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Selection of stevia elite genotypes aiming at the development of cultivars adapted to the Brazilian Savanna

Selección de genotipos élite de estevia con el objetivo de desarrollar cultivos adaptados a la Sabana Brasileña

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***Stevia rebaudiana* (Bert.) growing at Embrapa Cerrados research fields (Distrito Federal, Brazil).**
Photo: F.C. Castro

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Short title: SELECTION OF STEVIA ELITE GENOTYPES ADAPTED TO THE BRAZILIAN SAVANNA

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Abstract

In this work, the objective was to evaluate morphoagronomic traits, correlate and select elite genotypes of *Stevia rebaudiana* (Bert.) Hemsley with high productivity, and adapted to Brazilian Savanna conditions. A total of 230 genotypes were evaluated, from the Embrapa Cerrados Working Collection, from 2018 to 2019, with 24 evaluations, carried out in six cuts, with each cut representing a certain moment of generation of the response data of the analyzed genotypes. Four traits were analyzed: green biomass production (g), plant height (cm), tillering number and juvenile period of each individual. The means of the characteristics of each genotype were correlated according to Pearson's correlation coefficient. The Mulamba and Mock index was applied, with a selection intensity of 10%, to choose the superior genotypes. There are high positive correlations between juvenile period, height and green biomass production. The selected population were 23 superior genotypes, based on the application of rank sum resulting in high selection gains for the traits of interest. Finally, the selection of elite stevia genotypes with high production of green biomass, greater insensitivity to length of the daily light period, as well as taller plants, are essential for the continuity of the stevia breeding program on the Brazilian Savanna.

Additional key words: *Stevia rebaudiana* (Bert.) Hemsley; breeding programs; correlation; yield increases; tillering; vegetative stage; plant height.

Resumen

En este trabajo, el objetivo fue evaluar las características morfoagronómicas, correlacionar y seleccionar genotipos étilite de *Stevia rebaudiana* (Bert.) Hemsley con alta productividad y adaptados a las condiciones de la Sabana Brasileña. Se realizaron 24 evaluaciones a 230 genotipos procedentes de la colección de trabajo de Embrapa Cerrados, de 2018 a 2019 en seis cortes, representando cada corte un determinado momento en la generación de datos de respuesta de los genotipos analizados. Se analizaron cuatro características: la producción de biomasa verde (g), la altura de la planta (cm), el número de macollas y el período juvenil de cada individuo. Las medias de las características de cada genotipo se correlacionaron según el coeficiente de correlación de Pearson. Se aplicó el índice de Mulamba y Mock, con una intensidad de selección del 10% para elegir los genotipos superiores. Existen altas correlaciones positivas entre el período juvenil, la altura y producción de biomasa verde. La población seleccionada fueron 23 genotipos superiores, con base en la aplicación de la suma de rangos, lo que resultó en altas ganancias de selección para los rasgos de interés. Finalmente, la selección de genotipos élite de estevia con alta producción de biomasa verde, mayor insensibilidad a la duración del período de luz diario, así como plantas más altas, son fundamentales para los futuros programas de mejoramiento de la estevia en la Sabana Brasileña.

Palabras clave adicionales: *Stevia rebaudiana* (Bert.) Hemsley; programa de mejoramiento genético; correlación; incremento del rendimiento; macollamiento; etapa vegetativa; altura de la planta.

INTRODUCTION

Stevia is a plant in which, through its leaves, natural sweeteners are extracted with zero calories. A *Stevia rebaudiana* (Bert.) Hemsley belongs to the Asteraceae family, characterized as an herbaceous plant, semiperene, found in the wild form in the border region between Paraguay and Brazil (De *et al*., 2013).

Stevia cultivation has been spreading in several countries such as China and Japan, which are the largest producers, India, USA, Canada, South Korea, Mexico, Russia, Indonesia, Tanzania, Argentina, Australia, Germany, Poland and in the countries of origin, Brazil and Paraguay (Yadav *et al*., 2011; De *et al*., 2013; Tavarini *et al*., 2018).

Yadav *et al*. (2011) pointed out that products such as stevia sweetener will grow due to consumer interest in natural products, being supported by improved varieties with high quantity and quality of diterpene and rebaudioside A, which do not taste bitter at the end or also know like “after taste”.

