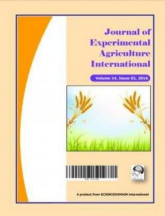

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Caryopsis Germination in Different Methodologies of Energy-sugarcane Crossing (*Saccharum officinarum* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. Author RSR elaborated the study, participated in all the steps of conducting, statistical analysis and writing the manuscript. Authors MFF and CRS were decisive in conducting the experiment, writing and correction phase. Author GCS was a work mentor. Authors LJOTM and DESN worked in the process of planning and supervisors of the experiment. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To compare and evaluate the germination index of caryopsis obtained by means of different crossing methods and to determine the quantitative of seeds to be used to compose the base population for selection among families by the Simplified Selection System (SSS) method.
Study Design: The experiment was conducted under a completely randomized design.
Place and Duration of Study: Devaneio Station of Sugarcane Flowering and Crossing and Experimental Sugarcane Station of Carpina, between February 2018 and December 2018.
Methodology: 27 crosses were performed, being eight biparental, ten polycrossing and nine self-

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fertilized. Germination tests were carried out in the greenhouse. From each crossing, four samples were sown with 0.5g caryopsis. The number of germinated caryopsis per box, five and thirty days after sowing, was evaluated to estimate the percentage of germination (% G).
Results: In the first evaluation, the C27 crossing (53.73%) presented the highest average. The caryopsis obtained by self-fertilization (12.82%) presented the highest germination average, followed by the polycrossing (10.88%) and the biparental crosses (9.99%). In the second evaluation, it was found that the crosses C8 (76.74%), C27 (61.02%), C3 (53.99%) and C13 (49.65%) presented with averages high. The highest percentage of germination was verified by the biparental method (31.10%), followed by the self-fertilizations (21.35%) and the polycrossing (20.43%).
Conclusion: Only crosses that show high germination are recommended for the composition of the families that will be evaluated through the Simplified System of Selection; caryopsis obtained through self-fertilization germinate rapidly compared to the methods of the polycrossing and biparental with respect to crosses between selected energy-sugarcane parents; biparental crosses between selected energy-sugarcane parents tend to have higher fertility of caryopsis than those obtained by means of polycrossing or self-fertilization; the families C1, C3, C8, C13, C26 and C27 can be used to compose the base populations in the SSS.

Keywords: Saccharum ssp.; caryopsis germination; crossing methods; simplified selection system; hybridization;

1. INTRODUCTION

The genetic improvement of sugarcane presents a long and expensive cycle, taking from 12 to 15 years until the release of a new variety [1]. According to Melo [2], it is important to invest in methodologies that can reduce the time until the release of a new variety.

For this purpose, the Simplified Selection System (SSS) was developed, which presents significant modifications in relation to the classical selection system. Among the main changes were: the method of obtaining and conducting the segregating population, the genetic selection of the family via Restricted Maximum Likelihood (REML) and Best Linear Unbiased Prediction (BLUP) and the early selection of sugarcane clones [2].

In both methods, the selection of new sugarcane genotypes by genetic improvement is carried out via plant populations obtained by means of seeds (caryopsis) [3]. However, in the SSS system, it is important to evaluate the fertility of caryopsis and to estimate the amount of caryopsis to be sown so that the populations present the same number of seedlings, unifying the competition between plants for space, water, light and nutrients.

The fertility of caryopsis may vary according to the method of crosses used, environmental, genetic and physiological factors [4,5]. The influence of such factors makes it difficult to obtain the seedlings, which will make up the base population for selection. Each crossing presents an intrinsic value of fertile caryopsis, making it necessary to perform previous germination tests.

The conduction of such tests is still quite disputed in the literature, since sugarcane caryopsis is not a commercial product and does not present standardized evaluation norms and described in the Rules for Seed Analysis [6]. Some studies were carried out by Cabral [4,7] and Caiero [8,9], who estimated the evaluation parameters of such seeds. According to Caiero [8], it is important to carry out studies aimed at understanding the physiological qualities of caryopsis.

In this context, the objective of this work is to compare and evaluate the germination index of caryopsis obtained by means of different crossing methods and to determine the quantitative of seeds to be used to compose the base population for selection among families by the SSS method.

