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CROP PROTECTION

Selection of sugarcane families and clones under cold stress

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ABSTRACT: *The purpose of this study was to select cold-tolerant sugarcane families and clones. Evaluations were carried out during three selection phases in the municipality of Pelotas, state of Rio Grande do Sul, Brazil. The experiments were arranged in an incomplete block design, with initially 4,452 seedlings of 53 full-sib families. Aside from the traits soluble solids content (BRX), tons of stalks per hectare (TSH) and tons of brixper hectare (TBH), the survival of the apical bud (ABS) was evaluated in the first selection stage (T1) of the breeding program. At the end of three selection phases, 15 clones of 14 of the 53 families evaluated in the first phase (T1) were selected for the experimental phase. Of these, the clones RS/PR126066, RS/PR126044, RS/PR126052, RS/PR126007 and RS/PR126033, had a good performance for apical bud survival in the first selection phase.*
Key words: *Saccharum spp., abiotic stress, plant breeding, frost.*

Seleção de famílias e clones de cana-de-açúcar sob estresse pelo frio

RESUMO: *O objetivo desse trabalho foi selecionar famílias e clones de cana-de-açúcar tolerantes ao estresse por frio. As avaliações ocorreram durante três fases de seleção no município de Pelotas, no estado do Rio Grande do Sul, Brasil. Os experimentos foram conduzidos no delineamento de blocos incompletos, sendo inicialmente composto por 4.452 seedlings oriundos de 53 famílias de irmãos completos. Além dos caracteres teor de sólidos solúveis (BRIX), tonelada de colmos por hectare (TCH) e tonelada de brix por hectare (TBH), foi avaliada a sobrevivência do meristema apical (GEM) na primeira fase de seleção (T1) do programa de melhoramento genético. Ao final de três fases de seleção, 15 clones pertencentes a 14 das 53 famílias avaliadas na primeira fase (T1) foram selecionados para a fase de experimentação, destes, destacam-se: RS/PR126066, RS/PR126044, RS/PR126052, RS/PR126007 e RS/PR126033, pois além de apresentar desempenho satisfatório para os caracteres TCH e TBH, foram avaliados com notas máximas (N4 e N5) para sobrevivência de gema apical no primeiro ciclo de seleção.*
Palavras-chave: *Saccharum spp., estresse abiótico, melhoramento de plantas.*

INTRODUCTION

Although, sugarcane is mostly cultivated in regions with a tropical climate, it is estimated that in 25% of the producing countries it is planted in areas where low temperatures can damage the crop and, consequently, cause economic losses (HALE et al., 2016). In Brazil, this scenario may affect 1.2 million hectares, with highest risks in the states of Rio Grande do Sul, Santa Catarina, Paraná, Mato Grosso do Sul, São Paulo and Minas Gerais, in this order (CONAB, 2016).

As pointed out by Verissimo et al. (2012), one strategy for cold tolerance in sugarcane is the use of chemical ripeners to anticipate harvesting to avoid winter frosts. A second alternative would be cold-tolerant varieties, which are not widely used in Brazil due to the low availability. This deficiency is a consequence of the lack of evaluations of the cold response of the currently available varieties for planting. For many cultivated species, the demand for studies involving varietal tolerance to address abiotic stresses has increased in recent years. Cold or frost tolerance tests of promising sugarcane varieties and clones are commonly performed in Australia, Canada, the United States of America and South Africa (FRIESEN et al., 2014; HALE et al., 2016; HEERDEN et al., 2010; WEAICH et al., 1993).

The development of sugarcane varieties involves several stages and the time from the cross to the release of a variety can last 15 years. The main stages of a breeding program can be summarized

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as follows: selection of parents; hybridizations to establish families; distribution of the caryopses to the test stations for the first assessment phase (T1), where highest variability is observed in the field evaluations of thousands of seedlings; second test phase (T2) with evaluations and selection in small plots; third test phase (T3) at few cultivation locations, with at least two replications; and finally, the experimental phase (EPh), at multiple locations and in different years (CASTRO et al., 2016).

Family selection has been used in different sugarcane breeding programs in several countries. These studies showed that selection based on the mean family performance was more effective for traits with strong environmental influence, i.e., with low heritability at the plant level, such as cane yield per hectare (BRASILEIRO et al., 2016; KIMBENG& COX, 2003; OLIVEIRA et al., 2016; RESENDE & BARBOSA, 2006). The knowledge generated in these family studies contributed with data to sugarcane breeding programs, to plan future crosses with a clearer identification of the best combinations and the best parents. Therefore, the objective of this study was to select cold-tolerant sugarcane families and clones, with a view to contributing to cane breeding programs.

MATERIALS AND METHODS

The sugarcane caryopses used for planting originated from 53 biparental crosses, performed at the breeding center of Serra do Ouro of the Federal University of Alagoas, located in Murici, Alagoas, Brazil.

Sowing was carried out on July 31, 2012, at the experimental station of Paranavaí, of the Federal University of Paraná, in Paranavaí, Paraná. Three months after sowing, 4.452 seedlings from 53 families were sent to an experimental station of Embrapa Temperate Climate (Brazilian Agricultural Research Company), in Pelotas, Rio Grande do Sul (latitude 31º41 'S, longitude 52º25' W; 50 m asl), and planted in the field at the beginning of December 2012. The experiment in the T1 phase were arranged in an incomplete block design. The soil of the experimental station was classified as ArgissoloVermelho-Amarelo and the regional climate, according to Köppen's classification, is Cfa, humid subtropical, with no defined dry season and hot summers (KUINCHTNER&BURIOL, 2001; SANTOS et al., 2018). The mean annual temperature is less than 16 °C, winter frosts are possible, and maximum temperatures exceed 30 °C in the warmest month.

