



Elite genotypes for the evaluation of crosses in sugarcane genetic breeding

Genotipos élite para la evaluación de cruces en la mejora genética en caña de azúcar

 Reynaldo Rodríguez Gross^{1*},  Yaquelin Puchades Izaguirre¹,
 Wilfre Abiche Maceo¹,  Héctor García Pérez²

¹Instituto de Investigaciones de la Caña de Azúcar (INICA Santiago de Cuba). Oriente-Sur. Carretera Central Km 2½, Los Coquitos, Palma Soriano, Santiago de Cuba, Cuba. C.P. 92610

²Instituto de Investigaciones de la Caña de Azúcar (INICA Habana). Carretera a la CUJAE km 1½, Boyeros, La Habana, Cuba. CP:55100

ABSTRACT: Efficient sugarcane breeding programs require availability of diverse germplasm and they are facilitated by formulation of appropriate selection strategies and procedures. The objective of the present study is the detection and use of genotypes or elite individuals as a criterion for the evaluation of crosses of the Sugarcane Breeding Program in the southeastern region of Cuba. The data from the selection evaluations of the stages of the genetic breeding scheme belonging to clonal propagation 1, 2 and replicated studies (period 2000-2014) were used. A classification algorithm was established to detect elite individuals on the variables diameter, stem length and refractometric brix compared to the control cultivar. The repeatability of the individuals and crosses where the elite individuals were classified from one stage to the other was determined. It was possible to detect the crosses that provide elite individuals, with significantly higher values in the clonal selection stage 2 to replicated studies than in the first clonal selection stage. The repeatability of the elite individuals in the clonal selection stages increases in the final stages of selection with respect to the initial one. The effectiveness of classification of these elite individuals in becoming recommended cultivars was 28.3 % when detected in clonal stage 1 compared to 85.3 % in clonal stage 2.

Key Word: selection, hybridization, cultivars.

RESUMEN: La eficiencia de los programas de mejoramiento genético en caña de azúcar requiere la diversidad del germoplasma. Esta es facilitada por formulaciones de estrategias y procedimientos de selección. El objetivo del presente estudio es la detección y uso de genotipos o individuos élite como criterio de evaluación de cruces del Programa de Mejoramiento Genético de la caña de azúcar en Cuba. Se utilizaron los datos de selección de las etapas del esquema de mejoramiento genético pertenecientes a la propagación clonal 1, 2 y estudios replicados (período 2000-2014). Se determinó el porcentaje de genotipos élite y la repetibilidad de esta clasificación de una etapa respecto a la otra. Se obtuvo un algoritmo de clasificación de genotipos élite con una efectividad de 85.3 %, para pronosticar cultivares comercializables en etapas tempranas de selección, cuando se detectan en la etapa clonal 2 respecto al 28.3 % en la etapa clonal 1. Se identificaron los 26 cruces con mayor aporte de genotipos élite a estudios replicados. De estos, el 72.8 % se corresponde con cruces de moderadamente comprobado a muy comprobado según la clasificación genética preliminar. Este resultado permite evaluar y pronosticar su respuesta a la selección y actualizar el programa de cruces para la mejora genética de la caña de azúcar en Cuba.

Palabras clave: selección, cultivares, hibridación.

*Author for correspondence. reynaldo.rodriguez@inicascazucuba.cu

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INTRODUCTION

The breeding of sugarcane cultivars has played a fundamental role in the development of the industry in the world (1). The selection process in breeding programs is complex and is developed along several breeding lines or objectives (2).

The efficiency of sugarcane breeding programs requires germplasm diversity, which is facilitated by formulations of selection strategies and procedures, as well as optimization of resources (3). The choice of parents and the prediction of their value is one of the most important actions and, therefore, the increase in selection and genetic gain is a measure of the success of these programs (4).

The Sugarcane Research Institute of Cuba (INICA) develops a genetic improvement program to respond to the obtention of new cultivars. The southeastern region of Cuba is included in this program with the annual evaluation and selection of 40 000 postures. This means that there is a group of clones under study in different stages and years of selection.