2. MATERIALS AND METHODS

Initially 27 crosses were performed, being eight biparental, ten polycrossing and nine self-fertilized, according to Table 1. Hybridizations were performed at the Devaneio Station of Sugarcane Flowering and Crossing (EFCCD), located in Amaraji Municipality, Zone of Atlantic forest South to the State of Pernambuco (latitude 08°19'8''S, longitude 35°24'893''W and altitude 514m a.s.l.). The average annual precipitation of

the Region is 2600 mm, with minimum and maximum temperatures of 18.92 ºC and 28.15 ºC, respectively. According to Köppen [10], the local climate is classified as tropical with dry season (As). This environment is considered propitious to the flowering of sugarcane, making it possible to carry out hybridizations [5,11,12,13,14].

Germination tests were carried out in the greenhouse at the Experimental Sugarcane Station of Carpina (EECAC), belonging to the Federal Rural University of Pernambuco (UFRPE), a member of the Interuniversity Network for the Development of the Sugar and Energy Sector (RIDESA), located in the municipality of Carpina-PE (latitude 07º51'03"S, longitude 35º15'17"W and altitude 184m a.s.l.).

The test was conducted in a completely randomized design, according to the model: $y_{(ij)} = \mu + \tau_{(i)} + \varepsilon_{(ij)}$, where: $y_{(ij)}$ is the data observed in plot *ij*; μ is a constant (general mean); $\tau_{(i)}$ is the effect of the treatment *i* and $\varepsilon_{(ij)}$ is the experimental error of plot *ij*. The F test ($P < 0.01$, $P < 0.05$), the Schott and Knott clustering test ($P < 0.05$) [15] and the Tukey test ($P < 0.05$) were performed using the GENES software application [16].

From each crossing, four samples were sown with 0.5g caryopsis in plastic boxes (50 x 30 cm) containing substrate (composed of sugarcane press mud/filter cake and straw), which were subsequently covered with napkins.

The number of germinated caryopsis per box, five and thirty days after sowing, was evaluated to estimate the percentage of germination (% G). The reference value proposed by Cabral [7] was used to estimate the amount of fertile caryopsis in 0.5 g. According to the author, in 2g of unselected caryopsis, it is possible to obtain 1152 seedlings. Thus, theoretically observed at 0.5g it is possible to obtain 288 seedlings.

Table 1. Identification of sugarcane crosses performed to evaluate the fertility of caryopsis

Crossings	Genitors		Origin
	Female	Male	
C1	PRBIO 392	PRBIO 215	EFCCD-EECAC-UFRPE/RIDESA
C2	PRBIO 273	PRBIO 163	EFCCD-EECAC-UFRPE/RIDESA
C3	PRBIO 302	PRBIO 298	EFCCD-EECAC-UFRPE/RIDESA
C4	PRBIO 298	PRBIO 150	EFCCD-EECAC-UFRPE/RIDESA
C5	PRBIO 392	PRBIO 011	EFCCD-EECAC-UFRPE/RIDESA
C6	PRBIO 221	PRBIO 215	EFCCD-EECAC-UFRPE/RIDESA
C7	PRBIO 264	PRBIO 182	EFCCD-EECAC-UFRPE/RIDESA
C8	PRBIO 353	PRBIO 273	EFCCD-EECAC-UFRPE/RIDESA
C9	RB027052	*	EFCCD-EECAC-UFRPE/RIDESA
C10	PRBIO 298	*	EFCCD-EECAC-UFRPE/RIDESA
C11	PRBIO 133	*	EFCCD-EECAC-UFRPE/RIDESA
C12	PRBIO 150	*	EFCCD-EECAC-UFRPE/RIDESA
C13	PRBIO 371	*	EFCCD-EECAC-UFRPE/RIDESA
C14	IN 84-58	*	EFCCD-EECAC-UFRPE/RIDESA
C15	Co285	*	EFCCD-EECAC-UFRPE/RIDESA
C16	RB892783	*	EFCCD-EECAC-UFRPE/RIDESA
C17	RB036066	*	EFCCD-EECAC-UFRPE/RIDESA
C18	PRBIO 225	*	EFCCD-EECAC-UFRPE/RIDESA
C19	PRBIO 298 ⊗	-	EFCCD-EECAC-UFRPE/RIDESA
C20	PRBIO 273 ⊗	-	EFCCD-EECAC-UFRPE/RIDESA
C21	PRBIO 163 ⊗	-	EFCCD-EECAC-UFRPE/RIDESA
C22	PRBIO 393 ⊗	-	EFCCD-EECAC-UFRPE/RIDESA
C23	RB036066 ⊗	-	EFCCD-EECAC-UFRPE/RIDESA
C24	MEX54-81 ⊗	-	EFCCD-EECAC-UFRPE/RIDESA
C25	PRBIO 212 ⊗	-	EFCCD-EECAC-UFRPE/RIDESA
C26	PRBIO 589 ⊗	-	EFCCD-EECAC-UFRPE/RIDESA
C27	PRBIO 392	-	EFCCD-EECAC-UFRPE/RIDESA

