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Environmental factors influence the production of flowers and fruits of cassava

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A R T I C L E I N F O

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A B S T R A C T

Cassava (*Manihot esculenta* Crantz) is a widely adaptable crop and plays a vital role in food security in many countries. Although farmers constantly demand improvements in crop productivity through genetic improvement, low flowering in the species can make it challenging to include some parents in crossing blocks or generate large populations. This study aimed to evaluate the influence of various environmental factors on cassava flowering by planting at different times of the year in the Recôncavo of Bahia region (Brazil). Thirty-five cassava accessions with contrasting flowering characteristics were planted in six different planting seasons, with a two-to-three-month difference between each planting date. Climate and flowering data were collected every two weeks during the experimental period, evaluating traits such as the number of days to the beginning of the first ramification (ND1B), flowering score (FS), score weighted by early flowering (SW) and height of the first branch (H1B). We found significant differences in all evaluated traits between planting seasons. The broad-sense heritability (h^2_a) and Cullis' heritability (h^2_c) were of medium (0.38 for FS) to high magnitude (0.98 for SW. Notably, the third planting season (February 2021), resulted in early flowering, approximately two months after planting, with SW of 10.40 and a higher flowering score of 0.56, representing a 52 % and 60 % increase compared to the first planting season, respectively. Our findings indicate that temperature and accumulated degree days are environmental factors correlated with increased flowering in cassava. Moreover, the dispersion of the genotypes in the two principal component analysis showed differences across the six planting seasons, indicating the environmental influence on the expression of flowering in this species. These findings can help breeders make reliable decisions when setting up crossing fields to accelerate the acquisition of improved progenies.

1. Introduction

Cassava (*Manihot esculenta* Crantz) has gained importance in recent decades due to its socioeconomic importance and for being an important source of energy for approximately one billion people (Latif; Müller, 2015). Beside human food, it also stands out for being used as a raw material in numerous industrial products and for animal feed. It is a crop that adapts to different soil and climate conditions, being cultivated in underdeveloped or developing countries, especially on African, Southeast Asia and Latin America continent (Ceballos et al., 2012). The cultivation of cassava enables the generation of direct jobs promoting income generation and favoring the global economy (USDA, 2022). The development of superior genotypes that meet the specific demands of small producers and the industry, is a constant demand, given that regardless of the level of technification of farmers, the variety is the main cultivation technology adopted.

For the selection of superior genotypes, it is necessary to increase and explore the genetic variability of the species, in search of more favorable combinations of traits that often have negative correlations. To this end, hybridization or artificial self-fecundation are performed to produce segregating populations. However, one of the major barriers in this process are the obstacles related to the flowering of the crop. The development of genotypes with erect plant architecture and absence of branches, to meet the demands of mechanized planting, tends to select genotypes with absence or late flowering. In cassava, the branching potential of the stems is directly correlated with flowering (Ceballos et al., 2017).

Another challenge imposed on the planning of crosses in cassava

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refers to the lack of flowering synchronization, because some clones flower early (4–5 months after planting - MAP), while others flower later (> 12 MAP) (Ceballos et al., 2015, 2017; McGarry et al., 2016; Adeyemo et al., 2018). In that situation, it is necessary to know the flowering phenology of the parents involved in the crosses.

The floral phenology, can be understood as the beginning of reproductive development of plants and an important phase of the general phenology, being the key point for the reproduction of the species (Cortés-Flores et al., 2016; König et al., 2017). Recently climate change has caused changes in important events in plant life cycle and biodiversity (Sun; Frelich, 2011; Davies et al., 2013; Wang et al., 2021). Environmental changes between different habitats can provide answers about which plant physiological traits combined with floral phenology enhance or reduce reproductive success (Freschet et al., 2013; Fontana et al., 2017). Temperature has long been considered the most consistent and dominant controller of floral phenology (Wolkovich et al., 2012). Studies have adopted temperature sensitivity (change in days per degree Celsius, d°C^{-1}) to characterize the responses of floral phenology to temperature changes (Menzel et al., 2006; Tooke; Battey, 2010; Diez et al., 2012). Although some other abiotic factors such as photoperiod and rainfall influence floral phenology, temperature has been noted as the dominant factor especially for temperate plants (Ellwood et al., 2013; Jochner and Menzel, 2015).

In recent years, studies have been conducted to understand the morphological, physiological, biochemical and environmental aspects that regulate flowering in cassava (Ha et al., 2012; Adeyemo et al., 2018; Ramos Abril et al., 2019; Pineda et al., 2020a; Bandeira and Sousa et al., 2021). Climatic factors, such as temperature and insolation period, have been reported as the main causes of variation in flowering phenology among species (Boyle; Bronstein, 2012; Davies et al., 2013). Knowing the influence of these factors, makes it possible to determine the best planting seasons to obtain a greater success in the production of flowers and seeds (Ceballos et al., 2011, 2017). In some genera such as *Arabidopsis*, planting at times with adequate climatic conditions allows for better synchronization of flowering between the parents and consequent realization of the desired crosses (Kim et al., 2009).

In cassava, temperature influences floral initiation, with delayed flowering in cultivars subjected to temperatures ranging from 22 to 34 °C (Adeyemo et al., 2018). On the other hand, higher flowering was observed at milder temperatures (22 to 28 °C) (Adeyemo et al., 2018). In a study with approximately 1000 cassava accessions, it was reported that although flowering is dependent on the genetic material, in the months with higher photoperiod (12.19 h) a higher production of flowers was observed (Souza et al., 2020). Photoperiod and temperature are involved in the regulation of genes related to floral expression, inhibiting or stimulating flowering in certain cassava genotypes (Adeyemo et al., 2018). In addition to these factors, relative humidity also has an important influence on flower and fruit production in cassava, when provided adequately it induces flowering and fruit production, while, high air temperatures (>30 °C) and low relative humidity repress flower and fruit production (Ravi and Ravidran, 2006).

Studies on the flowering phenology of cassava are important because they allow the identification of genotypes that are favorable to flowering and seed production, beside determining the best time of year to plant the crossing blocks. Thus, it will be possible to plan more efficiently the crosses in breeding programs. This study aimed: i) to evaluate the influence of different planting seasons on flowering time; and ii) to evaluate the effect of climatic variables on flowering, and agronomic characteristics of cassava.

2. Material and methods

2.1. Plant material

We selected 35 cassava accessions from the Genebank (BAG) of Embrapa Mandioca e Fruticultura (Cruz das Almas, Bahia, Brazil), which exhibit diverse flowering characteristics (Table 1).

2.2. Experimental design

The experiments were conducted in field conditions in Cruz das Almas, Bahia (12°40′19′' S, 39°06′22′' W, and altitude of 220 m). We used cutting pieces of 16–18 cm long with an average of eight buds, obtained from the middle third of 12-month-old health plants. Soil preparation was done by one plowing and two harrows, followed by opening the planting furrow at 0.90 m between rows with a cassava planter. Fertilization was carried out based on soil analysis, and cultivation was done according to the recommendations of Souza et al. (2006). We implemented the experiment in six planting seasons with a 2- to 3-month interval between each planting time, with the first planting in September/2020 (Table 2).

For each planting seasons, we used a randomized complete block design with two repetitions. The plots comprised of two rows with four plants each, totaling eight plants per plot. The planting spacing was 0.90 m between rows and 0.80 m between plants. We collected daily climatic data, including rainfall averages, maximum, minimum, and average temperatures, and air relative humidity (%), during the evaluation period of the experiment from the meteorological station of Embrapa Mandioca e Fruticultura (Fig. 1). We calculated the accumulated degree day (ADD) using the equation $DD = \left\{\frac{Tmax+Tmin}{2}\right\} - Tb$, where DD represents the degree day (°C), *Tmax* represents the maximum day temperature (°C), *Tmin* represents the minimum day temperature (°C), and *Tb* represents the base temperature for cassava cultivation (°C). The ADD was estimated based on a 365-day cycle for cassava. The base temperature was considered according to the method proposed by Arnold (1959) considering the period from planting to flowering, using

Table 1
List of the 35 cassava genotypes evaluated for the influence of environmental factors on flowering and fruit production, their ways of obtaining and collection sites.

