

# Morpho-horticultural potential of seven strawberry cultivars for two consecutive cycles

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ABSTRACT: Maintaining plants for two consecutive cycles in strawberry cultivation makes it possible to reduce costs with the acquisition of daughter plants and allows fruit production throughout the year. Thus, the objective of this study was to investigate whether strawberry cultivars grown for two consecutive cycles in a greenhouse change their horticultural potential. The research was carried out in the municipality of Passo Fundo, State of Rio Grande do Sul, Brazil. We tested seven strawberry cultivars (classified as short days and neutral days in terms of flowering) in two production cycles. The cultivars morphology was characterized, and the fruit production and quality were evaluated. The morphological descriptor that most contributed to the divergence among cultivars corresponded to anthocyanin coloration of stipules. 'Aromas' stood out for the greater amount of fruit produced in both production cycles. In the first cycle 'Albion', 'Camino Real', and 'Monterey' produced truits with the highest average fresh masses. Phenolic compounds of the fruits were superior in the second cycle of cultivation in relation to the first cycle. The total aflavonoid content was higher in 'Aromas', 'Camarosa', 'Camino Real', and 'Monterey'. 'Camarosa' and 'San Andreas' had the highest concentration of total polyphenols. The total antioxidant capacity was higher in 'Aromas' and 'San Andreas' had the highest vertices who want to stagger their crops should choose 'Aromas' to obtain greater productive potential (in both cycles) and 'Camarosa' to meet the demanding consumer market in fruit quality.

Key words: descriptors; Fragaria x ananassa Duch.; multivariate analysis; phenolic compounds; production

# Potencial morfo-hortícola de sete cultivares de morangueiro

## por dois ciclos consecutivos

**RESUMO:** A manutenção das plantas por dois ciclos consecutivos no cultivo do morangueiro possibilita reduzir os custos com a aquisição de mudas e permite a produção de frutos durante todo o ano. Assim, o objetivo deste estudo foi investigar se cultivares de morangueiro conduzidas por dois ciclos consecutivos em estufa alteram seu potencial hortícola. A pesquisa foi realizada no municipio de Passo Fundo, Rio Grande do Sul, Brasil. Testamos sete cultivares de morangueiro (classificadas como de dias curtos e dias neutros em relação ao florescimento) em dois ciclos de produção. A morfologia das cultivares foi caracterizada e a produção e qualidade de frutos foram avaliadas. O descritor morfológico que mais contribuiu para a divergência entre as cultivares correspondeu à coloração antociânica das estípulas. Aromas se destacou pela maior quantidade de frutos foram avaliadas. O descritor morfológico que mais contribuiu para a divergência entre as cultivares correspondeu à coloração antociânica das estípulas. Aromas se destacou pela maior quantidade de frutos gorados em ambos os ciclos de produção. No primeiro ciclo Albion, Camino Real e Monterey produziram frutos com as maiores massas frescas médias. Os compostos fenólicos dos frutos foram superiores no segundo ciclo de cultivo em relação ao primeiro ciclo. O conteúdo de flavonoides totais foi superior em Aromas, Camarosa, Camino Real e Monterey. Camarosa e San Andreas apresentaram maior concentração de polifenois totais. A atividade antioxidante foi mais elevada em Aromas e Camarosa. Conclui-se que cultivares de morangueiro conduzidas por dois ciclos consecutivos em estufa apresentam variações no seu potencial hortícola. Produtores que desejam escalonar suas lavouras devem optar por Aromas para obter maior potencial produtivo (nos dois ciclos) e Camarosa para atender mercado consumidor exigente em qualidade de frutos.

Palavras-chave: descritores; Fragaria x ananassa Duch.; análise multivariada; compostos fenólicos; produção



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### Introduction

In the Brazilian subtropics, strawberry (*Fragaria* x *ananassa* Duch.) cultivation is migrating from open field cultivation to greenhouse production in order to improve their productivity. Generally, this protected cultivation takes place in structures with a ceiling height of 3 m, a width of 5 to 10 m and a length of 25 to 50 m, using substrate as the growing medium (<u>Chiomento et al., 2023</u>). Knowledge about the agronomic performance of strawberry cultivars in protected agroecosystems makes it possible to provide information to producers about the best performance of the cultivars (<u>Chiomento et al., 2021</u>).