** unknown genitor; ⊗ self-fertilization.*

3. RESULTS AND DISCUSSION

According to the F test, there was a significant difference at the 1% probability level for the source of variation caryopsis fertility. The coefficients of variation were 13.95% and 10.66% for the first and second evaluation, respectively, being considered medium, which demonstrates good experimental precision [17]. Such precision, in this case, is closely associated with good homogenization of caryopsis before sowing, as described in the Rules for Seed Analysis (RAS) of the Ministry of Agriculture Livestock and Food Supply (MAPA) [6] (Table 2).

Due to the significant effect for the source of variation caryopsis fertility, the method of Schott and Knott [15] was applied to form the groupings of the means. In the first evaluation, ten distinct groups were formed by the method of Schott and Knott [15]. It was verified that the crossing C27 (53.73%), with a germination index above 50%, obtained through self-fertilization, presented the highest germination average of the caryopsis, as opposed to the data obtained by Cabral [4], which concluded that the highest percentages of fertile spikelet's and caryopsis with higher physiological potential were obtained from multiple crossing. The highest mean germination of caryopsis, obtained through polycrossing, was also verified in the study presented by Caiero [8] (Table 3).

In turn, in the second evaluation, eleven groups were formed. It was found that the crosses C8 (76.74%), C27 (61.02%), C3 (53.99%) and C13 (49.65%), although they were statistically different, presented with averages high. It was observed that the C8 crossing, obtained through the biparental method, presented the highest germination average of the caryopsis, followed by the C27 crossing, which was acquired through self-fertilization, once again diverging from the considerations made by Cabral [4] and Caiero [8], where both stated that the highest averages come from polycrossing (Table 3).

Table 2. Result of the analysis of variance of the caryopsis fertility averages of the 27 families evaluated

Source of variation	Degrees of freedom	Average square 1st evaluation	Average square 2nd evaluation
Fertility of caryopsis	26	513.1869**	1507.6761**
Residue	81	2.4271	6.4899
Overall average		11.17	23.90
Coefficient of variation (%)		13.95	10.66

*** significant at the 1% probability level by the F test (P < 0.01)*

Table 3. Grouping of mean values of caryopsis fertility of crosses according to the Schott and Knott test [15]

Crosses	Averages of the 1st evaluation	Averages of the 2nd evaluation	Crosses	Averages of the 1st evaluation	Averages of the 2nd evaluation
C27	53.73a	61.02b	C26	7.20g	44.18e
C3	27.08b	53.99c	C25	5.21h	8.42j
C10	21.27c	25.61g	C4	4.77b	23.78g
C17	21.09c	21.27h	C2	3.91i	12.67j
C13	18.66d	49.65d	C9	3.47i	3.82k
C19	15.63e	26.39g	C11	3.21i	4.60k
C6	15.28e	23.09g	C21	2.95i	3.73k
C15	14.84e	25.09g	C1	2.69i	39.84f
C14	14.24e	26.82g	C22	2.34j	2.52k
C24	13.98e	11.37j	C20	1.04j	17.45i
C23	13.28e	17.10i	C16	0.35j	1.04k
C7	12.41f	18.40i	C18	0.35j	25.00g
C12	11.37f	21.44h	C5	0.26j	0.26k
C8	10.94f	76.74a			

Averages followed by the same letter do not differ by the Schott and Knott test [15]

Once that there were divergences in the literature regarding the crossing method of *Saccharum* spp. which can generate more fertile offspring, proceeded a comparative analysis of averages between the methods that were worked out, according to Table 4.