Genotype	Obtained by	Place of collection or generation
2011–34–41	Improvement	Cruz das Almas, BA, Brazil
2011–34–45	Improvement	Cruz das Almas, BA, Brazil
2011–34–69	Improvement	Cruz das Almas, BA, Brazil
BGM-0062	Local Variety	Cruz das Almas, BA, Brazil
BGM-0195	Local Variety	Valença, BA, Brazil
BGM-0590	Local Variety	Itambé, BA, Brazil
BGM-0593	Local Variety	Lajeado, RS, Brazil
BGM-0598	Local Variety	São Sebastião do Cai, RS, Brazil
BGM-0630	Local Variety	Pacatuba, CE, Brazil
BGM-0702	Local Variety	Acre State, Brazil
BGM-0707	Local Variety	Alagoinhas, BA, Brazil
BGM-0935	Local Variety	Maracas, BA, Brazil
BGM-1118	Local Variety	Brumado, BA, Brazil
BGM-1128	Local Variety	Itiruçu, BA, Brazil
BGM-1511	Local Variety	Capim Grosso, BA, Brazil
BGM-1722	Local Variety	Santo Antônio de Jesus, BA, Brazil
BGM-1760	Local Variety	Chapadinha, MA, Brazil
BRS Caipira	Improvement	Cruz das Almas, BA, Brazil
BRS CS-01	Improvement	Cruz das Almas, BA, Brazil
BRS Dourada	Local Variety	São Felipe, BA, Brazil
BRS Formosa	Improvement	Cruz das Almas, BA, Brazil
BRS Gema de Ovo	Local Variety	Amazonas State, Brazil
BRS Kiriris	Improvement	Cruz das Almas, BA, Brazil
BRS Mulatinha	Improvement	Cruz das Almas, BA, Brazil
BRS Novo Horizonte	Improvement	Cruz das Almas, BA, Brazil
BRS Tapioqueira	Improvement	Cruz das Almas, BA, Brazil
BRS Verdinha	Improvement	Cruz das Almas, BA, Brazil
Cigana Preta	Local Variety	Amargosa, BA, Brazil
Correntão	Local Variety	Laje, BA, Brazil
Corrente	Local Variety	Oliveira dos Brejinhos, BA, Brazil
Equador72	Local Variety	Cali, Colombia
Eucalipto	Local Variety	Boa Vista do Tupim, BA, Brazil
Nega Maluca	Local Variety	Laje, BA, Brazil
Salangor	Local Variety	Laje, BA, Brazil
Vassoura Preta	Local Variety	Santo Antônio de Jesus, BA, Brazil

Table 2
Description of the planting seasons used to evaluate the flowering and fruit production in 35 cassava genotypes and accumulated rainfall and degree-day data.

Planting seasons	Planting date	Days after planting (DAP)		
		1–120	121–240	241–365
S1	18/Sep/2020	1403.54 °C accumulated 162.40 mm rainfall	2831.48 °C accumulated 477.40 mm rainfall	3944.17 °C accumulated 709.50 mm rainfall
S2	30/Nov/2020	1501.46 °C accumulated 103.80 mm rainfall	2678.46 °C accumulated 560.20 mm rainfall	3980.20 °C accumulated 1004 mm rainfall
S3	09/Feb/2021	1384.46 °C accumulated 318.8 mm rainfall	2471.23 °C accumulated 687.1 mm rainfall	3878.07 °C accumulated 1009.5 mm rainfall
S4	15/May/2021	1090.25 °C accumulated 377 mm rain	2482.17 °C accumulated 958 mm rainfall	3976.17 °C accumulated 1531 mm rain
S5	10/Aug/2021	2867.58 °C accumulated 416 mm rain	3017.46 °C Accumulated 900 mm rainfall	2727.7 °C Accumulated 1530.3 mm rainfall
S6	18/Nov/2021	3028.04 °C accumulated 455.4 mm rain	2789.7 °C accumulated 1074.3 mm rain	2690.1 °C accumulated 1456.2 mm rainfall

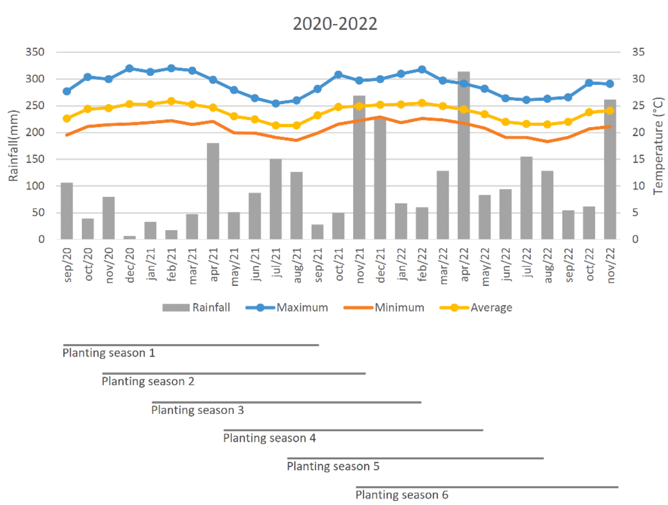

Fig. 1. Average rainfall, minimum, maximum and average monthly temperature, recorded from September 2020 to November 2022 in the experimental area of Embrapa Mandioca e Fruticultura (Cruz das Almas, Brazil, Bahia state).

the value of 13 °C according to the model for cassava cultivation (Mithra et al., 2018).

2.3. Data collection

To collect data, biweekly evaluations were conducted for a period of 12 months, starting from the beginning of flowering until harvest. The assessments included various traits such as: (i) flowering score by branching tier, using a scale from 0 to 3, as described by Souza et al. (2020), where, 0 = plant has no inflorescences, 1 = very poor flowering with only up to two female flowers and/or 20 male flowers, 2 = medium flowering, with up to four female flowers and/or 20–30 male flowers, and 3 = abundant flowering, with more than four female flowers and more than 30 male flowers; (ii) number of days to start first branching (ND1B); (iii) number of branching tiers (NOB); (iv) height of the first branching (H1B). Based on the biweekly flowering data, we calculated the: (1) average flowering score (FS) for each genotype using the formula $FS = \frac{\sum_{i=1}^{q} n_i}{q}$ where n_i represents the flowering score assigned biweekly i, and q corresponds to the number of biweekly evaluations; (2) genotype early flowering weighted score (SW) using the formula $SW = N_{24Q}x2 + N_{23Q}x4\ldots + N_{2Q}x42 + N_{1Q}x44$ .The weight assigned to each genotype is determined by its flowering time, with the highest weight (weight 44) assigned to the access with earlier flowering and the lowest weight (weight 2) assigned to the access with late flowering (Souza et al., 2020).

2.4. Statistical analysis

Initially, an individual analysis of variance was performed for each planting season using the average of the plots in randomized block design. This analysis was carried out to determine if there was genetic variability among the plots and to estimate the genetic and non-genetic parameters for each planting season. Then, a joint analysis of variance was performed for all six planting seasons to obtain the variance components for genetic, planting seasons, and genetic × planting season interaction effects. The model used for this analysis was a mixed-effects model that considered fixed effects for planting season and repetition, and random effects for genotype and the interaction between genotype and planting season.

The phenotypic observations for each genotype i in the jth repetition within planting season k were modeled using the equation: $Y_{ijk} = \mu + s_k + g_i + \left(\frac{r}{es}\right)_{jk} + (g * s)_{ik} + \epsilon_{ijk}$, where μ is the overall mean, s_k is the fixed effect of planting seasons k, g_i is the random effect of genotype i, $(r/es)_{jk}$ is the fixed effect of the repetition j nested in planting seasons k, $(g * s)_{ik}$ is the random effect of the interaction between genotypes and planting seasons, and ϵ_{ijk} is the random residual effect of genotype i in the jth repetition at time k. In both analyses, the effects of genotypes were considered random. However, in the pooled analysis, the effect of planting season was considered fixed, assuming it remained constant across all six planting seasons.

The variance components were estimated using the REML (Restricted Maximum Likelihood) method through the sommer package (Covarrubias-Pazaran, 2016). This package provides two functions: mmer (matrix-based) and mmer2 (formula-based), which allow the user to select one of the four supported ML/REML methods. The best variance structure was determined and then used to estimate the adjusted mean BLUPs (best linear unbiased predictions) and genetic parameters. The Cullis' heritability - h_c^2 (Cullis et al., 2006) was estimated in the analysis of individual variance, and the broad-sense heritability - h_a^2 was estimated using the following equation: $h_a^2 = \frac{\sigma_g^2}{\left(\sigma_g^2 + \frac{\sigma_{gxs}^2}{r} + \frac{\sigma_e^2}{rxk}\right)}$), where σ_g^2 represents the genotypic variance, σ_{gxs}^2 is the variance of the interaction between genotype and planting seasons, σ_e^2 is the variance of the error between plots, r is the number of repetitions, and k is the number of planting seasons. Tukey's test ($p \leq 0.05$) was used to analyze the differences between the overall means of the treatments and for each genotype. This was done using the rstatix package (Kassambara, 2023). The data were analyzed using the R program version 4.1.2 (R Development Core Team, 2021).

2.5. Principal component analysis

To analyze the data on flowering traits across different planting seasons and genotypes, we employed principal component analysis (PCA) using the kinship matrix for each genotype. The K-means function was then utilized to group individuals with similar behavior in relation to the characteristics associated with flowering in each planting season.

2.6. Analysis of climatic variables

To investigate the relationship between flowering scores and climatic conditions, we obtained climatic data for each biweekly evaluation, including average temperature, accumulated degree days, and rainfall. We then correlated these data with the average flowering scores. Using Pearson's correlation coefficient (r), we estimated the relationship between biweekly flowering data and climatic variables. All analyses were performed using the R program version 4.1.2 (R Development Core Team, 2021).