The second test phase (T2) consisted of 529 clones of 44 families in the T1 phase. Selection in phase T1 and planting of T2 occurred in August 2013. The experiment was arranged in an incomplete block design, where each clone was represented by one plot and each block consisted of 40 plots. Each experimental plot consisted of one 2 mrow (groove), at a row spacing of 1.40 m. For the planting, 18 buds per meter were used.

In August 2014, at the end of the plant cane of phase T2, the population was assessed, resulting in the selection of 72 clones. These, together with the early maturing RB966928 and the medium-late maturing RB867515, both commercial clones, constituted the third (T3) test phase. The multiplication of the clones for phase T3 was carried out via pre-sprouted seedlings (PSS) at the Experimental Station of Embrapa Temperate Climate, using individual buds of the middle third of stalks for planting in dibble tubes containing commercial substrate.

Thereafter, the seedlings were placed in a greenhouse for sprouting and acclimatization for transplanting. Approximately 60 days after planting, the seedlings were taken to the field in November 2014 and planted in phase T1, at a spacing of 0.50 m between seedlings and 1.4 m between rows. The experiment was arranged in an incomplete block design with two replications per clone and different numbers of representatives (replications) per family, ranging from one to 10 clones. Each experimental plot consisted of two 10-m rows.

The effect of low temperatures on the crop was monitored in all test phases. Temperature data were collected in situ, at mid-canopy height, with a RHT10 USB Data logger and measured at the Meteorological Station of Embrapa Temperate Climate, from April to August (period of possible occurrence of cold and frost) in 2013, 2014 and 2015.

Mainly in the first test phase (T1) from 2012 and 2013, severe frosts were recorded, killing seedlings in the experiment. The seedling response to cold tolerance was evaluated on a scale of apical bud (meristalk) survival (ABS) as proposed by Verissimo et al. (2018). Thirty days after the first frosts, the scores were applied according to the survival rate and stalk lesion (measured with a graduated ruler, in mm), being: Score1 (Sc1) - dead apical bud and necrotic lesion (length>5.0 cm) below the apical bud caused by cold, and dead/absent lateral shoots; Score 2 (Sc2) - dead apical bud, but with lesion immediately below the bud (2.0 - 5.0 cm), visible lateral shoots; Score 3 (Sc3) - dead apical bud, with lesion below the bud (0.5 - 2.0 cm), abundant lateral shoots; Score 4 (Sc4) - apical bud

with small lesion (<0.5 cm) and presence of live tissue with growth return in the central axis of the stalk and without visible lateral shoots; Score 5 (Sc5) - live bud, with no visible lesion and no lateral shoots.

In all test phases in August, the total soluble solids content in the juice (BRX) was evaluated. All plants of the plots were assessed during T1, sampling one stalk per plant, and three shoots per plot in phases T2 and T3. To sample the juice, the mid third of the stalk was perforated with a probe and readings performed with a portable digital refractometer (Atago® -PAL 1), with automatic temperature compensation.

The experiments of the three test phases (T1, T2 and T3) were harvested in August 2013, 2014 and 2015, respectively. The harvest of T1, occurred 9 months after planting and of the following phases after 12 months. After weighing a sample of 10 stalks per plot (W10S) and counting the number of stalks per plot (NS), the trait tons of stalks per hectare (TSH) was calculatedas: $TSH = \frac{NSx\left(\frac{W10S}{10}\right)x\,10}{EA}$, where: EA is the evaluated area per plot in m^2; and 10 is the constant used to convert the plot weight into tons per hectare;

Based onTSH and BRX, the trait tons of brix per hectare (TBH) was calculated by: $TBH = \frac{TCHxBRIX}{100}$.

The data of the first test phase were analyzed by restricted maximum likelihood and best linear unbiased prediction (REML/ BLUP), according to the following statistical model:$y=Xr+Za+Wp+Sf+Tb+e$,where y = data vector; r = vector of the replication effects, assumed as fixed and added to the overall mean; a = vector of the individual additive genetic effects; assumed as random; p = vector of plot effects, assumed as random; f = vector of genetic effects of dominance associated with full-sib families; assumed as random; b = effects of incomplete blocks, assumed as random; e = vector of errors or residues; and X, Z, W, S and T represent the incidence matrices for the above effects.

For the data analysis of the traits evaluated in T2, the statistical model $y=Xr+Zf+Wb+Sc+ewas$ used, where: y = data vector; r = vector of the replication effects, assumed as fixed and added to the overall mean; f = vector of family effects of full sibs, assumed as random; b = vector of the environmental effects of incomplete blocks, assumed as random; c = vector of clone effects within a full-sib family, assumed as random; e = error or residue vector, and X, Z, W, and S are the incidence matrices for these effects. All analyses were performed using SELEGEN-REML/ BLUP software (RESENDE, 2016).

RESULTS AND DISCUSSION

The evaluations in the first selection phase (T1) were preceded by extreme weather events in the winter of 2013. The parameters estimated for the traits evaluated in this phase (Table 1) indicated the possibility of genetic gains for the traits evaluated with selection among and within families, particularly with regard to apical bud survival (ABS), for which high heritability with an accuracy>0.80 in the family selection was observed (Table 1). This information indicated the relevance of the proposal of this study, since based on the ABS scores, the families can be discriminated in classes of cold response, and more tolerant clones can be selectedearly.

