



Article Adaptability and Stability Analyses of Improved Strawberry Genotypes for Tropical Climate

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Abstract: Strawberries are grown worldwide, and the fruit is known for its flavor, pleasant aroma, and the presence of important nutraceutical compounds. Under temperate conditions, the species is octaploid and presents a complex inheritance. Exploring polyploidy in varietal crosses is the main alternative to developing genotypes of high-temperature regions; thus, breeding programs must evaluate the interaction based on parameters, such as the heritability, stability, easy propagation, and adaptability to different soil and climate variations. To estimate the stability and temporal adaptability of pre-selected triple hybrids of day-neutral strawberries, thirty-six experimental genotypes, three commercial genotypes ('Albion,' 'Monterey,' and 'Dover'), and four single hybrids ('RVFS07,' (RVFS06,' 'RVDA11,' and 'RVCA16') were evaluated in a protected cultivation from August to February under tropical climate conditions (southern hemisphere) using the mixed linear model (MLM). The genotypes RVFS07M-34, RVFS07M-24, RCDA11M-04, RVFS07M-154, RVFS07M-36, RVFS07M-33, RVFS07M-80, RVFS07M-10, RVDA11M-21, RVDA11M-13, and RVFS06AL-132 had the highest values of total fruit mass, adaptability, and stability. The mean predicted genotypic values of the selected genotypes was 138% higher than the mean of the controls. Therefore, these genotypes have the potential to be released as cultivars.

Keywords: Fragaria × ananassa Duch; heritability; propagation; low cold requirement; neutral photoperiod

1. Introduction

Strawberry production in the southern hemisphere is totally dependent on cultivars developed by breeding programs in the United States and Spain [1]. In addition to low adaptability and stability in tropical regions, these cultivars increase production costs, since commercial nursery plants are multiplied and imported in dollars [2]. Another recurring problem concerns the physiological and phytosanitary quality, where several crops were affected by pathogens that did not exist in the producing regions, and they were probably introduced by imported nursery plants.

Climate change has promoted an increase in the temperature and in the concentration of carbon dioxide (CO_2) in the atmosphere [3,4]. High temperatures limit the culture, as strawberry plants require cold hours for floral induction. Thus, it can negatively affect the yield of cultivated plants, interfering with the balance of morphophysiological and hormonal processes [5,6].



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). The development of adaptable and stable tropical cultivars can be a solution to overcome this issue. Therefore, it is important that genetic breeding programs are stimulated to develop productive cultivars and adapted to a wide range of latitudes, allowing cultivation area expansion [7]. Several studies in tropical regions have demonstrated the potential of new genotypes with low chilling requirements and higher yields, compared to well-established cultivars in the market [2,8–10].

The environment interferes with agronomic characteristics [11] and the quality of the fruit post-harvest [12]. Considering the wide latitude of strawberry cultivation worldwide, studies of adaptability and stability should be carried out both in relation to the location and throughout the harvest period [1].

Studying the genotype \times environment interaction aims to identify the behavioral variation that genotypes undergo when exposed to varied environmental conditions [13]. One way to measure these interaction effects is through correlation studies between the characteristics of interest [14]. Correlations estimated among variables provide strategic information for studies to improve adaptability and stability in strawberries [15]. In addition, these studies allow genotype identification with predictable behavior, as well as those responsive to environmental variations, under specific conditions [16,17].

The variability of the genus Fragaria is wide, and it is classified according to the ploidy level, in which the basic number of chromosomes is equal to seven (x = 7) [18]. Among the twenty-five known species, thirteen are diploid (2n = 2x = 14), five are tetraploid (2n = 4x = 28), one is petaploid (2n = 5x = 35), one is hexaploid (2n = 6x = 42), three are octaploid (2n = 8x = 56), and two are decaploids [18]. The cultivated species (*Fragaria* × *ananassa*), classified as octaploid, is derived from immediate ancestors (*F. chiloensis* and *F. virginiana*), considered allopolyploids [19], which, in turn, are derived from two or more different diploid ancestors [20]. The octaploid commercial strawberry genome comprises 813.4 megabases (Mb) that are distributed across 28 pseudochromosomes, with 108,087 protein-coding genes and 30,703 RNA-coding genes [21]. Therefore, this wide variability generates expectations of obtaining genotypes adapted to a low chilling requirement.

Most polyploids obtained by chromosome duplication have characteristics of vigorous plants and larger fruits [22]. Eukaryotic polyploids also show strong resistance to biotic and abiotic factors [23].