Currently, the classification of strawberry cultivars in relation to their flowering divides the plants into three groups: short-day (SD), neutral-day (ND) cultivars and summer plants (UC Davis, 2021). The strawberry cultivars used in the southern hemisphere are generally those classified as ND and summer plants (Chiomento et al., 2020). In these regions, SD cultivars are sensitive to the duration of the night, being induced to flower in a photoperiod of less than 14 hours (light per day), with low temperatures, below 15 °C (Shalit et al., 2009). ND and summer plants cultivars, in these same growing regions, are insensitive to photoperiod and induced to flowering by the main effect of temperature (Durner, 2015).

Traditionally this horticultural crop is managed as an annual. The annual transplant of daughter plants the use of plastics as a cover for beds and the labor involved in the implementation of new crops make strawberry cultivation expensive (Chiomento et al., 2021). However, maintaining plants for two or more consecutive cycles is a promising strategy to reduce these costs inherent to cultivation (Zeist et al., 2019). In addition, this phytotechnical tool makes it possible to keep the plants producing fruits for a longer period, which would improve the income of producers mainly by supplying berries to the consumer market in the off-season periods.

However, the scarce literature on the horticultural performance of strawberry cultivars maintained for two consecutive seasons, in response to the micrometeorological conditions of the production agroecosystem, limits the choice of cultivars by producers, who may choose less productive materials. Still, without this information on fruit production and quality, producers may have difficulties to plan the staggering of cultivars in the establishment of their crops. Thus, the following question arises: how is the horticultural potential of strawberry cultivars conducted for two consecutive cycles in a greenhouse regarding morphological, productive, and phenolic compounds responses of fruits?

Therefore, based on the hypothesis that the productive and qualitative performance of strawberry is different among cultivars and crop cycles, here we investigate whether strawberry cultivars grown for two consecutive cycles in a greenhouse change their horticultural potential. Our study provides an insight into the morphology of seven cultivars, through morphological descriptors of culture, yield and berry biomolecule content.

## **Materials and Methods**

#### Plant material and research site

In this study, bare-rooted strawberry daughter plants from the Llahuén/Chilean Patagonia nursery (33° 50' 15.41" S; 70° 40' 03.06" W) were used, which constituted the plant material for the experiment. The research was carried out in the municipality of Passo Fundo (28° 15' 41" S; 52° 24' 45" W), State of Rio Grande do Sul, Brazil. The experiment was carried out from May (autumn) 2018 to January (summer) 2020, in a greenhouse (510 m<sup>2</sup>) with a semicircular roof, installed in the northeast-southwest direction, in a galvanized steel structure covered with a low-density polyethylene film, with anti-ultraviolet additive and a thickness of 150 microns.

#### **Experimental design**

The treatments studied were seven strawberry cultivars ('Albion', 'Aromas', 'Camarosa', 'Camino Real', 'Monterey', 'Portola', and 'San Andreas'), evaluated in two production cycles (2018/2019 and 2019/2020). The experimental design was in randomized blocks, with treatments arranged in plots divided in time (7 cultivars as plots and 2 cycles as subplots), with six replications and ten plants per plot, totaling 60 plants per treatment. The first production cycle took place from September (spring) 2018 to March (autumn) 2019 and the second cycle from July (winter) 2019 to January 2020.

Two strawberry cultivars classified as short days (SD) in terms of flowering ('Camarosa' and 'Camino Real'), four cultivars with neutral days (ND) ('Albion', 'Aromas', 'Monterey', and 'San Andreas') and a cultivar classified as a summer plant ('Portola').

#### Cultivation techniques and environmental monitoring

The cultivation system adopted was the conventional one (soil planting). The daughter plants were transplanted from May to June (winter) 2018, as they were received from the Chilean nursery, in beds covered with black mulching (thickness of 30 microns), with dimensions of 15 m long  $\times$  1.0 m wide. The daughter plants, with crown diameter greater than 8 mm, were planted at a spacing of 0.30  $\times$  0.30 m, in two rows per plot.

The chemical characterization of the soil is presented in Table 1.

The irrigation used was localized, using drip tapes (drippers spaced every 20 cm), with a flow rate of  $1.2 \text{ L} \text{ h}^{-1}$  per dripper. Soil moisture content was monitored by tensiometers at 20 cm depth. Irrigation was started when the content was less than -20 kPa. The fertilization provided to the plants was carried out according to the needs of the crop (CQFS, 2016).