For total soluble solids (BRX), the heritability and accuracy were 0.64 and 0.80, respectively (Table 1). For the yield traits TSH and

Table 1 - Genetic parameters estimated in sugarcane clones evaluated during the first test phase (T1) in 47 sugarcane full-sib families evaluated in the under cold stress.

Parameters*	Traits			
	ABS	BRX	TSH	TBH
h^2_{fam}	0.68	0.64	0.64	0.63
Selectionaccuracy	0.83	0.80	0.80	0.80
Overall mean	3.46	19.07	58.17	11.27

*Broad-sense heritability at the family averages level (h^2_{fam}); apical bud survival (ABS); total soluble solids content (BRX); tons of stalks per hectare (TSH) and tons of brix per hectare (TBH)

TBH, the heritability estimates were high (0.64 and 0.63, respectively) and accuracy was 0.80, indicating the possibility of gain in the family selection process (Table 1). These results corroborated those of SILVEIRA et al. (2015), who reported values of heritability (0.59 to 0.85) and accuracy (0.76 to 0.92) for the traits TSH and TBH, respectively, indicating a high correlation between the predicted genotypic means and the true values an allowing an efficient selection of the best families. In this sense, the genetic variability of the segregating population grown at low temperatures in southern Brazil was sufficient and heritability at the family level was high (Table 1).

At harvest, in August/September, plants with apical buds ranging from live (Sc5) to necrotic (Sc1) were observed, with lesions below the bud covering a large part of the stalk (Figure 1A). In this sense, seedlings with the highest ABS score were observe data a frequency of 11% in the population, i.e., classified in the category of highest cold tolerance (Sc5) (Figure 1B). In general, results showed that 19% of the evaluated plants scored Sc4 and Sc5 for ABS.

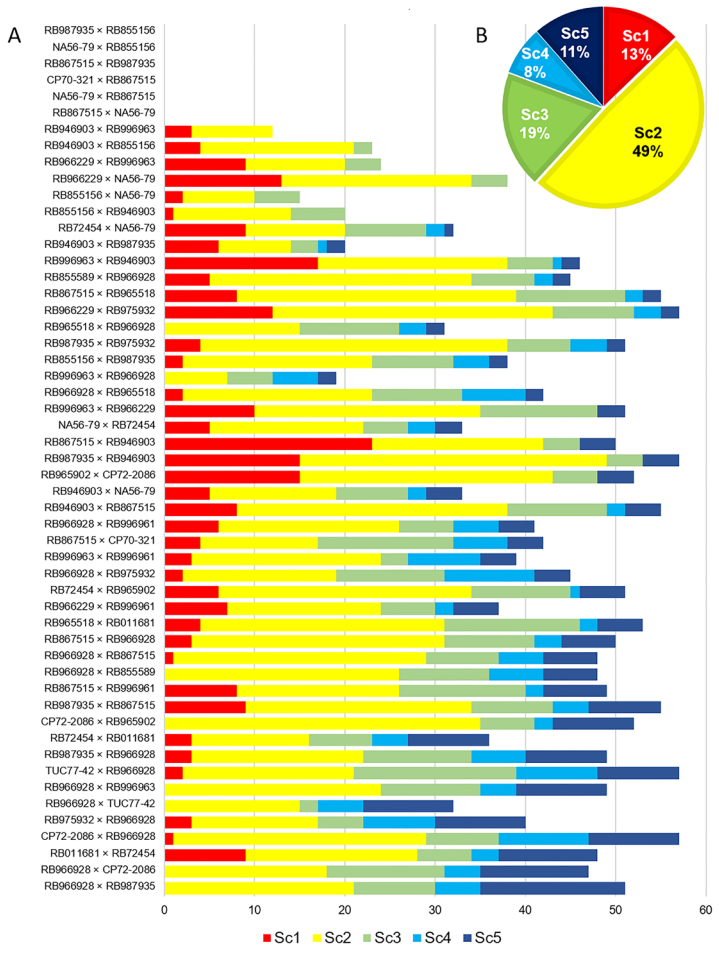

Figure 1 - Number of clones in different score classes of apical bud (ABS) in 53 sugarcane families evaluated in the first test phase (T1) (A) and overall percentage of the scores in the population of the first selection phase (B). Apical bud scores Sc1 (dead apical bud and necrotic lesion), Sc2 (dead apical bud, but with lesion immediately below the bud, visible lateral shoots), Sc3 (dead apical bud, with lesion below the bud, abundant lateral shoots), Sc4 (apical bud with small lesion and presence of live tissue with growth return in the central axis of the stalk and without visible lateral shoots) and Sc5 (live bud, with no visible lesion and no lateral shoots).

Therefore, these seedlings with higher scores are cold tolerant, in contrast to the other seedlings graded with scores Sc1, Sc2 and Sc3, which accounted for 81% of the evaluated plants.

