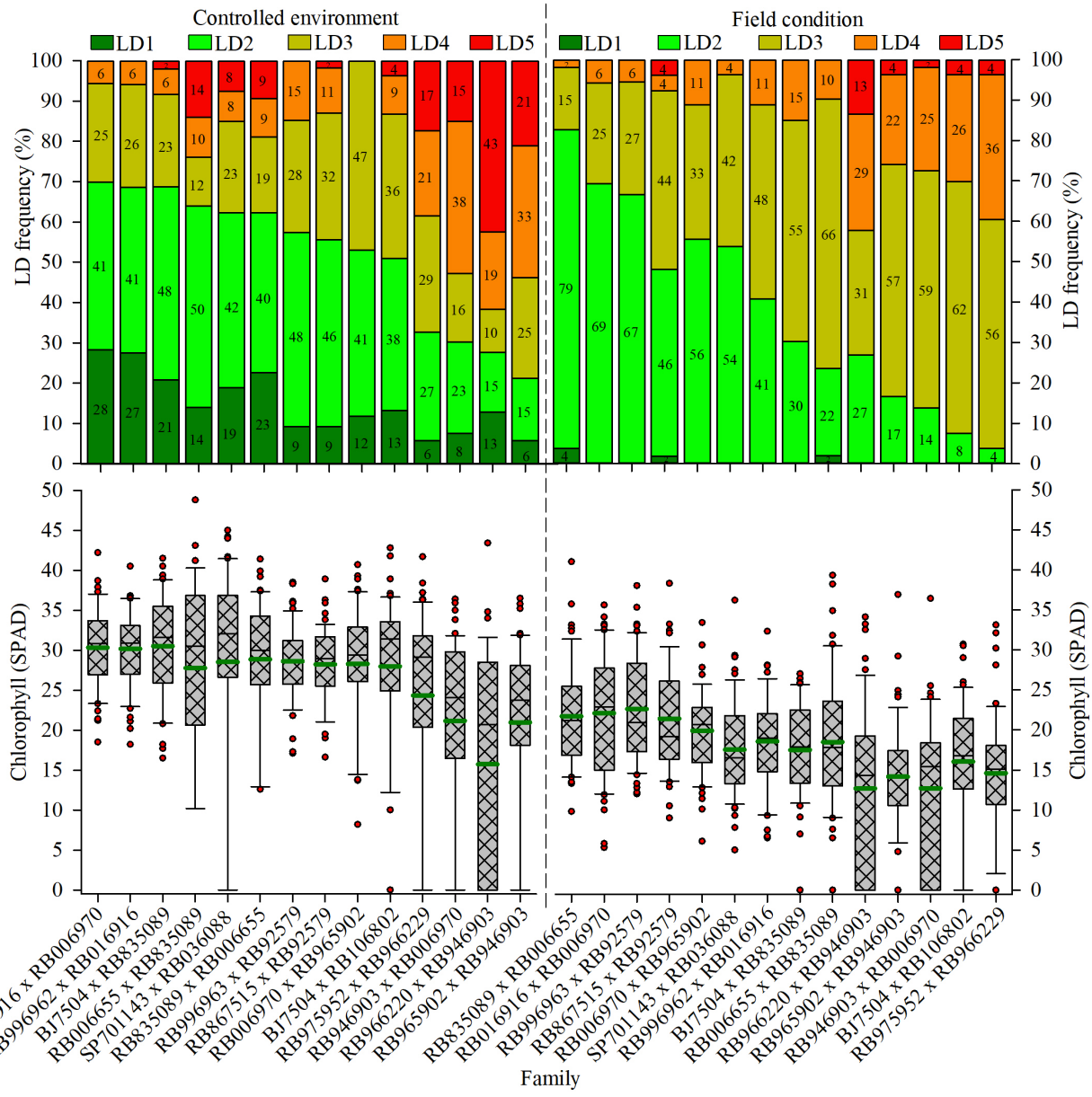

Figure 2. Frequency of the cold tolerance by leaf damage class (LD - %) and distribution of chlorophyll contents (CHL – SPAD), in 14 sugarcane (*Saccharum* spp.) families evaluated under simulated (controlled environment) and natural (field) conditions of cold stress.

observed in crosses involving the parent RB996963, with a frequency of cold-tolerant clones greater than 50% (Verissimo et al., 2020).