Mengesha *et al*. (2014) stated that until 2009, stevia was used in more than 6 thousand products such as soft drinks, food, medicines, among others, thus, aiming to become a commodity like sugar, and replace it in 20% of its world market.

The development of stevia cultivars adapted to the Brazilian Savanna involves the selection of genotypes with high phytomass production and with a long juvenile period.

Therefore, the evaluation of agronomic characteristics such as the number of days for flowering, phytomass production, plant height and tillering of genotypes of *S. rebaudiana* are important features in order to direct advances in selection and recombination cycles.

The Brazilian Savanna has been recommended as an interesting biome for stevia cultivation. It is a tropical, semi-humid climate, with two well-defined seasons: a rainy one in the summer and a dry one in the winter (Pelá and Castilho, 2010).

Stevia studies developed in this biome can produce valuable information and culminate in the development of varieties highly adapted to the conditions of the Brazilian Savanna, as well as to large-scale cultivation, constituting an alternative for producers in this important region of Brazil.

Correlation studies are of great importance in breeding programs, especially when the selection of a desirable trait presents difficulties, because it is a trait of low heritability and/or because of measurement and identification difficulties. Simple correlation allows assessing the magnitude and direction of the relationships between two characters, being useful for improvement, as it allows evaluating the viability of the practice of indirect selection, which, in some cases, can lead to faster progress (Cruz, 2013).

The selection of stevia genotypes best adapted to the Brazilian Savanna involves the combination of different characteristics of interest such as phytomass productivity, tillering capacity, long youthful period, among others. To combine different characteristics of interest in the same genotype, selection indexes have been used. One of the indexes used is that of Mulamba and Mock (1978), based on the sum of a ranking of genotypes based on the characteristics of interest. This sum of the "ranks" determines the classification of the genotypes based on the analyzed variables (Cruz *et al*., 2014).

The objective was to evaluate morpho-agronomic characteristics, correlate and select elite genotypes of *S. rebaudiana* with high productivity, long youthful period and adapted to the conditions of the Brazilian Savanna.

MATERIAL AND METHODS

The study was carried out at Embrapa Cerrados, in Planaltina-DF (15°36'19"S, 47°42'56"W and altitude of 1,024 m), latossolo vermelho (Santos *et al*., 2018), during the period of 2018 to 2019. The climatological data, during the conduction of the research were: minimum, average and maximum air temperature of 16.5, 22.1 and 29.3°C, respectively;

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minimum, average and maximum air humidity, correspondingly, of 40.5, 67.9 and 88.4%; 1,502 mm of precipitation, 18.3 MJ m^{-3} of solar radiation.

A total of 230 genotypes from the Embrapa Cerrados Working Collection were studied, originating from seeds obtained by the natural crossing between elite genotypes. Sowing was carried out at the Embrapa Cerrados greenhouse in April 2018 and field planting at the end of May 2018, when the seedlings were approximately 25 cm tall. The spacing used was 50 cm between rows and 20 cm between plants. In the greenhouse the average temperature ranging from 15 to 20ºC night temperature and 25 to 30ºC day temperature, relative humidity ranging from 60 to 65%.

There were 24 evaluations, carried out in six cuts, with each cut representing a certain moment in the generation of the response data of the analyzed genotypes. Since the interaction between the genotypes in the different cuts is a result already expected in the present study, the evaluation was directed towards a comparative analysis of the "genotype" factor at each moment, in order to subsequently highlight the best genotypes over time, as it is expected that there will be a relationship of responses regarding the daylight length. The six cuts were made at 10/19/2018, 12/13/2018, 3/28/2019, 6/25/2019, 9/30/2019 and 12/19/2019 to evaluate four characteristics.

The characteristics evaluated were: production of green phytomass of the aerial part in (PHM) (g), height of plant (HGT) (cm) and tillering (TILL) of each plant. The juvenile period was evaluated based on the number of days until the beginning of flowering (NDF) and when the plants presented a chapter with the presence of open flowers. The number of the day (NDF1) is based on the counting of days from the planting of seedlings in the field. The others evaluation is counting from the day of each cut for the other NDF periods (2, 3, 4, 5 and 6).