Of the 53 families in the first test phase (T1), six (RB987935 × RB855156; NA56-79 × RB855156; RB867515 × RB987975; CP70-321 × RB867515; NA56-79 × RB867515 and RB867515 × NA56-79) were not graded with ABS scores, since there were no surviving seedlings at the time of evaluation (Figure 1A). The high mortality was probably related to the low cold tolerance of the progenies of these crosses. In one of the few studies on this subject in Brazil, BASTOS et al. (1983) reported that after frost (-1 ºC) occurrence, plants were not damaged and the hypothesis formulated was that, when close to maturation, the protection factor of the varieties would change, assuming that the higher the density of liquid contained in the plant cells, the greater the plant resistance to frost effects. Based on the data collected at the study location, a minimum temperature of -2.2 °C was observed on July 24, 2013. The climatic conditions recorded during the experimental period were favorable for the selection of families and plants with cold tolerance, especially with regard to apical bud survival.

Based on the performance of the families with regard to the studied traits, 529 seedlings were selected in 44 of the 53 evaluated families (Table 1). Most plants were selected from families with above-average performance for yield traits and from highly cold-tolerant families, as indicated by ABS.

The reduction in the number of families in T3 was a consequence of the high frequency of clones with symptoms of the main sugarcane diseases such as leaf scald (*Xanthomonasa lbilineans*), smut (*Ustilago scitaminea*), brown rust (*Puccinia melanocephala*) and orange rust (*Puccinia kuehnii*). In this phase, susceptibility to these diseases resulted in the complete elimination of some families during the advancement of the generations. In T2, the coefficients of variation (CV%) were low to moderate (RESENDE, 2002), indicating good experimental precision, especially for BRX (CV%≤7.08), and highest for TSH and TBH (CV%≥31.38) (Table 2).

The individual genotypic variances among clones were significant for all studied traits by deviance analysis at 5% probability, indicating the presence of genetic variability in the populations even during the progression of the generations. In an evaluation of the yield performance of sugarcane families in different selection stages, Oliveira et al. (2016) reported similar results. Conversely, most of the individual phenotypic variance affecting the traits was due to environmental causes (Table 2).

The individual broad-sense heritability in phase T2 were low for BRX, TSH and TBH (h^2_{clon}≤0.30); as a consequence, the accuracy values for clonal selection were less than 70%. This condition is unfavorable for selection, due to the low correlation between predicted and true genetic values (Table 2) (RESENDE, 2002). Results for heritability at the family level in T2 phase were lower than in T1, as expected, since the representativeness of most families tends to decrease from one generations to the next (Table 1 and Table 2). The genetic parameters could not be estimated in T3 in the same way adopted in T2 due to the reduced number of families evaluated in this phase. Consequently, the random model cannot be applied (Table 2).

Contrary to what might be expected with the progress of the selection phases, the overall means observed for TBH and TSH were lower in the T3 phase compared to the T2 phase (Table 2). The visual selection process, which does not take into account the genotypic means of clones and families, and the discarding of clones due to incidence of diseases, can be pointed as factors for the decrease in the overall mean.

The number of clones per family indicated by the expression n_k corresponds to the number of individuals selected in the first test phase (T1) and which composed the second (T2) (Table 3). Of the 12 families that contributed most to the number of selected clones (n_k≥17), five were classified (Clas) among the 10 highest genotypic means (Vg) for the traits TSH and TBH (RB867515 × RB946903, RB965518 × RB011681, 7RB867515 × RB966928, RB966928 × CP72-2086 and CP72-2086 × RB966928), demonstrating a higher probability of containing clones with a superior genetic potential for simultaneous gains in TSH and TBH (Table 3).

Of the 72 clones derived from 30 families evaluated in the third test phases (T3), 15 clones of 14 families were selected for the experimentation phase (Table 4). Of these 14 families, only four (CP72-2086 × RB965902, RB946903 × NA56-79, RB946903 × RB867515 and RB946903 × RB987935) were not classified (Clas) among the 15 with highest genotypic means for TBH (Table 4). The ranking based on the genotypic values of the families during T2 and T3 for TBH included CP72-2086 × RB966928, CP72-2086 × RB965902, RB011681 × RB72454, RB867515 × RB966928 and RB867515 × RB946903 among the 10best families in both phases (Tables 3 and 4).

Table 2 - Genetic and environmental parameters estimated in sugarcane clones evaluated during the second and third test phases (T2 and T3).

Phase	Parameters*	BRX	TSH	TBH
T2	V_{fam}	0.58	174.93	0.87
	V_{clon}	0.83	390.69	8.87
	V_e	1.63	674.49	22.23
	V_f	2.79	999.87	33.43
	h^2_{fam}	0.21	0.02	0.03
	h^2_{clon}	0.30	0.23	0.27
	Ac	0.55	0.48	0.52
	CV%	7.08	31.64	31.38
	Overall mean	18.04	82.08	15.03
T3	CV%	5.36	22.45	22.44
	Overall mean	18.26	58.06	10.53

*V_{fam}: genotypic variance between families; V_{clon}: individual genotypic variance; V_e: residual variance; V_f: individual phenotypic variance; h^2_{fam}: broad-sense heritability of families; h^2_{clon}: individual broad-sense heritability; Ac: selection accuracy; CV%: experimental coefficient of variation; BRIX: total soluble solids in juice; TSH: tons of cane per hectare and TBH: tons of brix per hectare.

Oliveira et al. (2016) and Brasileiro et al. (2016) reported similar results, as they also observed a high correlation between the number of clones selected per family and their genotype means in the earlybreeding stages. This indicated that selection among and within the best families in the first test phases may contribute to the development of promising clones that will perform better in the final stages of the breeding program.