As to chlorophyll content, a median value above 28.0 SPAD in the controlled environment, and 15 SPAD in the field occurred for most families, except for the families RB966220 x RB946903, RB946903 x RB006970 and RB965902 x RB946903 in both environments, as well as RB975952 x RB966229 in the field, which showed a lower level for cold tolerance. The assessment of chlorophyll content has been used in studies on cold stress in sugarcane and represents an important variable for the characterization of different levels of tolerance (Tang et al., 2015; Zhang et al., 2015; Li et al., 2018; Kar et al., 2019).

Estimates of heritability in the narrow sense (h^2_a) were greater than 0.23 for all variables (Table 3). According to Resende's classification (Resende, 2002), estimates of h^2_a above 0.15 are considered of moderate magnitude, which allows of the application of selection strategies using individual information associated with that of families. The estimates of the genetic coefficient of variation were superior to 13.0%, which is therefore higher than the 10% threshold – an indicator of genetic variability between progenies – and confirms the existence of variability in the population for all variables (Resende, 2002). When simulating cold stress in a controlled environment, Kaiser & Sacks (2015) observed a high variability within and between progenies of *Miscanthus* spp. in the seedling stage, which indicates the applicability of this type of methodology for early screening.

The heritability estimates for family averages (h^2_{mf}) were of a high magnitude that are superior to the other estimates of this parameter, and they ranged from 0.762 (LD_{CE}) to 0.891 (DF_{FC}). Similar results were observed for cold tolerance in sugarcane families (Verissimo et al., 2018) and in eucalyptus (Moraes et al., 2016). In

Table 3. Estimates of the components of variance, through the restricted maximum likelihood (REML), for 14 sugarcane (*Saccharum* spp.) families evaluated under simulated (controlled environment) and natural (field) conditions of cold stress, for leaf damage (LD) and chlorophyll content (CHL, SPAD).

Parameter[(1)]	Controlled environment (CE)		Field condition (FC)	
	LD_{CE}	CHL_{CE}	LD_{FC}	CHL_{FC}
σ^2_a	0.375	25.745	0.163	15.417
σ^2_{parc}	0.254	14.043	0.063	8.229
σ^2_d	0.002	0.139	0.062	0.385
σ^2_e	0.722	60.979	0.296	40.710
σ^2_y	1.352	100.906	0.584	64.740
h^2_a	0.277±0.078	0.255±0.075	0.278±0.078	0.238±0.072
h^2_g	0.283	0.261	0.704	0.262
h^2_{mf}	0.762	0.778	0.891	0.781
σ^2_p	0.189	13.011	0.143	8.093
PEV	0.045	2.886	0.016	1.772
SEP	0.212	1.699	0.125	1.331
CV_f (%)	43.834	37.685	27.179	45.305
CV_g (%)	16.399	13.532	13.467	16.018
CV_e (%)	22.458	17.696	11.536	20.771
CV_r	0.730	0.765	1.167	0.771
AC_{fam}	0.873	0.882	0.944	0.884
Overall mean	2.653	26.655	2.812	17.760

Parameters: σ^2_a, additive genetic variance; σ^2_{parc}, variance between plots; σ^2_d, genetic variance of dominance among full-sib progenies; σ^2_e, residual variance between plots; σ^2_y, individual phenotypic variance; h^2_a, individual heritability in the strict sense – additive effects; h^2_g, individual heritability in the broad sense – total genotypic effects; h^2_{mf}, heritability between progeny means; σ^2_p, genotypic variance between families; PEV, prediction error variance of genotypic values; SEP, standard deviation of predicted genotypic value; CV_f, phenotypic coefficient of variation; CV_g, genetic coefficient of variation; CV_e, environmental coefficient of variation; CV_r, relative coefficient of variation; and AC_{fam}, selective accuracy among families.

this scenario, the selection of families is indicated to the detriment of individual selection (Resende, 2002; Oliveira et al., 2013). However, as the values of h^2_a were of moderate magnitude, it is also possible to adopt selection strategies combining individual and family information (Resende, 2002).

Considering the precision in the inference of the estimates of genetic parameters, it is highlighted that the selective accuracy values (AC_{fam}) were higher than 0.87, a value above 0.50 that is considered as an important indicator for the efficiency of the selection process (Resende & Duarte, 2007). Comparing the environments, the residual variance estimates (σ^2_e) in the field were lower for both variables; however, the genotypic variance between families (σ^2_p) was proportionally higher in a controlled environment, reflecting on similar estimates to h^2_a and h^2_{mf}, a result that is considered satisfactory, as it is possible to practice the selection and obtain gains in both environments. It is important to note that tests in a controlled environment standardize the environmental conditions, avoiding problems observed in field trials, such as spatial variability and seasonality of stress events (Parra-Londono et al., 2018).