No phytosanitary products were used to control pests and diseases. Were carried out weeding was performed biweekly to control weeds. Technical recommendations for irrigation and fertilization were used according to Lima *et al*. (2004).

The plants were harvested by cutting up to 5 cm from the ground, and the entire PHM was individually weighed. The HGT was measured from the ground to the apex of the plant. The number of tillers per plant was measured. Probably due to the characteristics of the species itself, *S. rebaudiana*, which presented an excellent performance in the Brazilian Savanna conditions, it was not necessary to use any product for pest or disease control. However, to achieve a better performance of the stevia plants in the field, specifically in relation to competition with weed species, it was necessary to perform manual weeding every two weeks, or as soon as the weeds started to reach five to 10 cm in height, they were eliminated manually with the use of weeding equipment such as hoes and hoes.

Firstly, descriptive analyzes were performed for the analyzed characteristics of each genotype, and Pearson's correlation coefficient was calculated to evaluate the correlation between the evaluated characters.

Due to its relative ease of construction, the Mulamba and Mock (1978) index was adopted, based on the sum of “ranks”, was used to select the superior genotypes and classify them in relation to each character. To calculate the index in question, a selection intensity of 10% was applied, which resulted in an effective selection size equal to 23 elite genotypes. In the sum of the classifications, an economic weight of 1 was assigned to all 24 evaluations of the 4 agronomic traits. All analyzes were generated using the Genes program (Cruz, 2013).

RESULTS AND DISCUSSION

It was found that the population of elite stevia genotypes, initially with 230 individuals, showed a high variation between genotypes for all characteristics studied in all six periods, with the exception of evaluations carried out in period three (NDF3, TILL3, and PHM3) (Tab. 1).

Table 1. **Selection gain estimates (GS and GS%) and averages of the initial population (X_0) and the selected population (X_s), obtained through the selection index based on the sum of ranks (Mulamba and Mock, 1978) of a population of genotypes elite of *S. rebaudiana* for the characteristics number of days to flowering (NDF), plant height (HGT), tillering (TILL), green phytomass (PHM), the numbers 1, 2, 3, 4, 5 and 6 relating to each cut. Embrapa Cerrados, Planaltina, DF. 2019.**

Characteristics	X_0	X_s	GS	GS %
NDF1	125.41	152.43	27.03	21.55
HGT1	41.80	59.91	18.11	43.33
TILL1	14.05	28.09	14.04	99.94
PHM1	331.42	543.23	211.80	63.91
NDF2	31.08	40.74	9.66	31.09
HGT2	49.27	70.48	21.20	43.03

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TILL2	39.73	78.26	38.53	96.96
PHM2	340.53	621.31	280.79	82.46
NDF3[#]	-	-	-	-
HGT3[#]	54.35	90.52	-	-
TILL3[#]	-	-	-	-
PHM3[#]	-	-	-	-
NDF4	28.02	50.52	22.50	80.29
HGT4	27.49	57.65	30.17	109.75
TILL4	9.57	30.09	20.52	214.40
PHM4	44.36	134.87	90.51	204.03
NDF5	26.03	45.43	19.40	74.52
HGT5	30.33	60.20	29.86	98.44
TILL5[#]	28.72	76.39	-	-
PHM5	257.39	702.00	444.61	172.74
NDF6[#]	28.44	48.48	-	-
HGT6	46.35	90.40	44.05	95.03
TILL6[#]	35.81	91.00	-	-
PHM6	434.55	1,109.96	675.40	155.43
Total gain			1,998.18	1,686.90

[#] characteristics marked with the symbol # show negative genetic variance.

Selection based on the Mulamba and Mock index, with a selection intensity of 10%, culminated in the selection of 23 highly superior elite genotypes. There was a high selection gain for all the agronomic characteristics analyzed (Tab. 1). Sayd *et al*. (2019), concluded that the Mulamba and Mock selection index was effective in the selection of three characteristics of interest for barley.

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The selection gains above 100% for the characteristics (HGT4), (TILL4), (PHM4), (PHM5) and (PHM6) stand out, with values in percentage of 109, 214, 204, 172, 155, respectively, displayed in the table 1.