3. Results

3.1. Multivariate analysis of variance and heritability

The h_a^2 and h_c^2 values ranged from medium to high in all planting seasons, indicating a strong genetic effect on the expression of phenotypes. For planting seasons 1 to 4, the values ranged from 0.38 (FS) to 0.89 (H1B). Planting seasons 5 and 6 had high heritability values for all traits, ranging from 0.80 (FS) to 0.98 (SW) (Table 3). In the joint analysis, the effects of σ_g^2 and σ_s^2 were similar for most traits, while the σ_e^2 ranged from 1.95 (H1B) to 12.09 (SW) times the σ_g^2. The variance of the genotype × planting season interaction (σ_{gxs}^2) showed smaller values, particularly for the traits ND1B, NOB, H1B, and SW, compared to σ_g^2 and σ_e^2.

Fig. 2 presents the averages of the traits evaluated in the six planting seasons (S1, S2, S3, S4, S5, and S6). For ND1B, significant differences were observed among the evaluated planting seasons. S1, S5, and S6 had the latest flowering, occurring at approximately 200 to 230 days after planting (DAP), while S2 and S3 allowed for earlier flowering, with an average of 150 days for flowering. S4 was intermediate, flowering at approximately 180 days (Fig. 2).

Table 3
Broad-sense (h_a^2) and *Cullis* (h_c^2) heritability estimates and summary of analysis of variance by planting season and joint analysis for flowering related-traits in cassava, evaluated in cassava genotypes in six planting seasons from September 2020 to May 2022.

Variance components / parameters	Planting Season	Traits				
		ND1B	NOB	H1B	FS	SW
		Multivariate analysis of variance by planting season				
σ_g^2	S1	2548.56	0.50	1359.24	0.01	6.07
	S2	935.24	0.62	1405.05	0.01	6.24
	S3	1469.89	1.29	1384.13	0.02	7.37
	S4	920.36	0.97	647.28	0.02	10.31
	S5	2577.14	1.31	2478.19	0.03	20.13
	S6	3424.28	1.11	3504.42	0.02	20.2
σ_e^2	S1	2349.41	1.68	1795.41	0.04	10.13
	S2	1445.08	2.14	1560.12	0.04	11.64
	S3	1079.71	2.54	803.80	0.04	11.79
	S4	3184.39	2.93	756.21	0.05	21.80
	S5	747.75	0.95	893.27	0.02	9.62
	S6	2033.70	1.81	806.64	0.03	9.94
h_a^2	S1	0.76	0.47	0.69	0.38	0.64
	S2	0.66	0.47	0.73	0.50	0.62
	S3	0.80	0.60	0.84	0.55	0.65
	S4	0.46	0.50	0.72	0.62	0.59
	S5	0.91	0.81	0.89	0.80	0.86
	S6	0.83	0.65	0.93	0.68	0.86
h_c^2	S1	0.88	0.87	0.88	0.86	0.88
	S2	0.68	0.89	0.88	0.92	0.88
	S3	0.79	0.95	0.88	0.93	0.90
	S4	0.67	0.93	0.75	0.96	0.93
	S5	0.98	0.97	0.96	0.98	0.98
	S6	0.95	0.96	0.97	0.97	0.98
		Joint multivariate analysis of variance				
σ_g^2	–	1218.39	0.48	1325.31	0.01	7.42
σ_s^2	–	948.33	0.62	703.17	0.01	2.51
σ_{gxs}^2	–	743.04	0.50	399.99	0.01	4.03
σ_e^2	–	1747.79	1.95	1213.41	0.03	12.09
h_a^2	–	0.82	0.66	0.89	0.66	0.81
Average	–	191.54	3.62	128.82	0.46	8.24

ND1B: number of days to branch initiation, NOB: number of branching tiers, H1B: height of first branch, FS: mean scores and SW: weighted flowering score.

There was a significant difference among the six evaluated planting seasons for the genotype early flowering weighted score (SW). S1 showed similar behavior to S5, with an average of approximately 4.75 and 4.88, respectively (Fig. 2). S3 and S4 presented the highest averages, with values of 10.4 and 9.5 SW, respectively, indicating earlier and more constant flowering throughout the crop cycle. The mean values for the average flowering score (FS) were similar for S2, S3, S4, and S6, ranging from 0.54 to 0.46. However, S1 and S5 had significantly lower mean values of flowering (0.35) compared to the other planting seasons.

The late flowering observed in S1 and S6 resulted in lower NOB compared to the other evaluated planting seasons (Fig. 2). S3 had the highest average NOB of 4.67, differing significantly from S1, S5, and S6 (3.24, 2.57, and 2.91, respectively). Despite the late flowering of S1, the development of the genotypes in this planting season was similar compared to the others for the H1B trait. S4 differed significantly from the other planting seasons, presenting lower plants with an average H1B of approximately 70 cm (Fig. 2).

3.2. Phenotypic performance of cassava genotypes for flowering related-traits in different planting times

Most genotypes, including 2011-34-41, BGM-1511, BGM-0707, BGM-0702, BGM-1118, Equador-72, BGM-0598, BRS Verdinha, Salangor, Vassoura Preta, BGM-0593, BGM-0590, BRS Caipira, and BGM-0062, exhibited early flowering in planting seasons S2 and S3, while Correntão and BRS CS01 flowered earlier in S4 (Fig. S1). However, the flowering initiation of BGM-1128 and Corrente remained unchanged throughout the six planting seasons, with an average of 130 and 260 DAP, respectively. Meanwhile, genotypes 2011-34-45, BGM-1722, Cigana, and Salangor flowered later in S6, at an average of 270 DAP.

In general, most genotypes did not show significant differences in NOB across the six planting seasons (Fig. S2). However, BGM-0195, BGM-0598, Equador-72, and Salangor exhibited higher NOB when grown in S2 (with an average of 4.40, 5.53, 5.15, and 3.80, respectively). In S3, the genotypes BRS Kiriris, BRS Novo Horizonte, BRS Verdinha, and Cigana had the highest NOB values (ranging from 5 to 8).

Most genotypes had low FS values throughout the crop cycle. However, S1 had the lowest flowering, while S2 and S3 showed the highest flowering for most genotypes. Unlike most of the other genotypes BGM-0707 and BGM1511 showed high FS in S4 (Fig. S3). Conversely, BRS-Caipira and Cigana had lower FS values in S6, averaging 0.38 and 0.33, respectively. In contrast, genotypes 2011-34-69, BGM-0062, BGM-0590, BGM-0593, BGM-0702, BGM-0935, BGM-1128, BGM-1722, BGM-1760, BRS Dourada, BRS Formosa, BRS CS01, Eucalipto, Nega Maluca, Salangor, and Vassoura Preta were not influenced by planting seasons.

On average, BGM-1760, BGM-1511, and BRS Kiriris had the highest best linear unbiased predictions (BLUPs) for SW, indicating earlier and more abundant flowering (Fig. S4). The greatest variation in SW among the six planting seasons was observed in genotypes BGM-0598, BGM-0707, BGM-1760, BRS Kiriris, BRS Novo Horizonte, BRS Verdinha, Cigana, and Equador72. In contrast, BGM-0593, BGM-0935, BGM-1128, BGM-1722, Nega Maluca, and Vassoura Preta showed no significant differences across the different planting seasons evaluated.

The study found significant differences in H1B among genotypes including 2011-34-41, BGM0195, BGM-0593, BGM-0630, BGM-1760, BRS Caipira, BRS Formosa, BRS Gema de Ovo, BRS CS01, Cigana, and Equador72 during at least two of the six planting seasons (Fig. S5). The genotypes 2011-34-45, 2011-34-69, BGM-0062, BRS- Dourada, BRS-Novo Horizonte, Correntão, and Vassoura Preta displayed the largest variations in H1B among the planting seasons. Although other