Genetic correlations were significant among all variables, and the values ranged from -0.596 (LD_{CE} x CHL_{CC}) to -0.881 (LD_{CE} x CHL_{CC}) (Table 4). Such results refer to the high correlation of genotypic values between the evaluation environments, as well as the efficiency of the leaf damage scales used in both environments to characterize different levels of cold tolerance. In a field study, Verissimo et al. (2018) suggested the use of damage scales to select sugarcane families under cold stress conditions. Leaf damage visual scales have also been used to characterize the cold tolerance in *Miscanthus* spp., with applicability for tests in controlled environment and field (Zub et al., 2012; Kaiser & Sacks, 2015), which can be used as a practice tool for mass selection.

The highest correlation coefficient between environments was observed for the variable CHL (0.705**), indicating the potential use of this character for screening cold tolerance at an early stage. Chlorophyll content is correlated with other physiological variables, such as photosynthetic rate, stomatal conductance, and electron transport efficiency, and it is considered an important trait for the characterization of cold tolerance in sugarcane (Tang et al., 2015).

In a controlled environment, 10 families showed prominence for genotypic values, with 8.5% and 7.0% gains, for LD_{CE} and CHL_{CE}, respectively (Table 5). In the field evaluation, eight families with DF_{FC} below 2.53, and 7 families with CHL_{FC} content above 18.96 SPAD showed gains of 9.6 and 12.1%, in that order. Considering the ranking of families, RB016916 x RB006970 showed an overall superior position to the other families, remaining between the first and second position for all variables. Families with genotypic values higher than the general average of LD_{CE}, and lower for CHL_{CE}, showed similar behavior in the field. This data confirms the lower cold tolerance level of RB975952 x RB966229, RB965902 x RB946903, RB946903 x RB006970, and RB966220 x RB946903, which showed genotypic values 16.4% below the average performance of families in the field.

The coincidence indices show the high association of results between the assessment environments (Table 6). There was a high efficiency of selection in a controlled environment for CHL_{FC}, with 100% coincidence considering $LD_{CE} \cap CHL_{FC}$ and $CHL_{CE} \cap CHL_{FC}$, in the 70% selection rate (TS). For the intersection of a field variable with both evaluated fields ($LD_{CE} \cap LD_{FC} \cap CHL_{FC}$ and $CHL_{CE} \cap LD_{FC} \cap CHL_{FC}$) in SR 70%, a high coincidence rate was also observed, with 90% and 75% for selection and disposal of families, in that order. The average coincidence of placement of families between the environments was 60%, for the selected group, and 80%, for the unselected group. According to Apiolaza (2009), screening techniques can be favorable for

Table 4. Genetic correlations for 14 sugarcane (*Saccharum* spp.) families evaluated under simulated (controlled environment) and natural (field) conditions of cold stress for leaf damage (LD) and chlorophyll content (CHL, SPAD).

		Controlled		Field	
		LD_{CE}[(1)]	CHL_{CE}	LD_{FC}	CHL_{FC}
Controlled	LD_{CE}	-			
	CHL_{CE}	-0.881**	-		
Field	LD_{FC}	0.626*	-0.640*	-	
	CHL_{FC}	-0.596*	0.705**	-0.864**	-

[(1)]CE, controlled environment; FC, field conditions. *, **Significant by the t-test, at 5% and 1% probabilities, respectively (N=14).

early selection aiming to eliminate populations with low genetic value, which is corroborated by our results, whose highest coincidence indices refer to the identification of families with low genotypic value.

In general, the set of families showing greater sensitivity to cold in a controlled environment had also a lower level of field tolerance, indicating the high efficiency of this methodology for early screening. A high association of cold tolerance in sorghum

Table 5. Genotypic value (Vg) and position (rk, ranking) of 14 sugarcane (*Saccharum* spp.) families evaluated under simulated (controlled environment - CE) and natural cold stress conditions (field conditions - FC), for leaf damage (LD) and chlorophyll contents (CHL, SPAD).