Table 2 contains the averages of the initial (Xo) and selected (Xs) populations for each characteristic over time, that is, in all available periods. It is observed that, with the increase of the juvenile period portrayed by the characteristic number of days to flowering (NDF), we obtained a very significant increase, more than double for the characteristic of production of green phytomass (PHM). The longer juvenile period of plants in which the economic interest is in the aerial part, as in the case of *S. rebaudiana,* may favor the producer to have greater flexibility of time for programming management activities, especially cutting the aerial part of the plants in the field. With a longer juvenile period the plants take more time vegetating, in other words, devoting all the energy spent to the growth of the aerial part, in other words, increasing the dry matter. With a reduced juvenile period, the plants will flower earlier, enter the reproductive phase, and thus, allocate the energy produced by photosynthesis to reproductive functions, and thus reduce or cease vegetative growth. For the producer of stevia in the Brazilian Savanna, it is interesting to have *S. rebaudiana* plants with a longer juvenile period, in order to have greater profitability with the greater vegetative growth of the plant and consequently the greater accumulation of dry matter of the plant.

Table 2. The average of the characteristics number of days to flowering (NDF), plant height (HGT) in centimeters, tillering (TILL), green phytomass in grams (PHM) of the initial and selected populations (X_o and X_s). Minimum and maximum values in relation to the 230 genotypes of *S. rebaudiana* and the numbers 1, 2, 3, 4, 5 and 6 relating to each cut. Embrapa Cerrados, Planaltina, DF. 2019.

Characteristics	Average X_o	Average X_s	Minimum	Maximum
NDF (2, 4, 5, 6)	28	46	21	80
HGT (1, 2, 3, 4, 5, 6)	41.60	71.53	15	118
TILL (1, 2, 4, 5, 6)	25.58	60.77	1	170
PHM (1 2 4 5 6)	281.65	622.27	1	1882

However, Serfaty *et al*. (2013), in Israel, reached approximately 130 cm in height of *S. rebaudiana* in the spring; in Iran, Taleie *et al*. (2012), reached 80 cm; in India Kimar *et al*.

Castro, F.C., F.G. Faleiro, J.S. Oliveira, R.F. Amabile, and J.V.P. Melo. 2022. Selection of stevia elite genotypes aiming at the development of cultivars adapted to the Brazilian Savanna. Revista Colombiana de Ciencias Hortícolas 16(3), e14727. Doi: https://doi.org/10.17584/rcch.2022v16i3.14727

(2014) reached a maximum height of 126.7 cm. In southern Brazil, Francisco *et al*. (2018) obtained a maximum height of 116 cm in one access. In comparison to the present study, the maximum height reached was 47.5% higher (118 cm), and the average of the selected population was 71.53 cm.

According to Francisco *et al*. (2018), the authors Lima *et al*. (2004); Serfaty *et al*. (2013) among others achieved lower yields of 4.3 t ha^{-1} of dry leaves. On the other hand, in Ethiopia, Btru *et al*. (2017) obtained a maximum height of 119.5 cm and yield of 7.8 t ha^{-1} of dry leaves, with a density of 125 thousand plants per hectare.

The estimates of Pearson's correlation coefficients between the means of the 24 characteristics of the 230 elite stevia genotypes, presented in tables 3, 4 and 5, show seven very strong correlations (> 0.9) between the characteristic HGT4 and HGT5, HGT6; NDF5 and HGT5, NDF6; HGT5 and NDF6, HGT6; and finally, NDF6 and HGT6, according to the classification of Carvalho *et al*. (2004). Therefore, the greater the height (HGT), the longer the juvenile period, that is, the greater the number of days to flowering (NDF). This fact can be verified because all the NDF and HGT characteristics in their corresponding periods are above 0.8, therefore, correlating positively and with high intensity, except for period three.

It appears that 105 correlations, which correspond to 38% of the total, are of intensities considered high (>0.6 and <0.9), according to Carvalho *et al*. (2004). It is noteworthy the existence of a behavior pattern of high and very high intensities between the NDF and HGT characteristics for periods 3, 4, 5 and 6 of these characteristics, as shown in Table 3,4 and 5. It is observed that, between the characteristics PHM and TILL, in periods 1, 5 and 6, there was a positive, significant correlation and above 0.8.