On the other hand, varietal development plans constantly require tactics for designing genetic improvement scenarios as efficiently as possible and interpreting collected data (3). From these procedures, correct inferences and conclusions can be drawn about the research question(s).

In the process of genetic improvement, many criteria are evaluated to choose the parents. Some authors consider the genetic value of a genotype as the ability to produce superior progeny when used as a parent and this value is used as a reference in plant and animal breeding (5).

The value of the crosses is also used to identify families that possess progenies with high value in certain characters, which are known as elite families (6). In the same way, elite individuals with superior characteristics to the rest of the progenies can be located, allowing the classification of outstanding families.

In the sugarcane genetic improvement scheme developed by INICA, individual selection is used as the basic method, to the detriment of family selection, which

makes it more difficult to estimate the value of parents and crosses. In addition, there is no methodology available for the identification and conduction of elite genotypes through the different stages of selection and that these at the same time contribute to the recommendation of new cultivars and the valuation of parents and families.

The aim of the present study is the detection and use of elite genotypes or individuals as criteria for the evaluation of crosses in the Sugarcane Genetic Breeding Program in the southeastern region of Cuba.

MATERIALS AND METHODS

The study was carried out at the Sugarcane Experimental Station of the southeastern region of Cuba, located in Santiago de Cuba province. Data from the selection evaluations of stages 2, 3 and 4 of the genetic breeding scheme were used according to the norms and methodological procedures of the Sugarcane Research Institute (7) (Table 1).

With the criteria established in the methodological norms and procedures, related to the selection of individuals in the clonal propagation stages, five algorithms for the classification of elite genotypes were programmed and simulated. For this purpose, the SASEL computer interface developed for information management of the sugarcane selection process in Cuba was used (8).

In the programming of the algorithms, the values of the variables brix refractometric, diameter and length of the stalks with respect to the control cultivar C87-51 were taken into account. In the case of stem length, the lowest selection pressure was used because it is the variable with the lowest heritability.

For the design of the algorithm, two strategies were used: i- prioritizing the sugar content of the selected individuals with refractometric brix superior or equal to the control and agricultural yield components similar to the control, ii- prioritizing the agricultural yield components (diameter and length of stems) superior or equal to the control and sugar content similar to the control.

Table 1. Stages, years and variables evaluated during the study period

Stages	Years	Variables	Evaluated harvests	Age (months)
Clonal propagation 1 (PC1)	2000-2014	• Refractometric Brix (%). • Stem diameter (cm). • Stem length (m)	First shoot	12
Clonal propagation 2 (PC2)	2000-2014	• Refractometric Brix (%). • Stem diameter (cm). • Stem length (m)	First shoot	12
Replicated studies (ER)*	2000-2012	• Number of individuals involved. • Recommended cultivars	• Cane plant • First shoot	18 12

*Years where variety trials were established

The following variables were determined in each year and selection stage:

- Percentage of elites with respect to those selected.
- Percentage of crosses where the elites were classified with respect to the total number of crosses of selected individuals.
- Percentage of repeatability of the elite status of one stage with respect to the other.
- Percentage of repeatability of elite crosses (families where the elites were detected and that repeat their condition from one stage to the other).

The means of the previous variables were tested by t-test for the comparison of means at 5% significance. From the elite genotypes in the PC1 and PC2 stages, the crosses or cultivar families established in the ER and the number of individuals were recorded. The genetic classification of these crosses was used to obtain a possible association between their categorization and the number of elites studied at this stage (9).

The number of varieties released to extension and their elite status classification in previous stages were determined to establish the effectiveness of the algorithm used through the percentage of recommended varieties versus preliminary elite classification.

RESULTS AND DISCUSSION

As a result of five designs and simulations, an elite ranking algorithm, not conceived in the methodological standards, was developed (Figure 1). This scheme detects the best traits in the selected genotypes from the evaluated variables and represents the objectives of genetic improvement in sugarcane for agricultural and industrial yield.