The figure 2 is formed by clones selected for the experimental phase (EPh) and the number of clones that were not advanced in the families during the first, second and third selection phases, NA(T1), NA(T2) and NA(T3); respectively, and the sums of the overlapping bars, allow us to obtain the number of clones present in the each phase can be computed: T1 (T1 = NA(T1) + NA(T2) + NA(T3) + EPh), T2 (T2 = NA(T2) + NA(T3) + EPh) and T3(T3 =NA(T3) + EPh).

In seven families, although, a high number of progenies were evaluated (T1≥50), no clones were selected for the experimental phase (EPh) (Figure 2). However, of the 14 families that originated clones selected for the experimental phase, 13 had 40 or more clones evaluated in the first selection phase, demonstrating that for families that had a low rate of surviving progenies in the first selection phase (T1≤40), the probability of having clones selected in EPh was low (Figure 2).

Although, only 19% of the clones were evaluated with favorable ABS scores (Sc4 and Sc5) in the first selection phase, the percentage of clones with favorable scores in T1, selected for EPh, was as high as 34% (Figure 3). The percentage of clones with the favorable scores 4 and 5 in the last selection phase was probably not higher because the temperatures in the other selection phases were not as low as in the first phase.

Most (60%) of the 15 clones selected in T3 belonged to the 14 families classified as best in relation to TBH in T1 (Table 5). According to SIMMONDS (1996), 10% of the best families concentrate approximately 60% of the best genotypes and little is gained by selecting more than 20% of the families. Therefore, due to the high number of families evaluated annually in many sugarcane breeding programs, it is preferable to select a smaller number of families and perform a comprehensive sampling within families i.e., the largest possible number of clones in the best families should be selected, to ensure that they are well represented in the following stages. In this way, the most promising families and plants can be evaluated again in the following phases (T2 and T3), by clonal selection with greater accuracy (BRASILEIRO et al., 2016).

Several authors have recommended selection by the individual simulated BLUP method (BLUPIS), a method that indicated the number of individuals to be selected within the best families, since the means of the selected individuals are

Table 3 - Clones selected per family in the first test phase T1 (n_k) and evaluated in the second test phase (T2), in 44 full-sib families.

Families	----------BRX*----------			----------TSH----------			----------TBH----------			n_k
	Vg	Clas	Ac	Vg	Clas	Ac	Vg	Clas	Ac	
RB867515 × RB946903	17.43	34	0.95	84.83	9	0.68	15.43	9	0.75	33
RB987935 × RB946903	17.87	27	0.93	80.69	34	0.58	14.62	35	0.65	26
RB946903 × RB867515	17.38	36	0.94	80.71	33	0.61	14.48	42	0.68	22
RB965518 × RB011681	18.29	18	0.93	85.15	6	0.58	15.86	6	0.66	21
RB987935 × RB966928	19.09	2	0.94	79.08	43	0.6	14.73	28	0.68	21
RB966928 × RB987935	18.75	8	0.94	79.69	41	0.6	14.7	30	0.67	20
RB867515 × RB966928	18.7	11	0.94	84.61	10	0.63	15.91	5	0.7	19
RB966928 × CP72-2086	19.66	1	0.93	86.02	2	0.6	16.7	1	0.67	18
RB987935 × RB975932	17.58	33	0.93	81.06	29	0.59	14.63	33	0.66	18
CP72-2086 × RB966928	18.87	6	0.94	85.22	5	0.61	16.07	2	0.69	17
RB965902 × CP72-2086	17.29	38	0.94	81.24	27	0.6	14.59	37	0.67	17
RB987935 × RB867515	18.01	22	0.93	80.37	35	0.57	14.58	38	0.64	17
RB867515 × CP70-321	18.38	16	0.91	82.12	19	0.51	15.12	15	0.59	16
RB966229 × RB975932	17.6	32	0.92	82.3	18	0.54	14.94	23	0.62	16
RB855156 × RB987935	18.91	5	0.93	79.83	40	0.6	14.95	22	0.67	15
TUC77-42 × RB966928	18.75	7	0.93	81.33	25	0.57	14.99	20	0.64	14
RB867515 × RB996961	17.91	25	0.94	85.75	4	0.6	15.83	7	0.68	13
CP72-2086 × RB965902	17.92	24	0.9	86.37	1	0.5	16.02	3	0.58	12
RB966229 × RB996961	17.41	35	0.9	82.99	15	0.5	15.04	19	0.58	12
RB996963 × RB966229	18.13	20	0.9	80.97	31	0.51	14.85	26	0.58	12
RB011681 × RB72454	17.87	26	0.9	85.86	3	0.5	15.92	4	0.58	11
RB72454 × RB965902	17.14	42	0.91	85.1	7	0.52	15.34	12	0.6	11
RB867515 × RB965518	17.71	29	0.91	83.06	14	0.51	15.11	16	0.58	11
RB966928 × RB867515	17.28	39	0.92	81.34	24	0.53	14.62	34	0.6	11
RB966928 × RB975932	18.46	15	0.89	83.17	13	0.48	15.39	10	0.56	10
RB996963 × RB946903	17.87	28	0.9	80.32	36	0.49	14.66	32	0.56	10
RB946903 × RB855156	17.63	31	0.85	84.91	8	0.39	15.61	8	0.46	9
RB966928 × RB996963	18.7	10	0.88	81.32	26	0.44	14.92	25	0.51	9
RB966928 × RB855589	18.52	14	0.89	82.89	16	0.47	15.36	11	0.55	8
RB966928 × RB965518	18.68	12	0.9	80.94	32	0.48	14.83	27	0.55	8
RB966928 × RB996961	18.99	4	0.9	79.91	39	0.49	14.72	29	0.56	8
RB966928 × TUC77-42	18.13	21	0.9	80.19	37	0.5	14.57	40	0.57	8
RB975932 × RB966928	18.34	17	0.88	76.01	44	0.45	13.61	44	0.52	8
RB855589 × RB966928	18.61	13	0.89	80.02	38	0.48	14.61	36	0.55	7
NA56-79 × RB72454	17.98	23	0.88	82.77	17	0.44	15.21	14	0.51	6
RB855156 × RB946903	16.85	43	0.87	81.58	21	0.42	14.57	39	0.49	6
RB946903 × RB987935	17.69	30	0.89	79.48	42	0.47	14.37	43	0.55	6
RB72454 × NA56-79	17.34	37	0.83	83.66	11	0.37	15.23	13	0.43	4
RB946903 × NA56-79	18.73	9	0.88	81.05	30	0.45	14.97	21	0.52	4
RB996963 × RB996961	16.24	44	0.82	81.42	23	0.34	14.53	41	0.4	4
RB72454 × RB011681	17.23	40	0.80	83.19	12	0.33	15.1	17	0.39	3
RB965518 × RB966928	19.06	3	0.83	81.9	20	0.36	15.09	18	0.43	3
RB966229 × RB996963	17.16	41	0.75	81.14	28	0.28	14.69	31	0.33	3
RB855156 × NA56-79	18.17	19	0.76	81.51	22	0.29	14.93	24	0.35	2