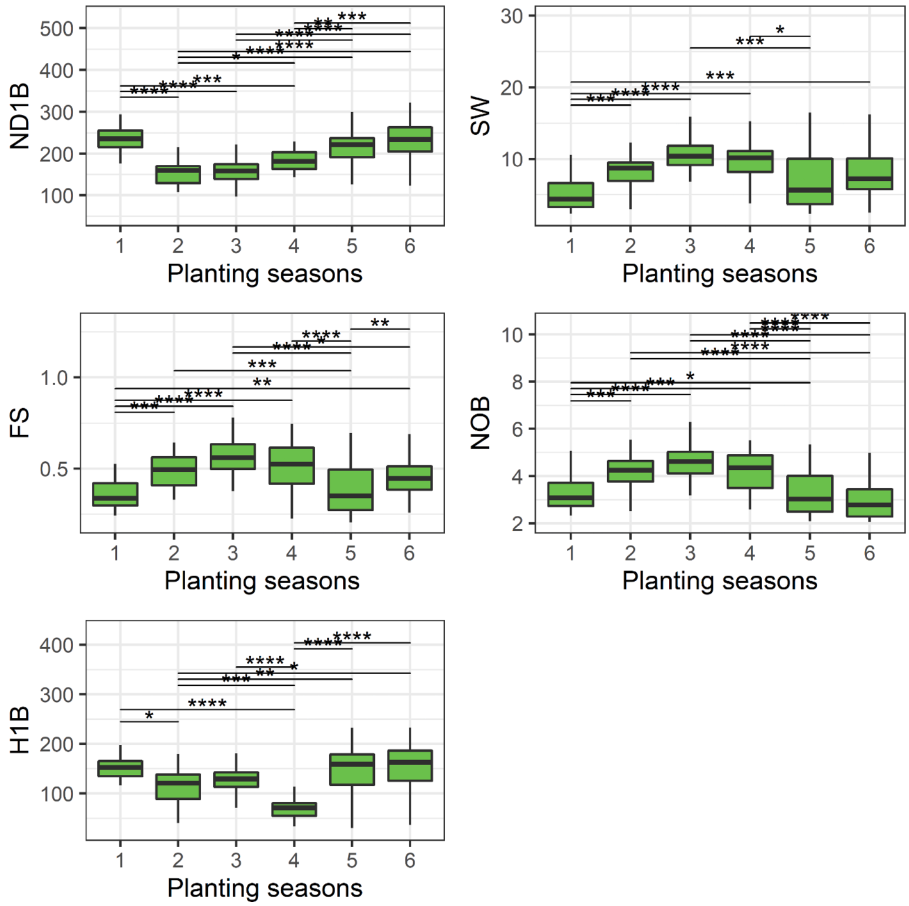

Fig. 2. Phenotypic means of 35 cassava accessions evaluated for several traits, including the number of days to start first branching (ND1B), genotype early flowering weighted score (SW), average flowering score (FS), number of branching tiers (NOB), and height of first branch (H1B), across six planting seasons. Upper lines represent Tukey's test ($p \leq 0.05$).

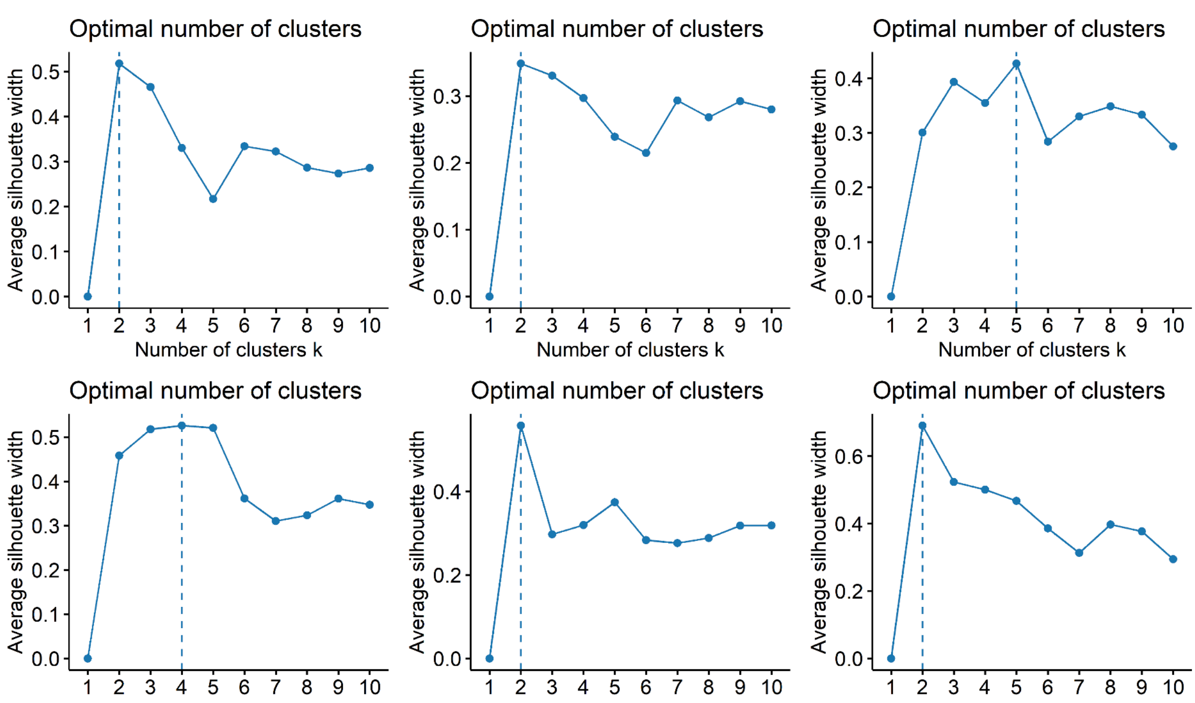

Fig. 3. K-means method used to identify the most likely number of clusters to represent the cassava genotype grouping according to the flowering traits.

genotypes showed some variation in H1B between planting seasons, the differences were not statistically significant.

3.3. Principal component analysis

The K-means method was utilized to classify the cassava genotypes into different groups based on their traits, with two groups (S1, S4, S5, and S6), three groups (S2), and four groups (S3) being identified (Fig. 3). The clones were then grouped in the PCA analysis according to these previous clustering (Fig. 4), with the first two principal components capturing a high percentage of the total variation associated with the phenotypic data of the cassava germplasm for each group (Fig. 4). Notably, high correlations were observed between ND1B and H1B traits, particularly for S1, S2, S3, S4, and S5. Both traits were located in the same quartile and provided opposite angulation to the others evaluated. Additionally, the traits FS, SW, and NOB were also found to be correlated in all six evaluated planting seasons (Fig. 4).

Further analysis revealed that the clusters for S1 were well separated in the biplot, with Group 2 showing a high association with desirable flowering traits, such as early and abundant flowering, with early branch plants (Figs. 5 and 6). For S2, which grouped the cassava genotypes into three clusters, Group 2 exhibited higher correlation with the traits SW, FS, and NOB (Figs. 5 and 6).

In the case of S3, Group 4 displayed high H1B and ND1B, while higher FS and NOB were mainly observed in Group 2 (Figs. 5 and 6). Similarly, for S5, Groups 1 and 2 displayed well-represented correlations between SW, FS, and NOB (Group 2) and H1B, ND1B (Group 1) (Figs. 5 and 6). Lastly, for S6, higher correlations were found for H1B and ND1B by Group 1 and NOB, SW, and FS by Group 2.

3.4. Effect of climatic factors on flowering induction

The pattern of flowering across the six planting seasons was observed in relation to climatic variables such as average temperature, accumulated degree days, and rainfall. Figs. 7–9 present the average biweekly flowering, average temperature, and rainfall respectively. The highest peak of flowering was observed during a period of increasing average temperature up to 23.43 °C (Fig. 7) with consistent rainfall between 100 and 250 mm (Fig. 9), and approximately 200 cumulative degree days (Fig. 8). In general, the highest flowering peaks occurred between 240 and 360 DAP for S1 and 240 to 360 DAP for S2, S3, S4 and S5, with the exception of S6, which showed greater flowering a little before 240 DAP. Despite providing late flowering (approximately four months), S2 showed higher flowering rates compared to the others.

The correlation between ND1B and mean temperature was significant and of median magnitude ($r = -0.59$, 0.50, -0.46 for S1, S4, and S6, respectively), indicating that temperature can affect early flowering depending on the planting seasons (Fig. 10). There was no significant correlation between rainfall and the onset of flowering. However, for cumulative degree days, significant and positive correlations were observed for S2, S3, S4, and S6 (r = 0.59, 0.53, 0.58, and 0.52, respectively).

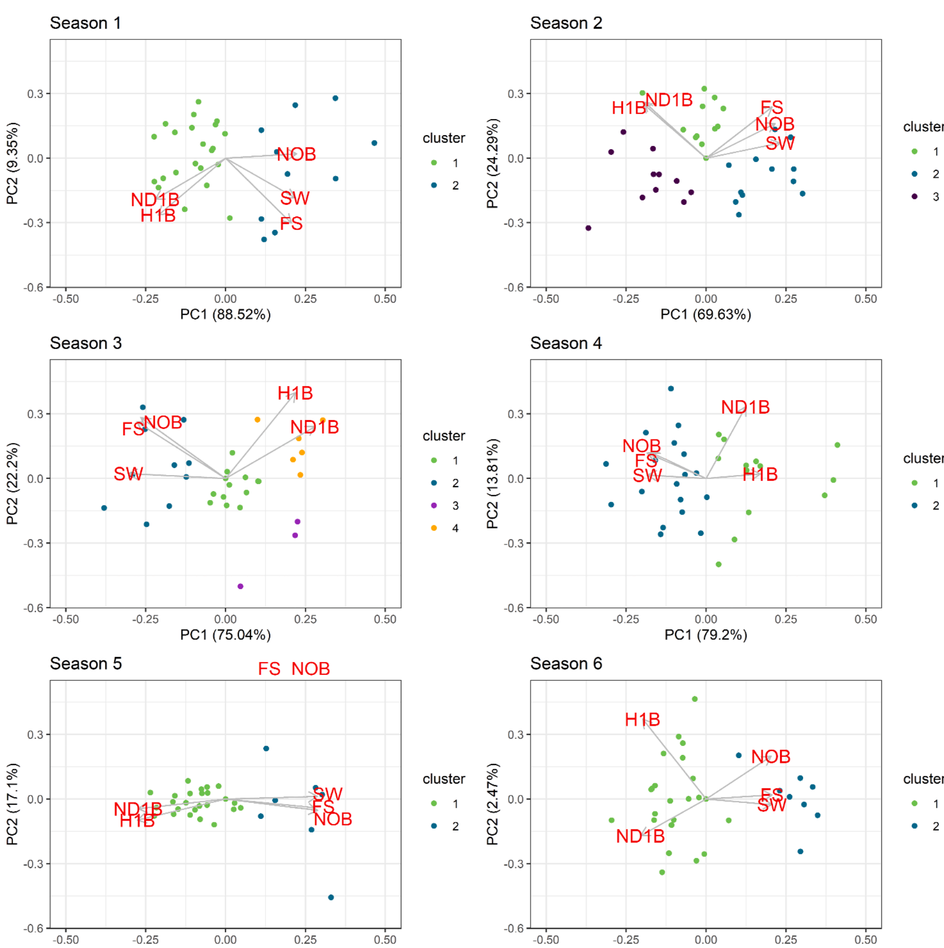

Fig. 4. Principal component analysis (PCA) of flowering-related traits of 35 cassava genotypes evaluated in six planting seasons. ND1B: number of days to start first branching; SW: genotype early flowering weighted score; FS: average flowering score; NOB: number of branching tiers; H1B: height of the first branch.