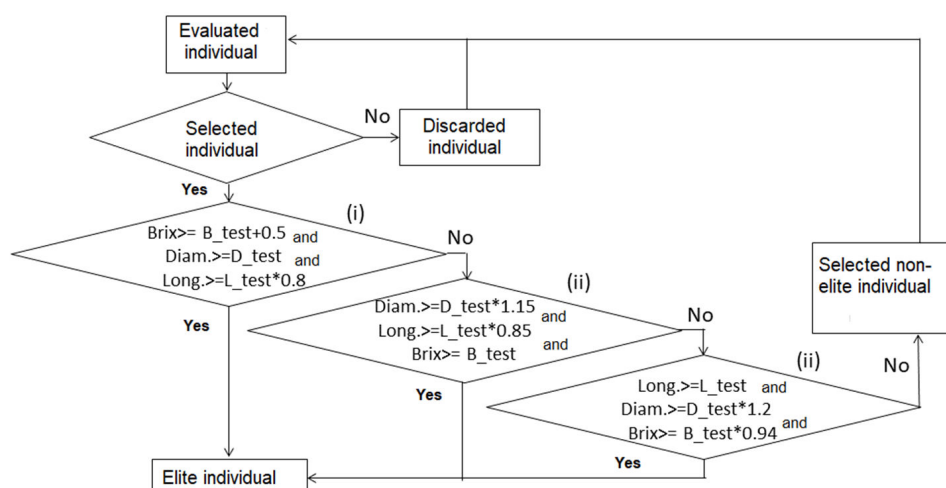
In the first strategy (i), when the variables were compared with the control C87-51, genotypes with high brix, higher by 0.5, stalk diameter equal or higher and stalk length equal or higher than 80 % were detected. The second strategy (ii) classified genotypes of equal or higher brix, equal or higher diameter by 115 % and stem length greater than or equal to 85 %. A second variant of this strategy revealed genotypes with brix equal or higher by 94 %, stem diameter equal or higher by 80 % and stem length equal or higher.

When applying the elite genotype classification algorithm, the results showed that, in all the series studied except for 2004, 2005 and 2008, the percentage of elite individuals with respect to those selected is lower in the PC1 stage than in PC2 (Figure 2a). The means of these percentages, in both stages, were 19 and 37 % respectively with differences significantly at 5 %. These results may be due to the fact that in stage 2 of selection the size of the plots is larger and with a previous selection process.

When the percentage of crosses with elite genotypes was analyzed, the value was higher in relation to the percentage of elite individuals, although without significant differences between the stages of clonal selection (Figure 2b). The means of the two stages were similar without statistical differences, with a slightly higher value for selection at stage 2 (51 %).

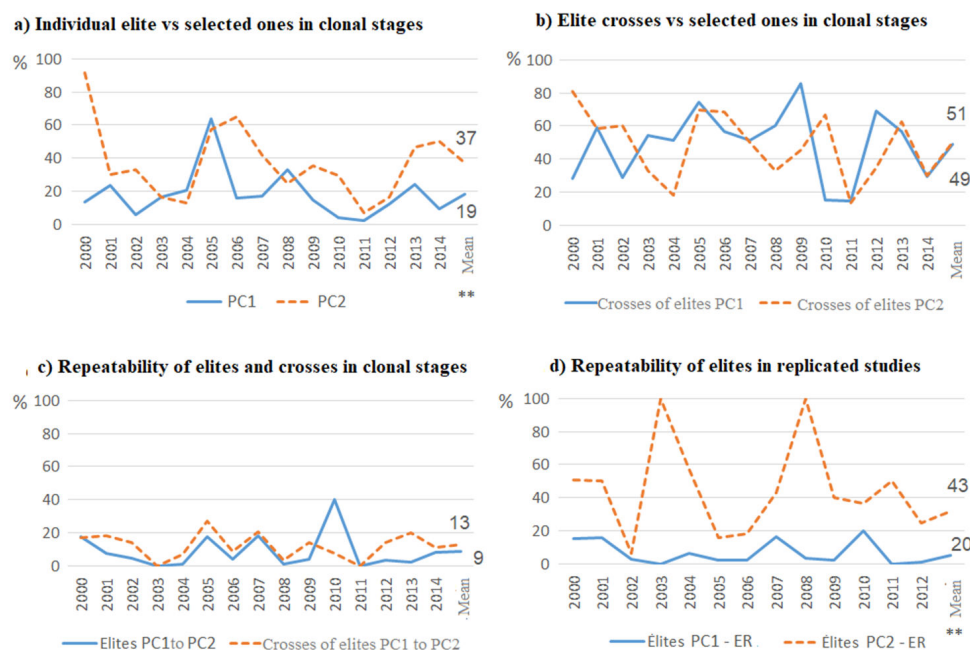
The repeatability of elites to be selected from one stage to the other was lower in the early stages of selection compared to the later stages (Figure 2c and 2d). In the later stages, there was a significant increase in the repeatability percentages of elites with the highest value from the PC2 to ER stage (43 %).