Mixed linear models consider genotype effects as modifications to estimate adaptability and stability, allowing a genetic effects analysis to be made using best linear unbiased predictors (BLUPs) [24]. However, mixed linear models are routinely prone to experimental inconstancy and the heterogeneity of environmental variations [25]. Therefore, in this method, the genotypic values (e.g., productivity), adaptability, and genotypic stability are analyzed simultaneously [26]. This methodology is based on the harmonic mean of the relative performance of genotypic values (HMRPGV) method, which considers the genotype mean and its variation in different environments.

The relationship between genotype and phenotype in different regions helps to anticipate more precise responses to the selection of individuals with heterogeneous habitats, whether spatial or temporal. If the genotype has phenotypic expression for a particular trait, depending on the environment, heritability measures can be altered according to variations in environmental conditions [27].

Thus, the objective of this work was to estimate the adaptability and the temporal stability, as well to select strawberry genotypes easy to propagate with lower cold requirements using a mixed linear model.

2. Materials and Methods

2.1. Location

The experiment was conducted at the University of Centro-Oeste, Guarapuava, Brazil (25°23'01" S, 51°29'50" W, elevation of 1025 m a.s.l.) from August 2019 to February of 2020. The soil was classified as typical dystroferric Latosol Bruno [28]. The climate was humid subtropical Cfb (temperate oceanic climate, warm summer, and without a dry season),

which includes hot summers and frosty winters. The average annual temperature was 17 °C, with maximum and minimum temperatures of 23.5 °C and 12.7 °C, respectively. The average annual rainfall was 1946 mm [28].

2.2. Experimental Genotypes

In our study, 36 genotypes (Table 1) were selected from 10 populations (total of 2000 F_1 plants) from crosses among genotypes pre-selected by our strawberry breeding program and commercial cultivars with responses to neutral photoperiods ('Albion' and 'Monterey'). The 36 genotypes were multiplied to produce the nursery plants for the trials.

Male Genitor Cultivars	Female Genitor Cultivar	Genotypes		
RVFS 07 (Festival \times Aromas)	Monterey	RVFS07M-24		
RVFS 07 (Festival \times Aromas)	Monterey	RVFS07M-36		
RVFS 06 (Festival \times Aromas)	Albion	RVFS06AL-132		
RVFS 07 (Festival \times Aromas)	Monterey	RVFS07M-34		
RVFS 07 (Festival $ imes$ Aromas)	Monterey	RVFS07M-179		
RVDA 11 (Dover \times Aromas)	Monterey	RVDA11M-04		
RVDA 11 (Dover \times Aromas)	Monterey	RVDA11M-21		
RVFS 07 (Festival $ imes$ Aromas)	Monterey	RVFS07M-02		
RVFS 07 (Festival $ imes$ Aromas)	Monterey	RVFS07M-32		
RVFS 07 (Festival $ imes$ Aromas)	Monterey	RVFS07M-38		
RVFS 07 (Festival $ imes$ Aromas)	Monterey	RVFS07M-05		
RVFS 07 (Festival $ imes$ Aromas)	Monterey	RVFS07M-33		
RVFS 07 (Festival $ imes$ Aromas)	Monterey	RVFS07M-10		
RVDA 11 (Dover \times Aromas)	Monterey	RVDA11M-13		
RVFS 07 (Festival $ imes$ Aromas)	Monterey	RVFS07M-154		
RVFS 07 (Festival $ imes$ Aromas)	Monterey	RVFS07M-47		
RVFS 07 (Festival $ imes$ Aromas)	Monterey	RVFS07M-31		
RVFS 07 (Festival $ imes$ Aromas)	Monterey	RVFS07M-16		
RVDA 11 (Dover \times Aromas)	Monterey	RVDA11M-25		
RVFS 07 (Festival $ imes$ Aromas)	Monterey	RVFS07M-113		
RVDA 11 (Dover \times Aromas)	Monterey	RVDA11M-32		
RVFS 07 (Festival $ imes$ Aromas)	Monterey	RVFS07M-30		
RVCA 16 (Camarosa \times Aromas)	Monterey	RVCA16M-01		
RVDA 11 (Dover \times Aromas)	Monterey	RVDA11M-28		
RVFS 07 (Festival $ imes$ Aromas)	Monterey	RVFS07M-151		
RVDA 11 (Dover \times Aromas)	Monterey	RVDA11M-10		
RVFS 07 (Festival $ imes$ Aromas)	Monterey	RVFS07M-124		
RVFS 06 (Festival $ imes$ Aromas)	Monterey	RVFS06M-29		
RVDA 11 (Dover \times Aromas)	Monterey	RVDA11M-29		
RVFS 07 (Festival $ imes$ Aromas)	Monterey	RVFS07M-42		
RVFS 07 (Festival \times Aromas)	Albion	RVFS07AL-28		
RVFS 07 (Festival \times Aromas)	Monterey	RVFS07M-48		
RVFS 07 (Festival \times Aromas)	Monterey	RVFS07M-88		
RVFS 07 (Festival \times Aromas)	Monterey	RVFS07M-80		
RVDA 11 (Dover \times Aromas)	Monterey	RVDA11M-03		
RVFS 06 (Festival \times Aromas)	Albion	RVFS06AL-36		