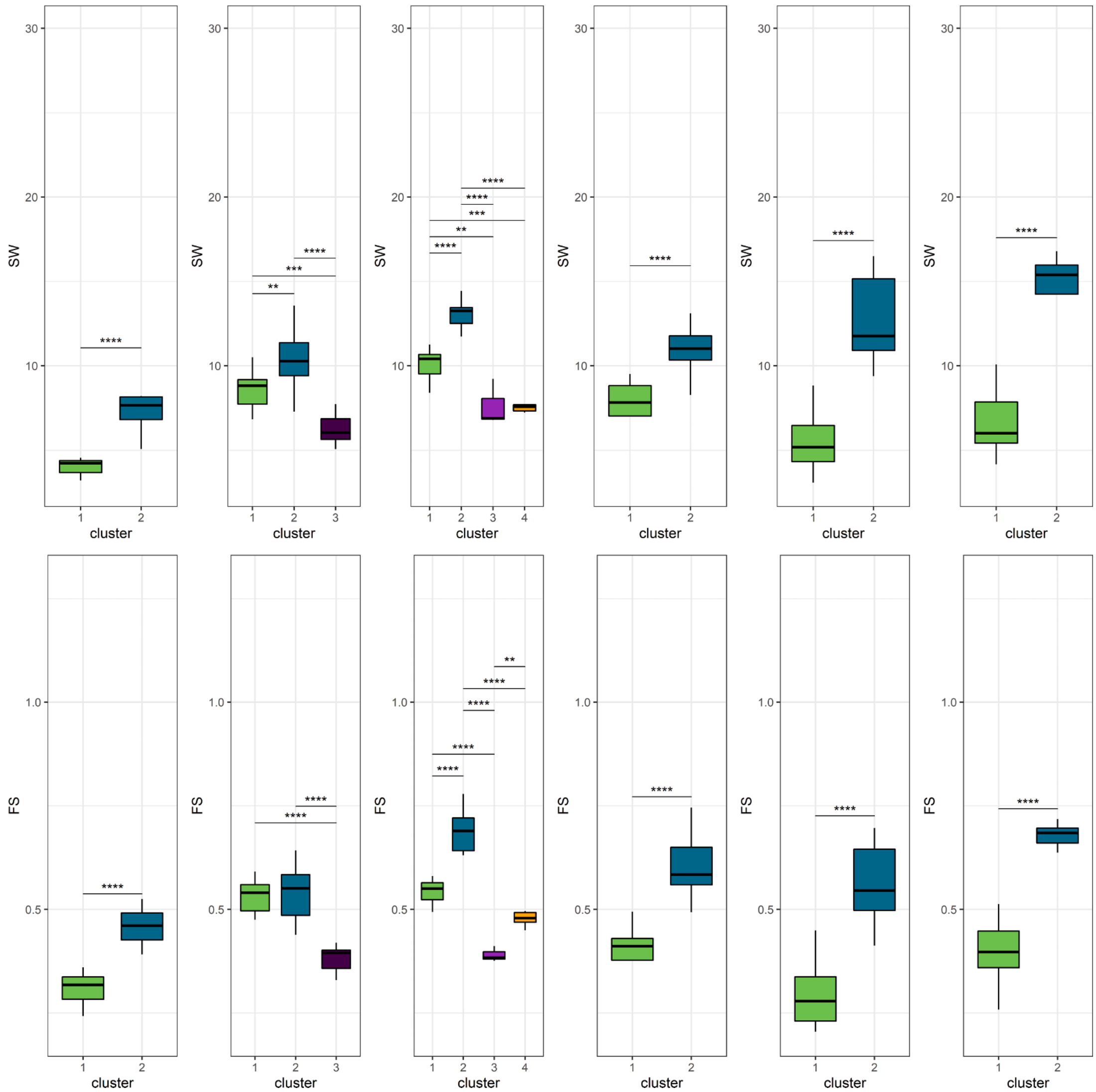

Fig. 5. Boxplots for the traits genotype early flowering weighted score (SW) and average flowering score (FS), considering the clusters obtained by the k-means method from principal component analysis (PCA) of 35 cassava genotypes, for six planting seasons.

4. Discussion

4.1. Influence of planting time on cassava flowering

The study found that the planting seasons significantly affected the onset and intensity of flowering in cassava. The experiment evaluated six planting seasons: S1: September/2020 (end of rainy cycle), S2: November/2020 (dry period), S3: February/2021 (dry period), S4: May/2021 (beginning of rainy period), S5: August/2021 (decrease of rainy period), and S6: November/2021 (dry period). Cassava accessions planted in S1 and S5 showed a lower intensity of flowering that occurred, on average, 260 days after planting (DAP). Hence, planting at the end of the rainy planting season is unfavorable if early flowering is desired. On the other hand, planting in dry periods (S2 and S3) and the beginning of the rains (S4) resulted in an earlier onset of flowering, with average flowering times of 160, 150, and 180 days after planting, respectively. Additionally, S3 and S4 displayed a greater intensity of flowering throughout the cycle.

To maximize the chances of obtaining numerous progenies and allow parents with shy flowering to participate in crosses, it is important to determine the best planting time to achieve a specific pattern of flowering according to different climatic conditions in target environments. It is crucial to recognize that the practical implications of the findings of this study go beyond purely scientific understanding. Understanding how different planting times influence flowering holds the potential to significantly impact agriculture and food production (DaMatta et al., 2010). The capability to control the timing and intensity of flowering empowers farmers to optimize and plan harvests more efficiently. Moreover, it provides the means to manipulate the reproductive process, leading to the acquisition of desirable traits in cassava varieties and driving forward the genetic enhancement of the crop (Ceballos et al., 2020; Andrade et al., 2019).

In general, the most favorable times for flowering in cassava are related to longer days and lower temperatures (22 °C) due to the stimulation of two FT homologs, MeFT1 and MeFT2 (Adeyemo et al., 2018). In this experiment, S2–S4 showed similar values for mean flowering, although S2, with mean temperatures around 23 °C, resulted in a greater increase in flowering. However, it's important to emphasize that the complexity of flowering regulation goes beyond climatic conditions. Optimal flowering periods are also influenced by endogenous and exogenous factors such as photoperiod, the presence of nitrates, and plant growth regulators (Srikanth et al., 2011; Romera et al., 2014; Olas et al., 2019; Oluwasanya et al., 2021) which were not captured in this study. Future investigations can delve deeper into the interactions among the mentioned factors and how they relate to different planting times, aiming to further enhance agricultural management strategies