Table 3. **Estimates of Pearson's correlation coefficients between characteristics: number of days to flowering (NDF: 1; 2), height (HGT: 1; 2) in centimeters, tillering (TILL: 1; 2), green phytomass in grams (PHM: 1; 2) of 230 elite genotypes of *S. rebaudiana (Bert.)*. Embrapa Cerrados, Planaltina, DF. 2019.**

	HGT1	TILL1	PHM1	NDF2	HGT2	TILL2	PHM2
NDF1	0.88*	0.76*	0.66*	0.79*	0.79*	0.60*	0.59*
HGT1	-	0.83*	0.78*	0.80*	0.85*	0.71*	0.68*
TILL1	-	-	0.85*	0.67*	0.73*	0.76*	0.67*
PHM1	-	-	-	0.60*	0.63*	0.62*	0.61*

NDF2	-	-	-	-	0.87*	0.65*	0.61*
HGT2	-	-	-	-	-	0.78*	0.79*
TILL2	-	-	-	-	-	-	0.80*

[*] significant at 1% probability by the t test.

Table 4. **Estimates of Pearson's correlation coefficients between characteristics: number of days to flowering (NDF: 1, 2, 3, 4), height (HGT: 1, 2, 3, 4) in centimeters, tillering (TILL: 1, 2, 3, 4), green phytomass in grams (PHM: 1, 2, 3, 4) of 230 elite genotypes of *S. rebaudiana*. Embrapa Cerrados, Planaltina, DF. 2019.**

	NDF3	**HGT3**	**TILL3**	**PHM3**	**NDF4**	**HGT4**	**TILL4**	**PHM4**
NDF1	0.00*	0.54*	0.00*	0.00*	0.51*	0.53*	0.44*	0.36*
HGT1	0.00*	0.61*	0.00*	0.00*	0.62*	0.62*	0.50*	0.36*
TILL1	0.00*	0.51*	0.00*	0.00*	0.52*	0.55*	0.47*	0.40*
PHM1	0.00*	0.43*	0.00*	0.00*	0.46*	0.49*	0.42*	0.33*
NDF2	0.00*	0.59*	0.00*	0.00*	0.56*	0.57*	0.49*	0.38*
HGT2	0.00*	0.69*	0.00*	0.00*	0.65*	0.69*	0.54*	0.44*
TILL2	0.00*	0.57*	0.00*	0.00*	0.59*	0.59*	0.50*	0.39*
PHM2	0.00*	0.56*	0.00*	0.00*	0.52*	0.59*	0.46*	0.41*
NDF3	-	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
HGT3	-	-	0.00*	0.00*	0.84*	0.87*	0.75*	0.57*
TILL3	-	-	-	0.00*	0.00*	0.00*	0.00*	0.00*
PHM3	-	-	-	-	0.00*	0.00*	0.00*	0.00*
NDF4	-	-	-	-	-	0.84*	0.66*	0.48*
HGT4	-	-	-	-	-	-	0.82*	0.72*
TILL4	-	-	-	-	-	-	-	0.80*

Castro, F.C., F.G. Faleiro, J.S. Oliveira, R.F. Amabile, and J.V.P. Melo. 2022. Selection of stevia elite genotypes aiming at the development of cultivars adapted to the Brazilian Savanna. Revista Colombiana de Ciencias Hortícolas 16(3), e14727. Doi: https://doi.org/10.17584/rcch.2022v16i3.14727

* significant at 1% probability by the t-test.