For all the series evaluated, except 2010, the repeatability of the crosses contributing elites from the PC1 to PC2 stage was higher, with an average of 13 %, which confirms that the families have greater consistency in the contribution of elites in the final stages of selection than that of the elite genotypes.



Brix-refractometric; Diam,- stem diameter; Long- stem length; B_test- Brix of the control; D_test- stem diameter of the control; i-strategy of prioritizing sugar content; ii-strategy of prioritizing agricultural yield; i_test- strategy of prioritizing sugar content; ii-strategy of prioritizing agricultural yield

Figure 1. Algorithm for detecting elite individuals in clonal propagation stages 1 and 2



PC1 - Clonal propagation stage 1; PC2 - Clonal propagation stage 2;
ER- Replicated studies stage; ** - means different at $p < 0.05$

Figure 2. Percentage of participation and repeatability of individuals and elite crosses

An analysis of the crosses that contributed the greatest number of elites to replicate studies showed that 72.8 % were classified as moderately proven to very proven crosses, with a prevalence of the proven classification (29.1 %) (Table 2). Only 5.1 % of the crosses with five or more individuals classified as moderately discarded, with no discarded or much discarded crosses.

Crosses with 14 or more elites to replicate studies classified as highly tested and tested were: C92-524 x Mex66-1235, C96-435 x Mex66-1235, CSG295-92 x B37161, C25-381 x CP70-1133, C229-84 x CP70-1133 and C86-12 x CP70-1133.

The effectiveness of the elite detection algorithm was evidenced by the results of the analysis of the series that completed the replicated studies and recommended cultivars for extension (Table 3). The usefulness of diagnosing superior genotypes at the PC2 stage was corroborated due to the percentage coincidence (85.7 %) with the cultivars recommended for extension and commercial use.

The system of proven crosses in sugarcane requires several years to determine whether a cross is considered elite or not. This system is based on the initial proportion of postures with respect to the genotypes that advance in later stages, which allows estimating the value of the cross (6). The results obtained in this work allowed characterizing the crosses that contribute genotypes to final stages of selection and therefore varieties to extension, which contributes to estimate its genetic value to incorporate it to the crossing program or to maintain it in the same as a proven cross.

The evaluation of parents with progeny data is more effective in plants with complex genomes such as sugarcane (10). Crosses with high germination percentage

and advances in subsequent stages of selection can be considered elites at the expense of new crosses or crosses with fewer standings (6).

Different authors have invoked the use of family selection followed by individual selection to produce gains more than those obtained by individual selection, especially in characters of low heritability (11). In this sense, the results of this study contribute to the identification of elite individuals and families, which allows a better choice of the combinations that participate in the crossbreeding and family selection program.

The objective of family selection is to identify elite families by focusing on the individual selection of genotypes (12) and is an important procedure to consider in the early stages of sugarcane breeding programs (13). These studies have shown that family selection is superior to individual selection, with high genetic gain for quantitative traits such as agricultural yield in the early stages of selection (10, 12). Therefore, crosses of elite parents should be more widely used in breeding programs because they provide a large proportion of genotypes with high expected yield (14). Although the present work is based on individual selection, it manages to extract information for the characterization of crosses.