Due to the significant effect for the crossing methods, the Tukey test at 5% probability was applied to identify the method that presented the highest percentage of fertile caryopsis, according to Table 5.

In the first evaluation, the caryopsis obtained by self-fertilization (12.82%) presented the highest germination average, followed by the polycrossing (10.88%) and the biparental crosses (9.99%). However, in the second evaluation, the highest percentage of germination was verified by the biparental method (31.10%), while the self-fertilizations (21.35%) and the polycrossing (20.43%) were not statistically different from each other. It is inferred that the germination of the caryopsis obtained through self-fertilization shows a higher germination speed in the first days after sowing, whereas the biparental and polycrossing present a slower germination speed. However, the biparental showed greater germination power at the end of the last evaluation (Table 5).

After estimating the percentages of caryopsis germination, it was possible to establish the quantitative data of the caryopsis to be sown and to relate the crosses that will enter the simplified selection system (SSS). According to Melo [2] the population density for composition and selection of families is one seedling per square centimeter. Taking into account that the sowing was carried out in boxes with dimensions of 40 cm x 30 cm, the total area corresponds to 1200 cm², making it necessary to obtain 1200 seedlings per family.

It is observed in Table 6 that a large amount of caryopsis will be necessary to compose the base population with the same number of plants, by the majority of the crosses. According to Simões-Neto [1], it is necessary 3 g of seeds to compose the basic populations for the classic genetic improvement, in which the physiological quality and the percentage of germination of caryopses are not measured. However, in the SSS method, it is important to estimate such parameters [2]. According to Ramos [18], the evaluation of the percentage of caryopsis germination can be done with 0.6 g of unselected caryopsis. The reduction of the seed quantity occurs due to the high density of the seedlings, which favors greater competition for space, water, light and nutrients.

Thus, in order to work with the study population, it will be necessary to reduce the density of plants per cm² so that the limit of 3 g per box is not exceeded. The exact number is still under study and soon more information will be available in the literature.

Table 4. Results of variance analysis of caryopsis fertility averages of biparental crosses, polycrossing and self-fertilization methods

Source of variation	Degrees of freedom	Average square 1st evaluation	Average square 2nd evaluation
Crossing methods	2	10.0951**	139.6752**
Residue	9	0.2096	0.5334
General average		11.12	24.29
Coefficient of variation (%)		4.12	3.01

*** significant at the 1% probability level by the F test(P < 0.01)*

Table 5. Results of the comparative test of the mean values of caryopsis fertility of the crossing methods evaluated according to the Tukey test.

Crossing methods	Averages of the 1st evaluation (%)	Averages of the 2nd evaluation (%)
Self-fertilization	12.82[a]	21.35b
Polycrossing	10.88b	20.43b
Biparental	9.99c	31.10a
DMS	0.90	1.44

Means followed by the same letter do not differ statistically by the Tukey test (P<0.05).

Table 6. Number of seedlings obtained in 0.5 g of caryopsis and estimation of the amount in grams of seeds per crossing required to compose the base population with 1200 seedlings in the Simplified System of Selection [2]

Crosses	Nº of plants	Estimate (g)	Crosses	Nº of plants	Estimate (g)
C8	221	2.71	C17	61	9.80
C27	176	3.41	C7	53	11.32
C3	156	3.86	C20	50	11.94
C13	143	4.20	C23	49	12.18
C26	127	4.72	C2	37	16.44
C1	115	5.23	C24	33	18.32
C14	77	7.77	C25	24	24.74
C19	76	7.89	C11	13	45.28
C10	74	8.14	C9	11	54.55
C15	72	8.30	C21	11	55.81
C18	72	8.33	C22	7	82.76
C4	69	8.76	C16	3	200.00
C6	67	9.02	C5	1	800.00
C12	62	9.72			

4. CONCLUSION

Only crosses that show high germination are recommended for the composition of the families that will be evaluated through the Simplified System of Selection.

Caryopsis obtained through self-fertilization germinate rapidly compared to the methods of the polycrossing and biparental with respect to crosses between selected energy-sugarcane parents.

Biparental crosses between selected energy-sugarcane parents tend to have higher fertility of caryopsis than those obtained by means of polycrossing or self-fertilization.

The families C1, C3, C8, C13, C26 and C27 can be used to compose the base populations in the simplified selection system.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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