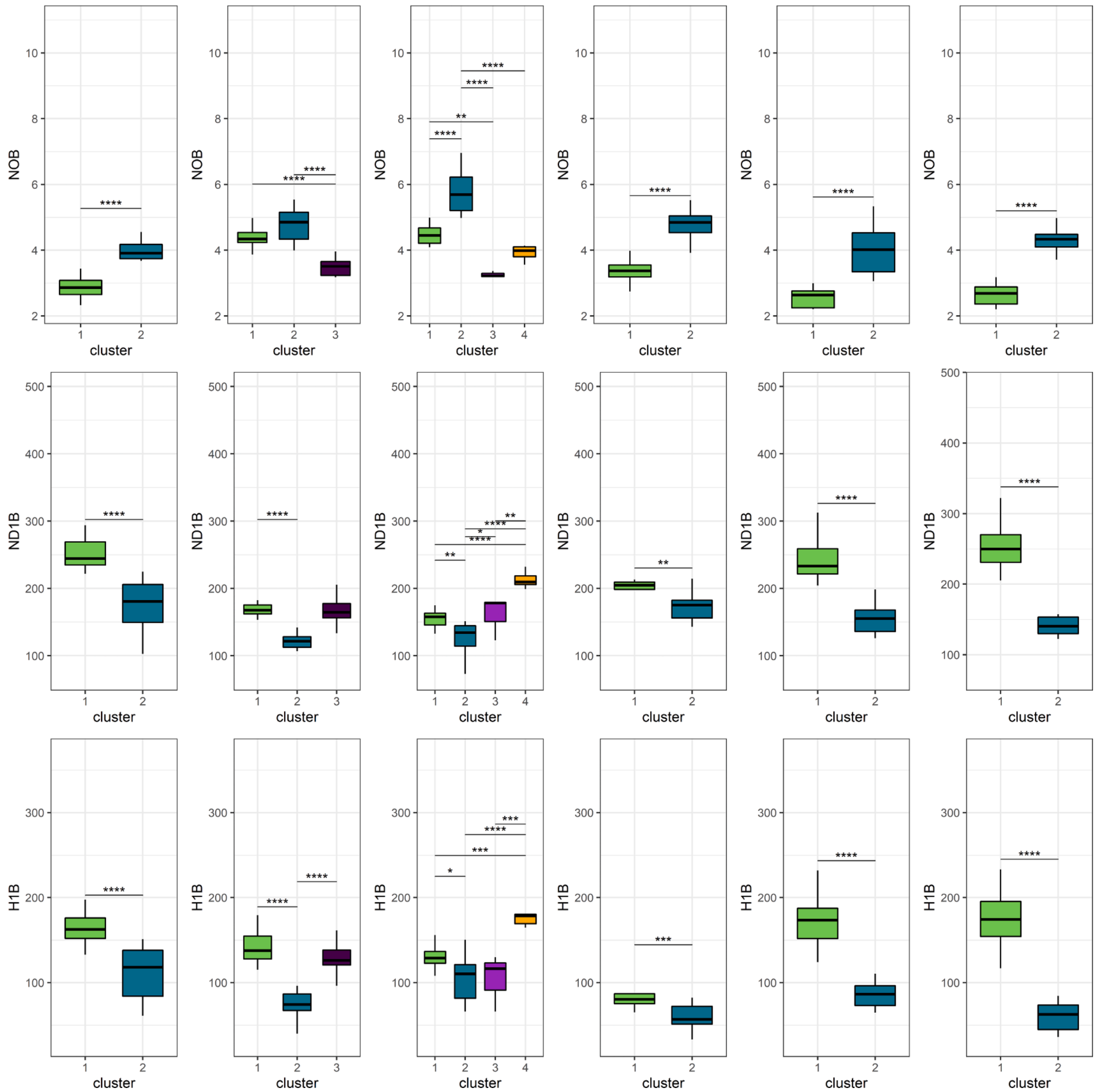

Fig. 6. Boxplots for the traits: number of branching tiers (NOB), number of days to start first branching (ND1B) and height of the first branch (H1B), considering the clusters obtained by the k-means method from principal component analysis (PCA) of 35 cassava genotypes, for six planting seasons.

and the selection of more suitable varieties for various scenarios.

4.2. Climatic factors and flowering intensity in cassava

The impact of climatic variables on cassava flowering was examined by correlating temperature, accumulated degree days (ADD), and accumulated rainfall data with biweekly evaluations of flowering. Higher rainfall (100–220 mm) in the previous fortnight led to greater flowering. For instance, S2 exhibited higher flowering rates in the period when there was a large accumulation of rainfall. On the other hand, S1 displayed lower intensity and late onset of flowering as there was less accumulated rainfall throughout the evaluation (~160 mm, 1–120 DAP). Similar results were obtained by Zhang et al. (2018), who found that rainfall had a greater impact on altering flowering dates than temperature, especially for late flowering species in the Guia Hill region, Macau. Lambert et al. (2010) also reported similar results, indicating that higher rainfall volumes induced flowering in *Erythronium grandiflorum*.

Rainfall is an important factor in inducing flowering in tropical or subtropical regions where the variation in rainfall is greater than that of temperature (Brearley et al., 2007; Bai et al., 2009;). Moreover, the dry period after high rainfall rates allows the relative humidity of the air to favor the development of fruits and seeds (Olani and Fikre, 2010). The availability of water for plants is influenced by a multitude of factors, with rainfall distribution playing a crucial role in shaping soil water content. When soil moisture is low, it encourages the growth of roots. However, as soil moisture decreases further, there is a noticeable drop in net photosynthetic rates. This reduction has a detrimental impact on flowering and maturation stages, and in certain crops, it can even lead to decreased productivity (Galon et al., 2010).

The ideal temperature and the presence of sunlight facilitate cross-pollination and seed formation in plants, thus indicating the importance of hydrothermal conditions in determining the flowering time (Olani and Fikre, 2010). Genetic variability for flowering-related traits exists in cassava, and climatic conditions induce adaptive responses of genotypes (Souza et al., 2020).

S1 and S6 showed lower flowering rates in periods of higher temperatures throughout the evaluations, which coincided with a more prolonged dry period as the experiment was set up without supplemental irrigation. Bandeira and Sousa et al. (2021) emphasized the role of environmental factors in the efficiency of the sexual reproduction of cassava, indicating that the ideal temperature for seed production varies between 22.5 and 24 °C, with a reduction of 21 % in seed production reported above this range.

Temperatures below 17 °C or above 37 °C inhibit the leaf growth of cassava, while temperatures below 20 °C decrease the photosynthetic

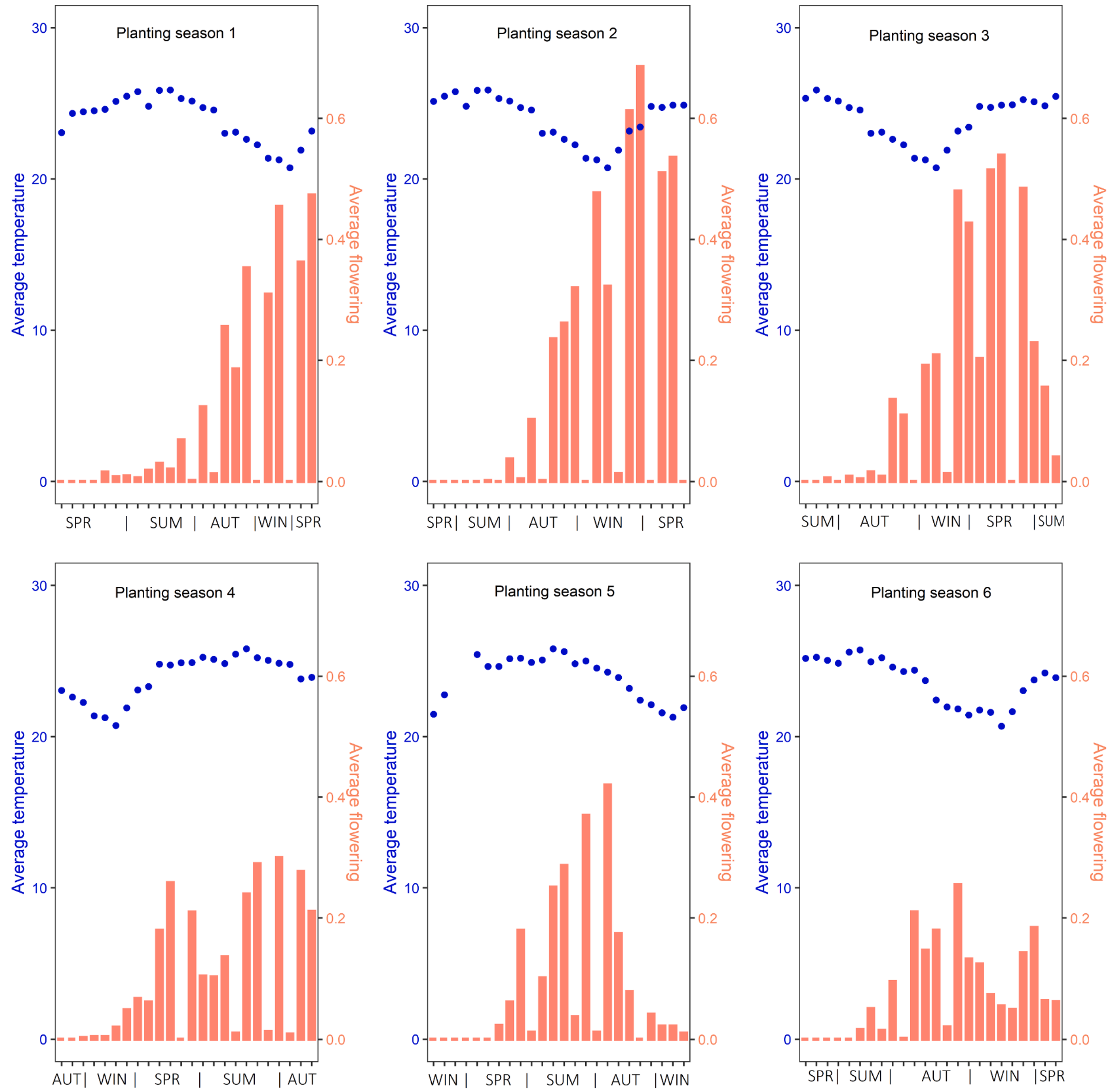

Fig. 7. Average performance of flowering taking into account the average temperature, during the six planting seasons, for the four seasons (biweekly data). The letters correspond to the seasons, SPR: spring; SUM: summer; AUT: autumn, WIN: winter.

rate (Keating and Evenson, 1979). The highest percentage of plants that flowered occurred after autumn and winter, which in Brazil occurs between March and October (240 to 345 DAP). Although they are not very defined planting seasons, they show higher levels of rainfall during the period in the Recôncavo of Bahia region (Brazil, Bahia state), corresponding with increased rainfall and decreased temperatures compared to the rest of the year. Multiple inflorescence-producing species like cassava are affected more by higher temperatures compared to species with a single inflorescence (Liu et al., 2012).