The best strategy in terms of genetic gain and cost is a combination of family selection and selection within families (12). However, potential problems showed significant correlation between visual grade and subsequent behavior in next clonal stages, even with sugar content (more vigorous stands tend to have high sugar content in next stages) (15).

Other authors consider that the use of family evaluation is justified in the seedling stage, followed by individual selection, restricted to what is selected in these families

due to the low heritability of quantitative traits together with the high environmental variation and small plot sizes present in this stage (6). However, according to the results

of this work, the individual selection of elite genotypes and the descendants that constitute it, contribute to the genetic improvement program of sugar cane developed in Cuba.

Table 2. Genetic classification versus crosses with contribution of five or more elites in replicated studies

Combination	Freq.	Elite clones	PNC	Classification*	%
C92-524 x Mex66-1235	1	21	FM	Very verified	21.1
C96-435 x Mex66-1235	1	15	M		
CSG295-92 x B37161	1	14	M		
C86-503 x CP70-1527	1	6	FM		
C25-381 x CP70-1133	1	23	M	Verified	29.1
C229-84 x CP70-1133	2	22	PNC		
C86-12 x CP70-1133	1	14	PNC		
C112-80 x CP70-1133	1	6	M		
C90-501 x Mex66-1235	1	6	PNC		
CP70-1133 x My5724	1	6	PNC		
C90-501 x C86-531	3	28	PNC	Moderately verified	22.6
CP36-13 x CP70-1133	1	10	M		
C90-501 x B45181	1	6	PNC		
C90-501 x CSG87-508	1	6	FM		
C323-68 x C84-474	1	5	F		
CP70-1527 x C86-502	1	5	FM		
C86-602 x C87-253	1	10	FM	Inconsistent	21.5
C90-501 x C85-507	1	10	FM		
C86-456 x CP56-59	1	9	PNC		
CP52-43 x CP70-1133	1	9	PNC		
C86-12 x C85-277	1	8	PNC		
CP72-2086 x Ja64-20	2	6	PNC		
Co421 x CP70-1133	1	5	PNC		
C323-68 x CP72-2086	1	5	PNC	Moderately Discarded	5.7
C86-407 x Ja64-11	1	5	PNC		
C90-501 x Ja64-11	1	5	PNC		
Total		265	14		

Freq. - Frequency; *- Genetic classification; %- Percentage of the genetic classification of the crosses according to the contribution of elites in replicated studies; PNC- National Crossbreeding Program; F- PNC female parent; M- PNC male parent

Table 3. Cultivars recommended to extension stage versus classification of elite individuals in previous selection stages

Serie	Extension	Progenitors		elite	
	varieties	Feminine	Masculine	PC1	PC2
2000	C00-575	C86-12	CP70-1133	No	Yes
2002	C02-554	C88-533	PC	No	Yes
2003	C03-551	C88-533	PC	No	No
2004	C04-553	ClonT96-40	CSG87-508	No	Yes
	C04-570	CP52-43	PC	Yes	Yes
2006	C06-559	C86-456	CP56-59	Yes	Yes
Total	7			2	5
%				28.7	85.7

PC1 - Clonal propagation stage 1; PC2 - Clonal propagation stage 2; % - Percentage of cultivars recommended to extension with elite classification in previous selection stages

CONCLUSIONS

- A classification algorithm of elite genotypes was obtained with an effectiveness, to predict marketable cultivars in early stages of selection, of 85.3 % when detected in clonal stage 2 with respect to 28.3 % in clonal stage 1.
- There were determined 26 crosses with greater contribution of elite genotypes to replicated studies, corresponding 72.8 % of them to the preliminary genetic classification from moderately proved to very proved, which allows evaluating and predicting their response to selection and perfecting the crosses program in the genetic improvement of sugarcane in Cuba.

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