Table 1. Genotypes pre-selected from the strawberry breeding program obtained from intravarietal crosses.

The mother plants were kept in a greenhouse with an average temperature of 25 ± 3 °C and a humidity of 75 ± 5 %. The nursery plants were obtained from runners (stolons) and transplanted into polypropylene trays, with 50 cells filled with substrate. After 50 days, they were transplanted to an experimental field for evaluation in a repeat trial.

2.3. Genotype Transplantation

The nursery plants were transplanted to the field in a low tunnel cultivation system that was spaced at 30×40 cm. Fertilization was composed of 1650 kg ha⁻¹ of simple superphosphate, 250 kg ha⁻¹ of potassium chloride, and 295 kg ha⁻¹ of urea and applied

to the soil. Irrigation was carried out by drippers with a spacing of 30 cm using two drip lines per bed spaced at 50 cm.

The experiment was performed in a randomized block design, with three repetitions and 10 plants per plot for each genotype for a total of 1320 plants. The commercial genotypes 'Albion', 'Monterey', and 'Dover,' and the single hybrids 'RVCA 16' ('Camarosa' × 'Aromas'), 'RVFS07' ('Festival' × Aromas), 'RVFS06' (Festival × Aromas), 'RVDA 11' (Dover × Aromas), and 'RVDA44' (Camarosa × 'Sweet Charlie') were used as controls.

Seven topdressing fertilizations were carried out at 15-day intervals. Each fertilization was composed of 30 kg ha⁻¹ of ammonium sulfate, 5.5 kg ha⁻¹ of potassium sulfate, and 7.5 kg ha⁻¹ of potassium chloride. At the beginning of flowering, boric acid and zinc sulfate were sprayed onto the leaves at 1 L 100 L⁻¹ and at 1 kg 100 L⁻¹, respectively. In the fruit production stage, 0.4% of calcium chloride was applied every 15 days. Phytosanitary control was carried out with preventive spraying, according to the specific techniques recommended for the culture. The biweekly sprays were interspersed among abamectin (75 mL ha⁻¹), thiametoxan (10 mL ha⁻¹), and fipronil (250 mL ha⁻¹) products. The control of fungal diseases was carried out with alternating applications of azoxystrobin (16 g ha⁻¹), tebuconazole (75 mL ha⁻¹), and mancozeb (250 g ha⁻¹).

2.4. Statistical Analyses

The total fruit mass (TFM g/plant) was obtained for each harvest from August 2019 to February 2020. Each month was considered a collection period to perform adaptability and stability studies.

Statistical analyses were performed using a linear mixed model methodology, where the genetic parameters were estimated using the restricted maximum likelihood method (REML). The genetic values were predicted using the BLUP method [29].

Data were subjected to individual and joint analyses of variance. To analyze the individual variance, the statistical model was adopted as follows:

$$y = Xb + Zg + e$$

where:

y = data vector;

b = vector of the fixed effects of the blocks added to the general mean;

g = vector of random data effects for genotypes;

e = effect of random vector errors;

and *X* and *Z* represent the incidence matrices for vectors *b* and *g*, respectively.

Data were standardized using the correction factor obtained for cases where the coefficients of variation of heritability were verified in a broad sense according to the following expression described by Resende (2007) [25]:

$$\sqrt{h^2}_{ik}/\sqrt{h^2}_t$$
,

where:

 h_{ik}^2 = the broad sense of individual heritability for characteristic *i* in the evaluation of *k*; and h_t^2 = the broad sense of individual mean heritability to evaluate *k* for characteristic *i*.

After standardizing the data, a joint analysis of variance was performed to consider the genotypes and harvesting according to the following statistical model:

$$y = Xb + Za + Wc + e$$

where:

b = vector of the block effects (assumed as fixed) added to the general mean;

a = vector of individual genotypic effects (random);

c = vector of plot effects (randomized);

e = error vector (aleatory);

and *X*, *Z*, and *W* represent the incidence matrices for the said effects (*b*, *a*, and *c*, respectively).