The increasing environmental changes observed worldwide have the potential to significantly impact flowering phenology. Reduced flowering in some crops directly affects seed quality and yield, as well as the interaction between plants and pollinators (Tun et al., 2021). The climate is changing rapidly, and greenhouse gas emissions are leading to successive changes in temperature, precipitation, and drought, which directly affect floral phenology (Ma et al., 2014; Zhang et al., 2017).

As the climate undergoes changes, there is the potential for shifts in seasonal patterns, affecting factors such as the duration of days and nights. For numerous plants, cassava included, photoperiod assumes a pivotal role in determining the optimal timing for flowering. At the molecular level, genes such as FT (Flowering Locus T), SOC1 (Suppressor of Overexpression of Constans 1), and LFY (Leafy) are intricately associated with cassava's flowering signaling and are particularly responsive to variations in photoperiod (Collani et al., 2019; Wigge et al., 2005). Consequently, alterations in seasons and day length have the capacity to disrupt cassava's photoperiodic response, thereby impacting its flowering dynamics (Pineda et al., 2020b; Hyde and Setter, 2022).

Furthermore, temperature plays a fundamental role in initiating the physiological processes that culminate in flowering. In the case of cassava, moderate temperatures (ranging from 22 °C to 34 °C) are conducive to flowering, as corroborated by this study and prior research (Adeyemo et al., 2018; Bandeira and Sousa et al., 2021). Temperature variations, whether consistent or fluctuating, have the potential to directly influence the phenology of flowering. This, in turn, can lead to either earlier or delayed flowering onset, disrupting synchronization among genotypes and ultimately resulting in the absence of fruit production (Souza et al., 2020).

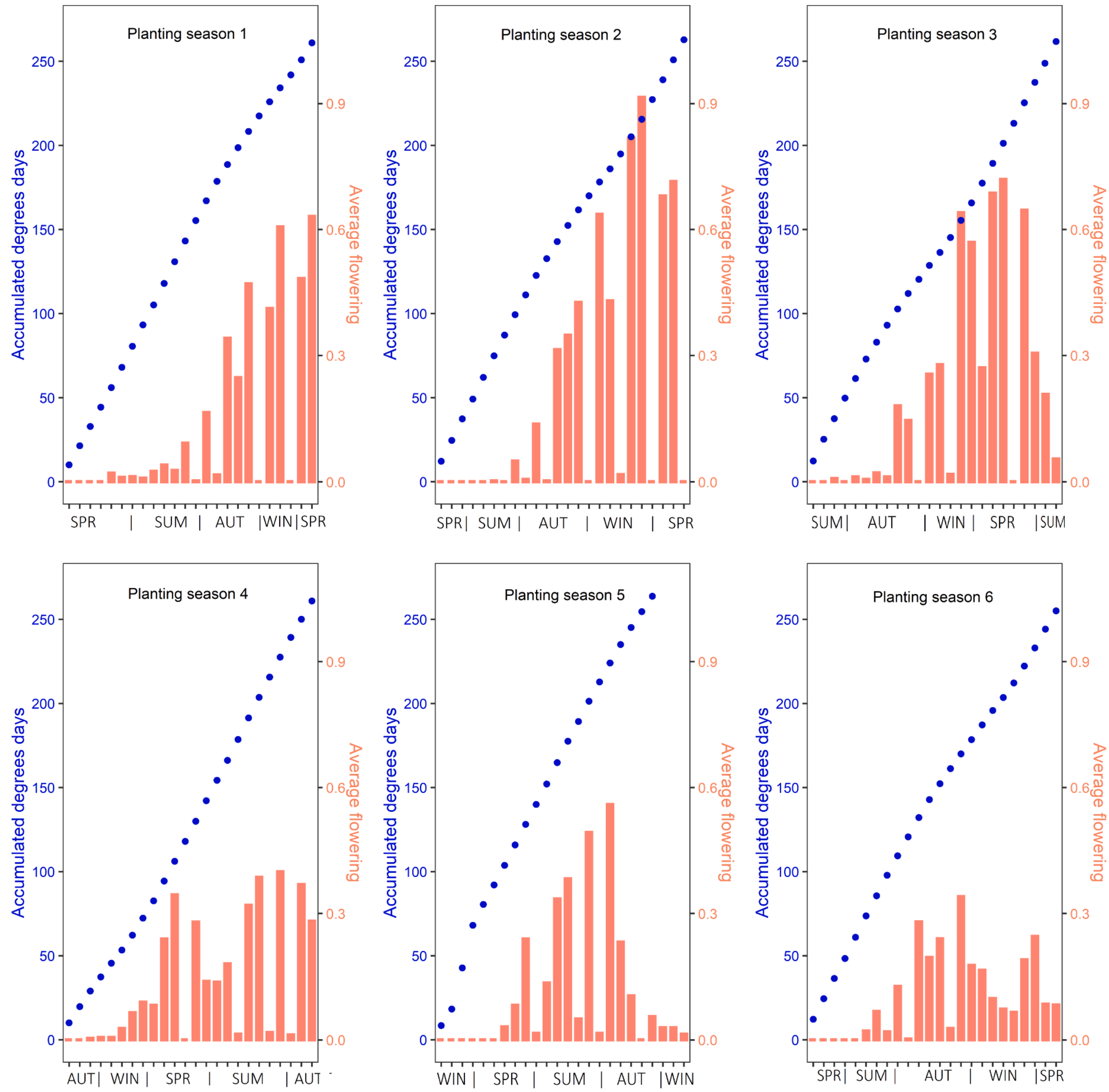

Fig. 8. Average performance of flowering taking into account the accumulated degree days, during the evaluated planting seasons, for the four seasons (biweekly data). The letters correspond to the seasons, SPR: spring; SUM: summer; AUT: autumn, WIN: winter.

4.3. Prospects for genotype clustering for flowering optimization in cassava breeding

Inducing and synchronizing flowering in cassava crossing blocks remains a significant challenge, particularly for including parents with reduced flowering and generating multiple breeding populations. Researchers have explored the impact of planting seasons and environmental factors on regulating flowering time in cassava to optimize flowering among different cassava parents. Understanding the influences of these factors could help improve our understanding of the flowering phenology of the crop and enable synchronization and optimization of flowering among different cassava parents.

In the Recôncavo of Bahia region, planting during S3 characterized by the dry period (1–120DAP) resulted in earlier flowering (~ at 2MAP), with significantly higher SW (52.40; 60 % compared to S1 and S5, which showed SW of 4.95; 4.88) and FS values (53; 60.71 % compared to FS 0.35; 0.22, respectively). These results suggest that choosing the appropriate planting seasons can have a significant impact on cassava flowering and should be considered when optimizing flowering among different cassava parents.

The genetic gain of breeders is affected by the time required for progeny generation, as shown by the equation $R_t = \frac{ir\sigma_a}{t}$, where R_t is the genetic gain over time, i is the selection intensity, r is the selection accuracy, σ_a is the genetic variance of the population, and t is the number of years per breeding cycle. Reducing the time required for progeny generation can increase genetic gain, as reducing breeding cycle time has a potentially greater impact than increasing heritability or selection intensity (Cobb et al., 2019). Although the increase in flowering rate is not directly related to the reduction of breeding cycle, generating progenies in sufficient numbers and in the shortest possible time can accelerate selection cycles of segregant populations. This can help advance selection cycles earlier or even outside of the normal planting season.

5. Final remarks

The study sheds light on the importance of understanding the influences of planting seasons and their environmental variations on the regulation of flowering time in cassava, a major problem for the species.