Family	Controlled				Field			
	LD_{CE}	rk	CHL_{CE}	rk	LD_{FC}	rk	CHL_{FC}	rk
RB996963 x RB92579	2.51	8[(1)]	28.27	6	2.43	3	21.59	1
RB016916 x RB006970	2.18	1	29.62	2	2.42	2	20.16	2
RB835089 x RB006655	2.51	6	28.31	4	2.25	1	20.00	3
RB867515 x RB92579	2.52	10	28.02	8	2.62	6	19.88	4
RB006655 x RB835089	2.51	7	28.28	5	2.79	8	19.56	5
RB006970 x RB965902	2.50	5	27.75	10	2.59	5	19.23	6
RB996962 x RB016916	2.19	2	29.74	1	2.70	7	18.96	7
SP701143 x RB036088	2.48	4	28.08	7	2.53	4	17.60	8
BJ7504 x RB835089	2.36	3	29.10	3	2.85	9	17.59	9
BJ7504 x RB106802	2.52	9	27.90	9	3.22	12	16.28	10
RB975952 x RB966229	3.05	11	24.87	11	3.34	14	15.49	11
RB965902 x RB946903	3.31	13	21.54	13	3.11	11	14.68	12
RB946903 x RB006970	3.16	12	21.96	12	3.10	10	14.34	13
RB966220 x RB946903	3.50	14	17.95	14	3.26	13	13.01	14
Média	2.65		26.66		2.81		17.76	

[(1)]Underlined numbers represent families with genotypic values below the overall mean, for the variable LD, and above the overall mean, for CHL.

Table 6. Coincidence index (%) for the intersection of leaf damage (LD) and chlorophyll contents (CHL, SPAD) between simulated (controlled environment - CE) and natural cold stress conditions (field conditions - FC), for different selection rates (SR, %) of 14 sugarcane (*Saccharum* spp.) families evaluated under cold stress.

Combination	Selection[(1)]	Coincidence index (%)							
		SR[(2)] (15%)		SR (30%)		SR (50%)		SR (70%)	
		S	NS	S	NS	S	NS	S	NS
$LD_{CE} \cap LD_{FC}$	S	50.0	50.0	50.0	50.0	71.4	28.6	90.0	10.0
	NS	8.3	91.7	20.0	80.0	28.6	71.4	25.0	75.0
$LD_{CE} \cap CHL_{FC}$	S	50.0	50.0	25.0	75.0	71.4	28.6	100.0	0.0
	NS	8.3	91.7	30.0	70.0	28.6	71.4	0.0	100.0
$CHL_{CE} \cap LD_{FC}$	S	50.0	50.0	50.0	50.0	71.4	28.6	90.0	10.0
	NS	8.3	91.7	20.0	80.0	28.6	71.4	25.0	75.0
$CHL_{CE} \cap CHL_{FC}$	S	50.0	50.0	50.0	50.0	71.4	28.6	100.0	0.0
	NS	8.3	91.7	20.0	80.0	28.6	71.4	0.0	100.0
$LD_{CE} \cap LD_{FC} \cap CHL_{FC}$	S	50.0	50.0	25.0	75.0	42.9	57.1	90.0	10.0
	NS	8.3	91.7	40.0	60.0	28.6	71.4	25.0	75.0
$CHL_{CE} \cap LD_{FC} \cap CHL_{FC}$	S	50.0	50.0	25.0	75.0	57.1	42.9	90.0	10.0
	NS	8.3	91.7	30.0	70.0	28.6	71.4	25.0	75.0

[(1)]S, selection; NS, no selection. [(2)]The selection rates at 15, 30, 50, and 70% represent the selection of 2, 4, 7, and 10 families, respectively, which showed the best rank positions for genotypic values of each variable.

(*Sorghum bicolor*) between growth chamber and field indicates that tests in a controlled environment can be used as an alternative or pre-selection method (Yu et al., 2004). In this sense, a methodology that enables the identification and exclusion of 30% of populations with the lowest tolerance level would represent a substantial gain in time and cost reduction in field evaluations, ensuring a greater agility to the breeding program.

The validation of early tests in a controlled environment with field results is important, since such methodologies can be adapted to automated phenotyping platforms, using some techniques, such as image analysis and sensors capable of predicting physiological characteristics of interest for selection (Humplík et al., 2015).

Conclusions

1. There is variability for cold tolerance between and within sugarcane (*Saccharum* spp.) families evaluated in a controlled environment and in the field.

2. There is a high correlation for cold tolerance between controlled environment and the field.

3. The methodology used is efficient in the early screening of sugarcane families for cold tolerance.

4. The coincidence between the tested environments shows a greater efficiency for the disposal of families with a low level of tolerance to cold.

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