Analysis of deviance (ANADEV) was performed to test the significance of the variance components according to the random effects of the model. The likelihood ratio test (LRT) was used to implement the variance components, in which the significance of the model was evaluated using the chi-square test with one degree of freedom [25].

The classification of genotypes simultaneously considering productivity and stability was performed using the harmonic mean of the genetic values (*HMGV*), which was obtained as follows:

$$HMGV_i = n/\Sigma_{ij=1}^n 1/GV_{ij}$$

where:

n = the number of months/harvests (n = 7) for which genotype i was evaluated;

and *GVij* = the genetic value of genotype *i* in month/harvest *j* expressed by the ratio of the mean in the month/harvest.

The genotypes, considering productivity and adaptability, were selected simultaneously by the performance of their genotypic values (*RPGV*) during the months/harvest obtained by the formula:

$$RPGV_i = 1/n \left(\frac{\sum_{j=1}^n GV_{ij}}{M_j}\right)$$

where:

Mj = fruit productivity means during the month/harvest *j*.

Strawberry genotypes were simultaneously classified in terms of productivity, stability, and adaptability through the harmonic mean of the relative performance of genotypic values (*HMRPGV*), obtained according to the following expression:

$$HMRPGV_i = rac{n}{\sum_{j=1}^n rac{1}{RPGV_{ij}}}$$

The values of $RPGV\mu$ and $HMRPGV\mu$ were obtained by multiplying RPGV and HMRPGV by the general mean of each characteristic and then considering all months/harvests. Thus, the mean values of the genotype were provided, penalized for instability, and capitalized by adaptability. Selective precision and selection gains were obtained according to Resende [29]. Statistical model 20 was adopted for individual analyses, which refers to the evaluation of unrelated genotypes obtained from randomized blocks containing five plants per plot. In addition, model 55 was used in the conjoint analysis for genotypes in an RBD, with stability and temporal adaptability for one place and seven months/harvests using the Selegen REML/BLUP program [29]. From the $HMRPGV\mu$ values, a box plot was generated using the R software with the ggplot2 package.

3. Results

A desirable strawberry cultivar should have good productivity, post-harvest characteristics, disease and pest tolerance, adaptability, and temporal stability, distributing production uniformly throughout the cultivation period. Adapted and stable genotypes are typically identified during the final selection cycles, and only those that demonstrate superiority are tested.

The climate conditions for the experiment cultivation period are shown in Figure 1. The minimum temperature (daily average) varied from 8.26 °C in July to 17.8 °C in March 2019. The maximum temperatures varied on a daily average from 20.11 °C in July 2019 to 28.4 °C in March 2020. The lowest rainfall value was observed in July (0.63 mm as the mean per day), and the highest was in December (5.04 mm per day). The monthly mean temperatures gradually increased during the cultivation period, ranging from 14.17 °C at the time of

transplantation (July 2019) to 21 °C in February 2020 at the end of the harvest period (Figure 1).

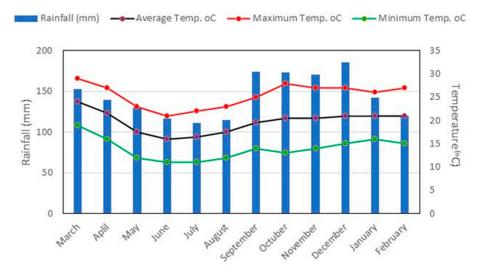


Figure 1. Rainfall and temperature data of the strawberry experimental location from March 2019 to February 2020.

For the analyses of adaptability and stability of the genotypes, the total mass of fruit was used for the analysis of the mixed models.

Significant differences were observed for total fruit mass in the sources of variation, genotypes, genotype interactions by harvest time, and permanent effects using deviance analysis based on the likelihood ratio test (Table 2). The interaction between genotype (G) and environment (E) showed variations in performance among different harvest periods (months).

Table 2. Deviance analysis (ANADEV) for total fruit mass in strawberry genotypes evaluated for seven months.

Variation Source	Deviance	LRT (X^2)		
Genotypes	8512.40	63.73 **		
Genotypes \times Months	9019.45	570.78 **		
Permanent effect $G \times E$	8492.63	43.96 **		
Complete model	8448.67	-		

** significant at 1% of probability by deviance analysis based on the LRT test (X²) with 1 degree of freedom (t_{table} = 6.63).

From the estimates of the variance components obtained using REML/BLUP, for the total mass of fruit of the genotypes, heritability in the broad sense was 33%. The mean heritability of the genotypes (79%) was superior to broad-sense heritability. The data presented an accuracy of 0.89, which was considered high, and the repeatability was 36% (Table 3).