*BRX: soluble solids content; TSH: tons of stalks per hectare; TBH: tons of brix per hectare; Genotypic means (Vg); classification (Clas) and accuracy (Ac).

Table 4 - Families evaluated in the third test phase (T3) and advanced to the experimental phase (EPh), in 30 full-sib families.

Families	T3										EPh
	BRX*			TSH			TBH			n_k	n_k
	Vg	Clas	Ac	Vg	Clas	Ac	Vg	Clas	Ac		
CP72-2086 × RB965902	18.0	18	0.86	57.59	18	0.63	10.45	18	0.55	9	2
CP72-2086 × RB966928	17.9	23	0.78	61.51	3	0.52	10.95	1	0.44	3	1
NA56-79 × RB72454	17.4	29	0.78	62.04	1	0.52	10.88	3	0.44	5	1
RB011681 × RB72454	17.7	26	0.80	61.69	2	0.52	10.84	4	0.44	3	1
RB855156 × RB987935	17.9	21	0.75	60.07	5	0.46	10.73	7	0.38	2	1
RB855589 × RB966928	19.5	1	0.70	58.39	12	0.41	10.73	8	0.34	1	1
RB867515 × CP70-321	17.6	28	0.80	60.37	4	0.52	10.71	9	0.44	4	1
RB867515 × RB946903	18.5	9	0.76	58.79	9	0.47	10.69	10	0.39	2	1
RB867515 × RB965518	18.9	5	0.77	57.98	15	0.48	10.61	11	0.40	3	1
RB867515 × RB966928	18.2	14	0.69	58.29	13	0.40	10.53	14	0.33	2	1
RB867515 × RB996961	18.9	4	0.74	57.15	21	0.46	10.50	15	0.38	2	1
RB946903 × NA56-79	17.9	22	0.69	57.50	19	0.40	10.39	21	0.33	2	1
RB946903 × RB867515	17.6	27	0.74	57.60	17	0.46	10.38	22	0.38	3	1
RB946903 × RB987935	17.3	30	0.74	56.92	23	0.46	10.24	24	0.38	3	1
RB965518 × RB011681	19.0	3	0.86	59.60	7	0.61	10.89	2	0.52	3	0
RB965902 × CP72-2086	18.4	11	0.80	59.58	8	0.52	10.75	5	0.44	2	0
RB966229 × RB975932	18.3	13	0.71	59.80	6	0.41	10.74	6	0.34	1	0
RB966229 × RB996961	17.8	24	0.58	58.77	10	0.30	10.55	12	0.24	1	0
RB966928 × CP72-2086	18.1	16	0.69	58.42	11	0.40	10.55	13	0.33	2	0
RB966928 × RB867515	18.5	8	0.57	57.64	16	0.30	10.49	16	0.24	1	0
RB966928 × RB965518	18.0	19	0.74	58.13	14	0.46	10.48	17	0.38	2	0
RB966928 × RB975932	18.3	12	0.58	56.99	22	0.30	10.42	19	0.24	1	0
RB966928 × RB987935	18.7	6	0.58	56.66	24	0.30	10.40	20	0.24	1	0
RB966928 × TUC77-42	17.8	25	0.82	57.35	20	0.56	10.37	23	0.47	4	0
RB987935 × RB867515	18.5	7	0.79	55.43	25	0.50	10.21	25	0.42	2	0
RB987935 × RB946903	18.4	10	0.76	55.27	26	0.47	10.20	26	0.39	1	0
RB987935 × RB966928	18.1	15	0.69	54.46	29	0.40	10.12	27	0.33	1	0
RB987935 × RB975932	18.0	17	0.58	54.60	28	0.30	10.10	28	0.24	1	0
RB996963 × RB996961	17.9	20	0.75	54.85	27	0.47	10.08	29	0.39	2	0
TUC77-42 × RB966928	19.3	2	0.77	53.70	30	0.48	10.06	30	0.40	3	0

*BRX: soluble solids content; TSH: tons of stalks per hectare; TBH: tons of brix per hectare; genotypic means (Vg), classification for the genetic value of the trait (Clas), accuracy (Ac) and number of clones (n_k).

strongly correlated with the true values estimated by individual BLUP (BLUPI), and can be used when the plants are cultivated for clonal tests by vegetative propagation in situations where individual evaluations in the first selection phase are unviable (BRASILEIRO et al., 2016; OLIVEIRA et al., 2011; SILVA et al., 2015).