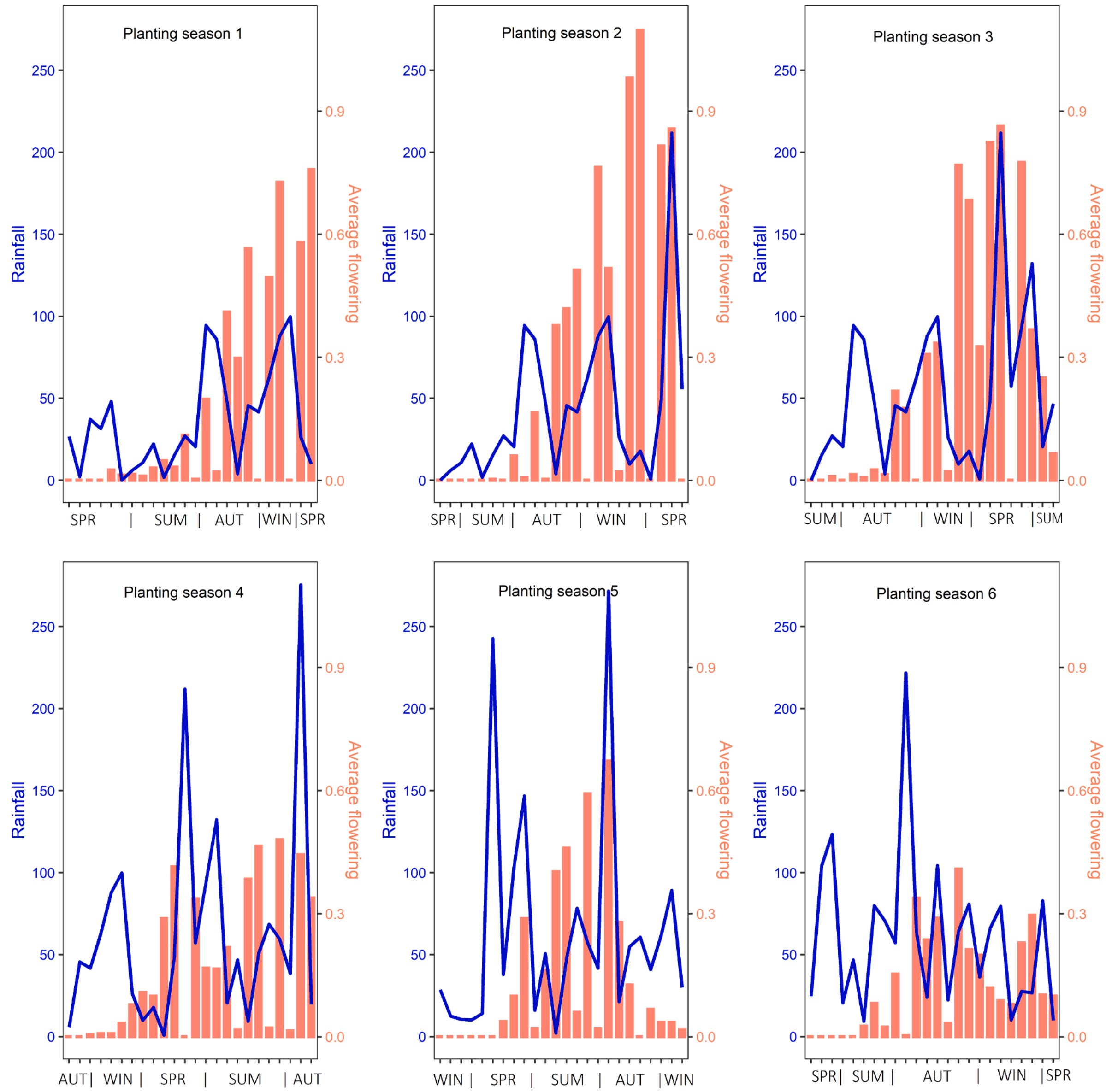

Fig. 9. Average performance of flowering taking into account the rainfall, during the evaluated planting seasons, for the four seasons (biweekly data). The letters correspond to the seasons, SPR: spring; SUM: summer; AUT: autumn, WIN: winter.

The study found that planting in S3 in the Recôncavo of Bahia region (Brazil, Bahia state) provided earlier flowering and similar flowering averages compared to S2, making it the optimal planting time for cross blocks. This information can be useful for cassava breeding programs to plan the crossing fields and reduce the time required to achieve the desired goals. Overall, the study contributes to the improvement of cassava breeding programs and ultimately benefits farmers and consumers who rely on this crop for their livelihood and food security.

Suplementary figures

Fig. S1. Best linear unbiased prediction (BLUP) of the number of days to start first branching (ND1B), for six planting seasons. Upper lines represent significant differences ($p \leq 0.05$) by Tukey test.

Fig. S2. Best linear unbiased prediction (BLUP) of the number of branching levels (NOB), for six planting seasons. Upper lines represent significant differences ($p \leq 0.05$) by Tukey test.

Fig. S3. Best linear unbiased prediction (BLUP) of the average flowering score (FS), for six planting seasons. Upper lines represent significant differences ($p \leq 0.05$) by Tukey test.

Fig. S4. Best linear unbiased prediction (BLUP) of the genotype early flowering weighted score (SW), for six planting seasons. Upper lines represent significant differences ($p \leq 0.05$) by Tukey test.

Fig. S5. Best linear unbiased prediction (BLUP) of the number of branching levels (H1B), for six planting seasons. Upper lines represent significant differences ($p \leq 0.05$) by Tukey test.

CRediT authorship contribution statement

Alexandra Damasceno Santos: Investigation, Data curation, Writing – original draft, Writing – review & editing. **Massaine Bandeira e Sousa:** Formal analysis, Writing – original draft, Writing – review & editing. **Alfredo Augusto Cunha Alves:** Conceptualization, Investigation, Formal analysis, Writing – review & editing. **Eder Jorge de Oliveira:** Conceptualization, Investigation, Formal analysis, Writing – review & editing.

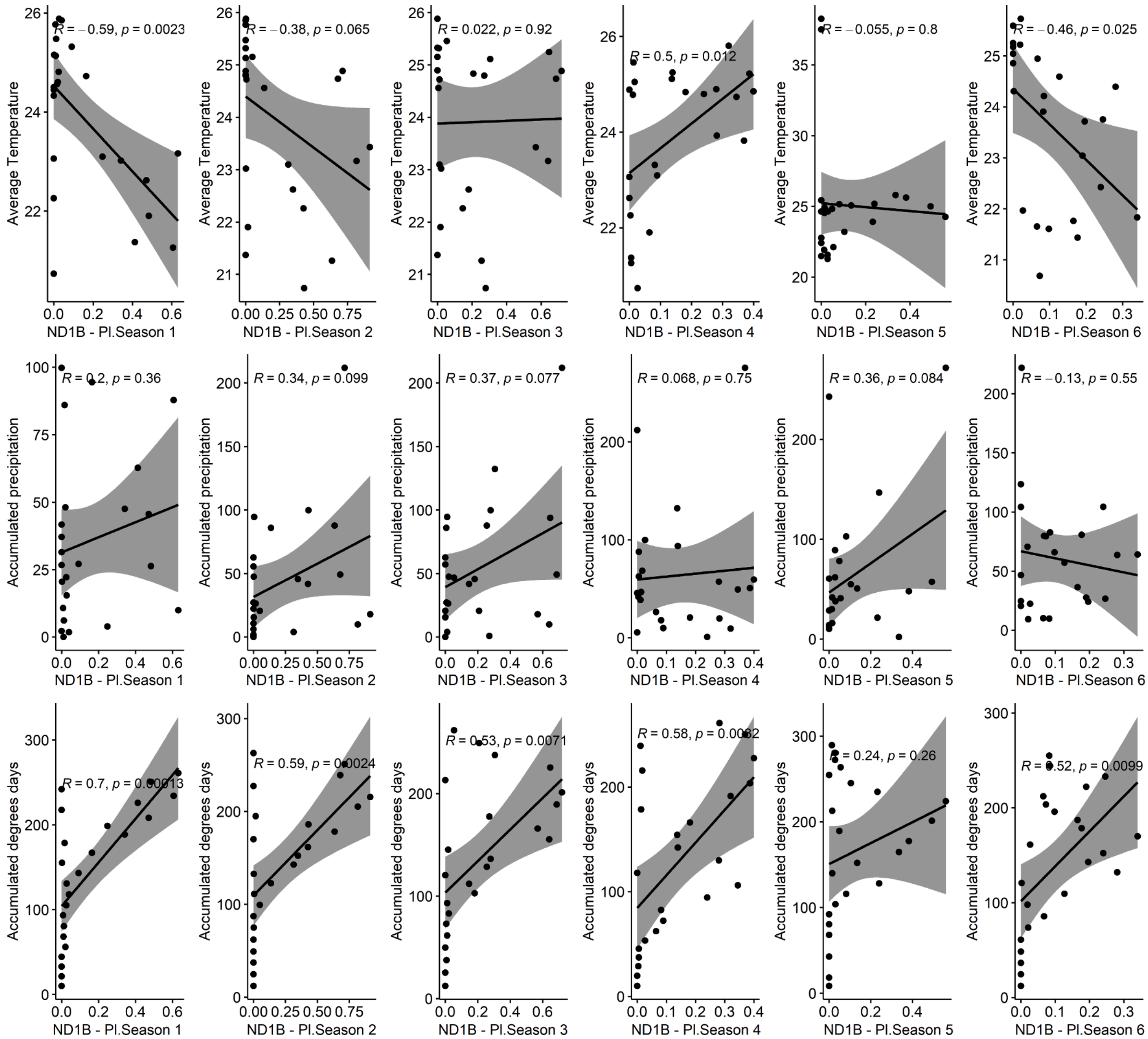

Fig. 10. Correlation between temperature, rainfall, and accumulated degree days and the number of days to onset of first branching (ND1B). Pl.Season: planting seasons.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Eder Jorge de Oliveira reports financial support was provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Fundação de Amparo à Pesquisa do Estado da Bahia, UK Foreign, Commonwealth & Development Office and Bill & Melinda Gates Foundation.

Data availability

Data will be made available on request.

Acknowledgments

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2023.112498.

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