Among the 44 genotypes evaluated, 17 showed positive genetic effects, and their predicted genotypic values ranged from 128.16 (RVDA11M-25) up to 278.02 (RVFS07M-34) (Table 4). Through the analyses based on mixed models, the genotypic values were considered to evaluate the strawberry genotypes for the general performance in all seven harvests analyzed and for the individual performance for each harvest. The 11 best genotypes selected for each harvest period are presented in Table 5. As for overall performance, the mean genotypic values ranged from 158.18 g/plant (RVDA11M-13) up to 311.86 g/plant (RVFS07M-34). In general, considering every harvest, genotypes RVFS07M-34 and RVFS07M-24 were among those selected with the highest values of total fruit mass. Among the evaluated controls, Monterey, RVCA44, and RVFS07 were selected only in some specific harvests. Monterey was a unique commercial cultivar (control) ranked among the

11 most productive genotypes but only in the last harvest (February), whereas, RVCA44 was selected in harvest 1 (August) and harvest 2 (September), and RVFS07 was selected in harvest 5 (December).

Table 3. Estimation of variance components for total fruit mass in strawberry genotypes evaluated for seven months of cultivation.

Individual REML	Value		
Genotypic variance (g^2)	3483.54		
Genotype month variance (gm^2)	5334.64		
Permanent effects variance (<i>perm2</i>)	337.04		
Temporary residual variance (e2)	1422.48		
Phenotypic variance (f2)	10,577.70		
Heritability in the broad sense in the plot $(hp2)$	0.33		
Repeatability (r)	0.36		
Mean heritability of genotypes (<i>ahg2</i>)	0.79		
Accuracy (A)	0.89		
General mean	123.33		

Table 4. Predicted genotypic values for total fruit mass obtained from 44 strawberry genotypes.

Genotype	$u + g^1$	CI ²
RVFS07M-34	278.02	[222.45; 333.59]
RVFS07M-24	244.71	[189.14; 300.28]
RVDA11M-04	239.54	[183.97; 295.12]
RVFS07M-36	191.39	[135.83; 246.97]
RVFS07M-33	183.14	[127.57; 238.71]
RVFS07M-05	182.46	[126.89; 238.03]
RVFS07M-154	180.29	[124.72; 235.86]
RVFS07M-31	167.46	[111.89; 223.03]
RVFS07M-80	155.91	[100.34; 211.48]
RVFS06AL-132	153.04	[97.47; 208.61]
RVDA11M-13	151.92	[96.35; 207.50]
RVDA11M-21	151.47	[95.90; 207.04]
RVCA16M-01	151.30	[95.73; 206.87]
RVFS07M-32	148.63	[93.06; 204.20]
RVFS07M-10	147.01	[91.44; 202.58]
RVDA11M-03	139.66	[84.09; 195.23]
RVDA11M-25	128.16	[72.59; 183.73]
RVFS07M-124	122.98	[67.41; 178.55]
RVDA11M-32	119.52	[63.95; 175.09]
RVFS07M-16	116.20	[60.63; 171.77]
RVCA16	115.89	[60.32; 171.46]
RVDA11M-10	115.69	[60.12;171.26]
RVFS07M-179	112.89	[57.32; 168.46]
RVFS07M-113	102.96	[47.38; 158.53]
RVCA44	101.42	[45.85; 156.99]
RVFS07M-38	98.73	[43.16; 154.3]
RVFS07	97.12	[41.55; 152.69]
RVFS06	94.68	[39.12; 150.26]
RVFS07M-47	94.41	[38.84; 149.98]
Monterey	93.23	[37.66; 148.80]
RVDA11M-29	91.97	[36.39; 147.54]
RVFS07M-02	91.76	[36.19; 147.33]
RVFS07M-88	91.36	[35.79; 146.93]
RVDA11M-28	90.90	[35.33; 146.47]
Albion	90.58	[35.01; 146.15]
RVFS06AL-36	89.62	[34.05; 146.19]

Genotype	$u + g^{1}$	CI ²
RVDA11	87.49	[31.92; 143.06]
RVFS07M-30	85.03	[29.46; 140.60]
RVFS07AL-28	69.84	[14.27; 125.41]
Dover	62.30	[6.73; 117.87]
RVFS07M-151	60.44	[4.87; 116.01]
RVFS06M-29	52.15	[-3.43; 107.72]
RVVFS07M-48	44.64	[-10.93; 100.21]
RVFS07M-42	38.72	[-16.85; 94.29]

¹ Predicted genotypic value; ² confidence interval.