It is suggested to improve the accuracy observed in T2 for the traits TSH and TBH by using BLUPIS-based selection. By this strategy, approximately 50 plants from the best families are selected. However, the breeder defines the percentage of families to be selected. As in phases T2 and T3 all plants of all plots can be weighed, BLUPI can be applied to estimate the genotype means. Thus, if the best plants of the best families are selected in T1, as proposed by BLUPIS, the probability of obtaining promising clones in the following phases increases. By selecting 50 genotypes of the 10 best families evaluated in this study, it would be possible to advance 500 clones derived from the best families to T2. With this number of replications per family, the accuracy for traits such as TSH and TBH would be high (OLIVEIRA et al., 2013; RESENDE& BARBOSA, 2006).

With the advancing selection phases, the 15 clones with the best yield performance were selected

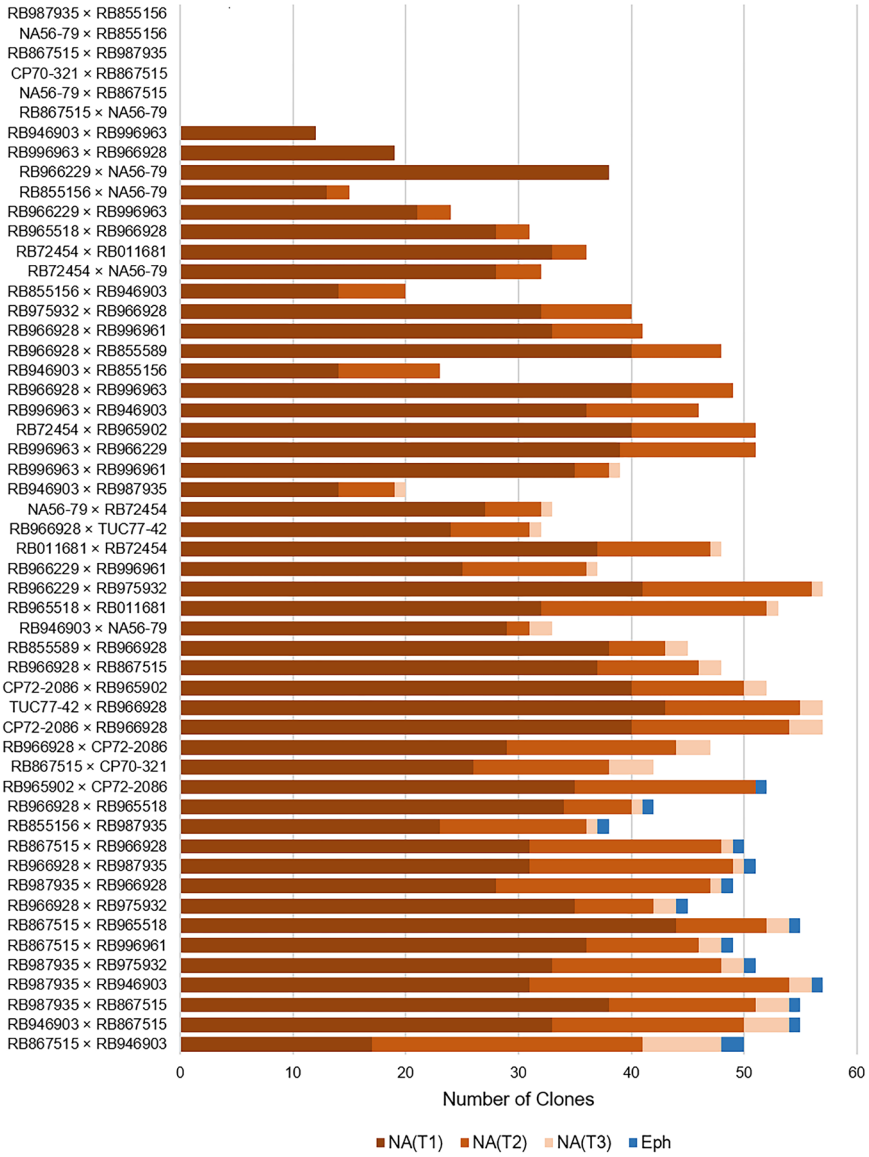

Figure 2 - Clones selected for the experimental phase (EPh) in each family and clones that were not advanced from the first (NA (T1)) to the second (NA (T2)) and third (NA (T3)) selection phases.

and will be evaluated in the experimentation phase (EPh). These clones have the potential to be exploited commercially and/or used in future crosses to increase cold tolerance. It is important to highlight that, despite the good agronomic characteristics of these clones, they must also have high agro industrial quality, tolerance to the main pests and diseases, maintenance of juice quality after freezing temperatures, and