Table 5. **Estimates of Pearson's correlation coefficients between characteristics: number of days to flowering (NDF: 1, 2, 3, 4, 5, 6), height (HGT: 1, 2, 3, 4, 5, 6) in centimeters, tillering (TILL: 1, 2, 3, 4, 5, 6), green phytomass in grams (PHM: 1, 2, 3, 4, 5, 6) of 230 elite genotypes of *S. rebaudiana*. Embrapa Cerrados, Planaltina, DF. 2019.**

	NDF5	HGT5	TILL5	PHM5	NDF6	HGT6	TILL6	PHM6
NDF1	0.51*	0.52*	0.41*	0.40*	0.52*	0.51*	0.40*	0.38*
HGT1	0.59*	0.63*	0.49*	0.48*	0.61*	0.60*	0.49*	0.44*
TILL1	0.54*	0.58*	0.51*	0.48*	0.54*	0.56*	0.47*	0.46*
PHM1	0.46*	0.52*	0.47*	0.45*	0.45*	0.47*	0.39*	0.39*
NDF2	0.55*	0.58*	0.46*	0.45*	0.58*	0.56*	0.44*	0.43*
HGT2	0.63*	0.68*	0.52*	0.52*	0.65*	0.67*	0.55*	0.50*
TILL2	0.59*	0.61*	0.52*	0.48*	0.57*	0.58*	0.50*	0.44*
PHM2	0.53*	0.60*	0.50*	0.52*	0.53*	0.61*	0.52*	0.54*
NDF3	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
HGT3	0.82*	0.87*	0.67*	0.67*	0.86*	0.85*	0.67*	0.62*
TILL3	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
PHM3	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
NDF4	0.84*	0.84*	0.66*	0.60*	0.86*	0.81*	0.65*	0.59*
HGT4	0.85*	0.94*	0.80*	0.79*	0.89*	0.92*	0.79*	0.74*
TILL4	0.70*	0.79*	0.72*	0.76*	0.73*	0.79*	0.75*	0.65*
PHM4	0.54*	0.66*	0.65*	0.71*	0.57*	0.67*	0.68*	0.68*
NDF5	-	0.90*	0.66*	0.64*	0.91*	0.86*	0.65*	0.60*
HGT5	-	-	0.79*	0.80*	0.93*	0.95*	0.79*	0.74*
TILL5	-	-	-	0.83*	0.68*	0.75*	0.81*	0.77*
PHM5	-	-	-	-	0.67*	0.79*	0.83*	0.86*
NDF6	-	-	-	-	-	0.92*	0.70*	0.63*

Castro, F.C., F.G. Faleiro, J.S. Oliveira, R.F. Amabile, and J.V.P. Melo. 2022. Selection of stevia elite genotypes aiming at the development of cultivars adapted to the Brazilian Savanna. Revista Colombiana de Ciencias Hortícolas 16(3), e14727. Doi: https://doi.org/10.17584/rcch.2022v16i3.14727

HGT6	-	-	-	-	-	-	0.81*	0.80*
TILL6	-	-	-	-	-	-	-	0.86*

* significant at 1% probability by the t-test.

It can be observed that, in tables 3, 4 and 5, there is a high and positive correlation between the characteristics NDF and PHM (1,2,5,6), with all these correlations being above 0.6. Thus, we can infer that, with the increase in the youthful period of the elite genotypes, there was concomitantly an increase in the production of green phytomass. Green phytomass is linked to leaf yield, which is the most commercially important part of the stevia.

Finally, the selection of elite stevia genotypes with high production of green or fresh phytomass, greater insensitivity to the short length of the day, which in turn, allows an increase in the youthful period; as well as high height without lodging, are fundamental for the improvement of *S. rebaudiana*, providing a production alternative for producers in the regions that present these conditions with emphasis on the Brazilian Savanna.

CONCLUSIONS

There are high positive correlations between the number of days for flowering, height and phytomass production.

The application of the selection index Mulamba and Mock, culminates in significant selection gains for all stevia characteristics.

The selection of 23 upper elite genotypes has an average of 46 d to start flowering; 71.53 cm in height; 60.77 tillers per plant and an average total green phytomass production of 622.27 g/plant.

Conflicts of interest

This manuscript was prepared and reviewed with the participation of all authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

Authorship contributions

Castro, F.C.: writing, assay implementation, sample collection, and results analysis, Faleiro, F.G.: writing, assay implementation, sample collection, and results analysis, Oliveira, J.da S.: writing, assay implementation, sample collection, and results analysis, Amabile, R.F.;

writing, assay implementation, sample collection, and results analysis, Melo, J.V.P.: writing, assay implementation, sample collection, results analysis, translation and final editing.

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