Table 5. Genotype selection in all harvests and in each harvest based on predicted genotypic values for the total fruit mass obtained from 44 strawberry genotypes.

General		Harvest 1 ¹		Harve	Harvest 2		Harvest 3	
Genotype	$u + g + gem^2$	Genotype	$u + g + ge^3$	Genotype	u + g + ge	Genotype	u + g + ge	
RVFS07M-34	311.86	RVFS07M-47	53.38	RVFS07M-80	119.64	RVFS07M-34	698.39	
RVFS07M-24	271.27	RVFS07M-34	38.59	RVFS07M-24	89.84	RVFS07M-05	414.05	
RVDA11M-04	264.97	RVDA11M-04	31.38	RVFS07M-36	88.04	RVFS07M-36	379.57	
RVFS07M-36	206.28	RVFS07M-24	29.96	RVFS07M-34	85.09	RVFS07M-124	350.38	
RVFS07M-33	196.22	RVCA44	29.35	RVFS07M-154	84.26	RVFS07M-179	334.93	
RVFS07M-05	195.40	RVFS07M-154	28.26	RVCA16M-01	75.27	RVFS07M-154	302.40	
RVFS07M-154	192.75	RVFS07M-80	27.30	RVDA11M-28	65.80	RVDA11M-04	293.37	
RVFS07M-31	177.11	RVFS06AL-132	25.62	RVDA11M-03	60.20	RVFS07M-10	263.49	
RVFS07M-10	163.04	RVFS07M-36	24.46	RVFS07M-32	58.46	RVDA11N-03	250.05	
RVFS06AL-132	159.54	RVFS07M-33	24.00	RVDA11M-21	54.19	RVFS07M-31	250.04	
RVDA11M-13	158.18	RVFS07M-113	21.23	RVCA44	50.91	RVFS07M-24	247.47	
Harvest 4 Harvest 5		Harvest 6		Harvest 7				
Genotype	u + g + ge	Genotype	u + g + ge	Genotype	u + g + ge	Genotype	u + g + ge	
RVFS07M-34	413.91	RVFS07M-34	563.86	RVDA11M-04	462.95	RVFS07M-24	400.41	
RVFS07M-31	405.00	RVFS07M-05	484.49	RVFS07M-24	340.98	RVFS07M-32	334.98	
RVDA11M-04	400.64	RVFS06AL-132	442.21	RVFS07M-33	264.74	RVDA11M-04	218.63	
RVFS07M-05	393.19	RVFS07M-24	416.42	RVDA11M-13	237.73	RVFS07M-80	200.98	
RVFS07M-24	373.76	RVDA11M-04	403.48	RVDA11M-21	218.94	RVFS07M-33	185.94	
RVFS07M-33	356.12	RVFS07M-31	397.62	RVFS07M-36	216.65	RVFS07M-34	172.44	
RVFS07M-36	332.83	RVCA16M-01	373.02	RVFS07M-34	210.74	RVFS07M-16	154.77	
RVFS07M-80	321.29	RVFS07M-36	349.95	RVCA16M-01	202.61	RVFS07M-154	153.83	
RVFS07M-154	312.29	RVFS07M-154	341.10	RVDA11M-25	186.88	Monterey	152.35	
RVDA11M-13	294.86	RVFS07M-33	331.10	RVDA11M-10	152.20	RVDA11M-21	128.65	
RVDA11M-21	289.38	RVFS07	317.73	RVDA11M-03	151.93	RVDA11M-25	125.97	

¹ Harvest 1 = August; harvest 2 = September; harvest 3 = October; harvest 4 = November; harvest 5 = December; harvest 6 = January; harvest 7 = February; ² mean genotypic value in 7 harvests that capitalizes the mean interaction with all evaluated harvests; ³ predicted genotypic value in each harvest, i.e., the genotypic values capitalizing the interaction with the harvests.

Mean genotypic values penalized by instability and capitalized by adaptability were obtained. Data dispersion showed the 11 genotypes that stood out from the others (Figure 2). They had the highest yields and were the most stable and adaptable: RVFS07M-34, RVFS07M-24, RCDA11M-04, RVFS07M-154, RVFS07M-36, RVFS07M-33, RVFS07M-80, RVFS07M-10, RVDA11M-21, RVDA11M-13, and RVFS06AL-132. This demonstrated the potential of theses genotypes as commercial cultivars.