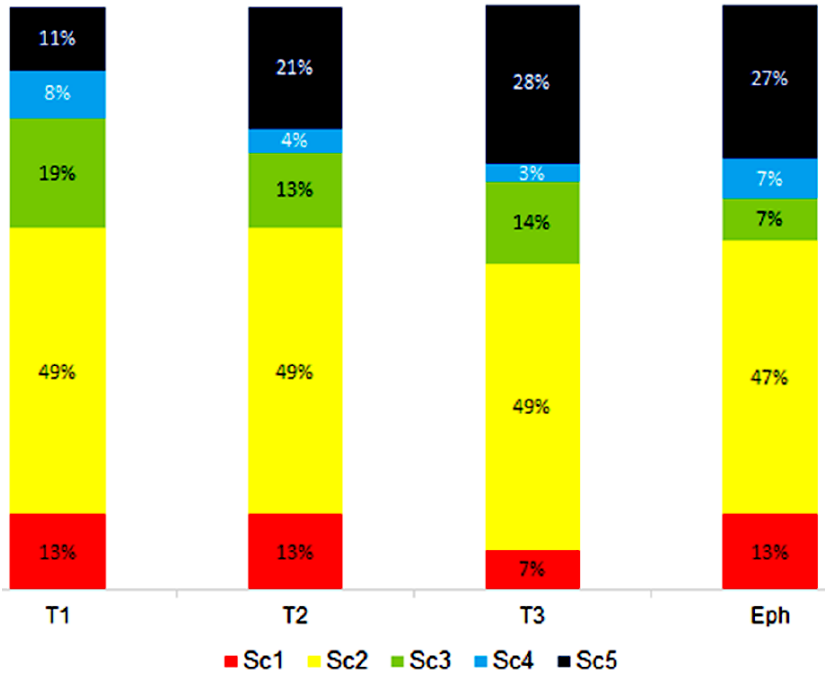

Figure 3 - Clones evaluated for the first (T1), second (T2), third (T3) and experimental phase (EPh). Percentage of apical bud scores Sc1 (dead apical bud and necrotic lesion), Sc2 (dead apical bud, but with lesion immediately below the bud, visible lateral shoots), Sc3 (dead apical bud, with lesion below the bud, abundant lateral shoots), Sc4 (apical bud with small lesion and presence of live tissue with growth return in the central axis of the stalk and without visible lateral shoots) and Sc5 (live bud, with no visible lesion and no lateral shoots).

longevity of ratoon cane. Therefore, these traits must be evaluated in the last selection phase (EPh).

Five clones with ABS with scores of 4 and 5 (RS/PR126066, RS/PR126044, RS/PR126052, RS/PR126007 and RS/PR126033) maintained a good level cold tolerance at low temperatures (Table 5). Considering the averages obtained for tons of brix per hectare (TBH) in the third selection phase (T3), two (RS / PR126052 and RS / PR126033) of the five clones of the highest scores for ABS in T1 presented a performance close to that obtained in commercial cultivars RB867515 and RB966928. However, in the third test phase low temperatures did not occur, being recommended that the five clones (and the other clones selected for the experimental phase) be evaluated during several harvests in places where the incidence of low temperatures is frequent before recommendation of a new commercial clone (Table 5).

CONSLUSION

At the end of three selection phases, 15 clones belonging to 14 of the 53 families evaluated in the first phase (T1) were selected for the experimental phase. Of these, the following stand out: RS/PR126066, RS/PR126044, RS/PR126052, RS/PR126007 and RS/PR126033, were evaluated with maximum scores (Sc4 and Sc5) for apical bud survival in the first selection phase.

Table 5 - Families evaluated in the first test phase (T1) that had clones selected for the experimentation phase and mean in the third test phase (T3).

Families*	Clone	----------T1----------			----------T3----------	
		ABS*	TSH	TBH	TSH	TBH
		Score	Clas(Vg)	Clas(Vg)	Mean	Mean
RB855156 × RB987935	RS/PR126066	5	6	3	63.09	10.84
RB867515 × RB946903	RS/PR126043	1	4	8	60.57	11.35
RB867515 × RB946903	RS/PR126044	5	4	8	56.09	9.75
RB867515 × RB965518	RS/PR126036	3	18	24	68.67	11.72
RB867515 × RB966928	RS/PR126063	2	12	14	57.67	10.38
RB867515 × RB996961	RS/PR126058	3	1	1	58.63	10.70
RB946903 × RB867515	RS/PR126052	5	24	29	64.08	11.67
RB965902 × CP72-2086	RS/PR126078	1	7	6	70.99	14.07
RB966928 × RB965518	RS/PR126015	2	34	33	59.85	10.89
RB966928 × RB975932	RS/PR126007	4	32	34	54.06	10.10
RB966928 × RB987935	RS/PR126025	2	10	10	56.56	10.38
RB987935 × RB867515	RS/PR126033	5	3	7	66.81	12.41
RB987935 × RB946903	RS/PR126069	2	30	35	56.61	10.11
RB987935 × RB966928	RS/PR126029	2	21	20	57.06	10.70
RB987935 × RB975932	RS/PR126074	2	9	13	61.62	10.89
RB72545 × ?	RB867515$	-	-	-	70.15	12.69
RB855156 × RB815690	RB966928$	-	-	-	64.20	12.65

*ABS scores: 1- dead apical bud and necrotic lesion; 2 - dead apical bud, but with lesion immediately below the bud, visible lateral shoots; 3 - dead apical bud, with lesion below the bud, abundant lateral shoots;4 - apical bud with small lesion and presence of live tissue with growth return in the central axis of the stalk and without visible lateral shoots and 5- live bud, with no visible lesion and no lateral shoots. TSH: tons of stalks per hectare; TBH: tons of brix per hectare and classification (Clas) of the families based on the genotype means.$ Commercial clones.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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