When considering the means of each harvest separately, the RVFS07M-34 (Figure 3) genotype stood out as always being among the seven best in all harvests. It had the highest predicted genotypic value (278.02 g/plant) and the highest mean genotypic value (311.86 g/plant) (Table 4 and Table 5, respectively). The RVFS07M-24 hybrid also showed high performance in almost all harvests, except in harvest 3 (October) (Table 5). In addition, RVFS07M-24 had the highest production stability in several harvesting periods, such as harvests 4 (November, 373.76 g/plant), 5 (December, 416.42 g/plant), 6 (January, 340.98 g/plant), and 7 (February, 400.41 g/plant).

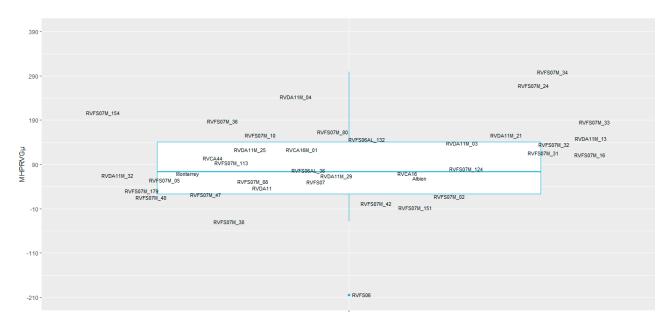


Figure 2. Box plot of adaptability and stability of the 44 evaluated genotypes of strawberry.

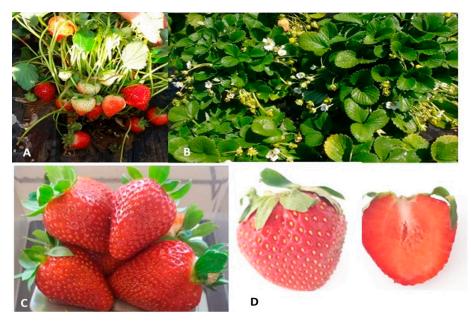


Figure 3. Morphoagronomic aspects of genotype RVFS07M-34 developed with high yield, stability, and temporal adaptability. (**A**) Production; (**B**) plant size, architecture, and flowering; (**C**) pseudo-fruit shape; (**D**) pseudo-fruit internal and external colors.

4. Discussion

Currently, strawberry cultivation in the southern hemisphere is based on cultivars from international breeding programs, which leads to market dependence and vulnerability [11,30], since the cultivars are not well adapted to the climate, and they do not express their maximum genetic potential. These cultivars have low productivity in tropical and subtropical conditions, due to their lack of adaptability and stability throughout the year, especially during the warmest periods [1].

To reduce dependence on imported cultivars and rescue the genetic sovereignty of the species, research has focused on the development of new strawberry genotypes classified as day-neutral and adapted to tropical and subtropical growing conditions [2].

The analysis of deviance in relation to total fruit mass in strawberry genotypes was significant for the effects of genotype, permanent effects, and the interaction between genotype and environment, demonstrating the presence of genetic variability among strawberry genotypes tested for the variable total fruit mass in response to harvest periods. Therefore, it was possible to carry out the selection of more stable genotypes for production throughout the year, which was also observed for strawberry genotypes with low chilling requirements [2], guaranteeing market fruit all year round and reducing the off-season period.

A significant interaction demonstrated the occurrence of a difference in the ranking of the genotypes in the different harvest months, indicating a complex interaction between the genotypes and the environment. According to Allard and Bradshaw [31], this type of interaction is caused by unpredictable variations in the environment, such as precipitation, temperature, relative humidity, the occurrence of pests and/or diseases, and even the production system used for cultivation. The significance in the interaction was evident when highlighting the climatic variation between the harvest months evaluated, when they occurred in three seasons of the year (winter, spring, and summer) in the southern hemisphere. The harvest seasons occurred from August (winter), September to December (spring), and February (summer), when temperatures were relatively high, and the photoperiod was long between the equator and the Tropic of Capricorn. Significant interaction results between strawberry genotypes and harvest environments have already been described in several studies [32,33]. The results underscored the importance of adaptability and stability studies in strawberry cultivars as a basic precept for recommending a cultivar developed by a breeding program [11,34,35].

The propagation of genetic material in a vegetative way in strawberry breeding programs makes it possible to take advantage of genetic variance, whether of an additive, dominant, or epistatic nature [36]. However, it is still important to consider the genotype \times environment interaction. The interaction generates uncertainties in heritability estimates, correlations (genetic, phenotypic), and expected gains with selection, mainly for quantitative traits, such as total fruit mass. The effect of the complex genotype \times environment interaction can be reduced by developing cultivars with greater stability and production adaptability [36], especially when the objective is to explore strawberry cultivation in a wide range of latitudes.

The broad-sense heritability of plot (hp2) of 33% was considered high ($h^2 > 0.3 =$ high) [26], demonstrating good genetic control of the quantitative characteristic total fruit mass. In Table 2, the representations of additive, dominant, and epistatic h^2 are observed. The cultivated strawberry species (*Fragaria* × *ananassa*) was octaploid; most characteristics were under polygenic control and were highly influenced by the cultivation environment [37,38]. In a study evaluating productivity and its main components in strawberry cultivated in a subtropical climate, the authors observed heritability values in the broad sense ranging from 0.01 to 0.63 [11]. Previous studies found broad-sense heritability results found in the present research corroborate several studies already described in literature.

Repeatability tends to be greater than broad-sense heritability, reaching the maximum value that broad-sense heritability can reach [39]. For example, one study observed heritability values of 74.81, 85.17, and 98.44% for the number of fruits per plant, mean fruit mass, and productivity, respectively [15].

Our results showed a high value for accuracy, demonstrating the experimental reliability of this research. Accuracy is the most suitable parameter for determining the proportions between variations of a genetic nature and residual quantitative traits [26].

Based on the harmonic mean of the relative performance of genotypic values (HMRPGV) method, which considers the genotype means and the variation of this mean along the environments, the 11 best genotypes were selected (Figure 2) for the variable total fruit production, as well as stability and adaptability throughout the harvest periods. The selected genotypes were RVFS07M-34, RVFS07M-24, RVDA11M-04, RVFS07M-154, RVFS07M-36, RVFS07M-33, RVFS07M-80, RVFS07M-10, RVDA11M-21, RVDA11M-13, and RVFS06AL-132 (Figure 2). Among them, only RVFS06AL-132 did not have Monterey as

a female parent. Furthermore, RCDA11M-04, RVDA11M-13, and RVDA11M-21 featured Dover in the male parent's genealogy, while the others had Festival in their genealogy.

Aromas was in the genealogy of all male parents used in this study. Camarosa and Aromas cultivars have been described as the most adapted and stable commercial strawberry cultivars [36]. These cultivars probably have additive alleles already fixed for stability and adaptability characteristics, which are inherited by the progeny, since the mean h² of the genotypes was 79%.

Productivity is a quantitative trait of polygenic inheritance with great influence from the environment [37], which can explain the variation in the ranking of genotypes among harvest periods. The experimental genotypes were superior to the commercial cultivars used in the experiment. Monterey was a unique cultivar ranked among the top 11 genotypes and only in the last harvest. The superiority of the experimental genotypes was due to the strawberry breeding program being developed in a soil and climate condition similar to the cultivation area. These results indicate the importance of plant selection occurring as closely as possible in the cultivation environment. A previous study evaluated advanced genotypes of day-neutral strawberries, where crosses were also performed with genotypes RVFS07, RVFS11, and Monterey. Our results agree with this study, as we obtained similar results [2].

The RVFS07M-34 hybrid was the most productive genotype, with greater stability and adaptability, and was classified among the five best genotypes in harvests 1–5 (August to December). A study evaluating strawberry genotypes in two seasons with three different environments obtained significant results for the components of environmental variations and interactions [35]. According to the same authors, abiotic and biotic factors, such as temperature variation, relative humidity, pests, and diseases, can interfere with the results, and they play an important role in the environmental variation for strawberry production.

Strawberry is a microclimatic crop, and the cultivars' behaviors can vary depending on many agronomical and environmental factors (climate conditions and season) [39], highlighting the importance of our study, which analyzed several harvest periods.

5. Conclusions

The use of the mixed linear model methodology to study adaptability and stability showed the superiority of 11 genotypes that had the potential to be released as cultivars. In addition, RVFS07M-34, being the most promising, will be chosen to follow the legal procedures with the official cultivar registration agency.

Author Contributions: D.A.N. performed the experiments and wrote the manuscript. Field management activities, collection, and data analysis were carried out by D.A.N., G.C.G., L.V.B.d.O. and G.F.d.P.G.; A.R.Z., S.T.I.-S. and K.H.M. helped with the experimental design, statistical analysis, and final review of the manuscript. S.R.R. assistance in the interpretation of post-harvest data and writing of the paper, J.T.V.d.R. was responsible for the Strawberry Breeding Program for tropical and subtropical climates (project coordinator and research creator) and obtained funding, assisted in writing, and reviewed the final manuscript. All authors have read and agreed to the published version of the manuscript.

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