During the experiment execution, through a mini meteorological station, the monitoring of the air temperature (minimum, average, and maximum) inside the greenhouse was carried out (Figure 1).

#### Table 1. Chemical properties of cultivated soil.

| Clay                   | рН       | SMP   | Р     | К     | ОМ  | Al    | Са    | Mg                                | H+AI           | CEC  | Sa    | turation ( | %)  |
|------------------------|----------|-------|-------|-------|-----|-------|-------|-----------------------------------|----------------|------|-------|------------|-----|
| (%)                    | $H_2O^1$ | index | (mg d | dm⁻³) | (%) |       | ()    | cmol <sub>c</sub> dm <sup>.</sup> | <sup>3</sup> ) |      | Bases | Al         | К   |
| 42                     | 6.5      | 6.8   | 63.4  | 330   | 4.8 | 0.0   | 9.8   | 4.4                               | 1.7            | 16.8 | 90    | 0.0        | 5.0 |
|                        | Sulfur   |       | Bor   | on    |     | Manga | anese |                                   | Zinc           |      |       | Copper     |     |
| (mg dm <sup>-3</sup> ) |          |       |       |       |     |       |       |                                   |                |      |       |            |     |
|                        | 8.0      |       | 0.    | 6     |     | 5.    | 1     |                                   | 10.6           |      |       | 1.7        |     |

<sup>1</sup> pH H<sub>2</sub>O - Hydrogen potential in water; SMP index - Shoemaker-Mac'Lean-Pratt index; P - Phosphorus; K - Potassium; OM - Organic matter; Al - Aluminum; Ca - Calcium; Mg - Magnesium; H + Al - Potential acidity; CEC - Cation exchange capacity.

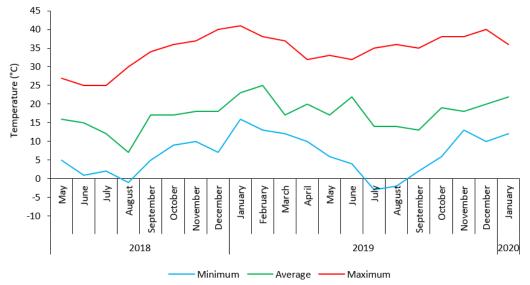


Figure 1. Monthly minimum, average, and maximum temperature during the experiment, from May 2018 to January 2020.

At the end of the first production cycle, in March 2019, renewal pruning was carried out on the plants (intensity of removal of 50% of leaves) in order to prepare them for the second production cycle. The evaluations were initiated after the issuance of leaves. Attributes related to the morphological characterization of the strawberry, to the fruit production and phenolic compounds were evaluated.

#### Strawberry morphological characterization

To characterize the different strawberry cultivars, the morphological descriptors of the culture were used (<u>UPOV</u>, <u>2012</u>). Eighteen morphological characters were considered

(Table 2) through two evaluations, carried out in June 2018 (first cycle) and in June 2019 (second cycle).

#### Fruit production

Yield was evaluated in terms of the total number of fruits per plant (TNF, number per plant), average fresh fruit mass (AFFM, g), and fruit production per plant (TP, grams per plant). As flowering and harvesting occurred concomitantly in part of the cycle, approximately twelve-monthly harvests were carried out, on average every three days. The fruits were harvested when they were fully ripe and then weighed on a digital scale. AFFM was determined dividing TP by TNF.

| Characteristics                                  | Description                                                     |  |  |  |
|--------------------------------------------------|-----------------------------------------------------------------|--|--|--|
|                                                  | Plant                                                           |  |  |  |
| Growth habit                                     | Jpright; semi-upright; spreading                                |  |  |  |
| Density of foliage                               | Sparse; medium; dense                                           |  |  |  |
| Position of inflorescence in relation to foliage | eneath; same level; above                                       |  |  |  |
|                                                  | Leaf                                                            |  |  |  |
| Color of stem                                    | Yellow green; light green; medium green; dark green; blue green |  |  |  |
| Color of leaf                                    | Yellow green; light green; medium green; dark green; blue green |  |  |  |
| Glossiness of leaf                               | Absent or weak; medium; strong                                  |  |  |  |
| Margin of terminal leaflet                       | Serrate; serrate to crenate; crenate                            |  |  |  |
| Anthocyanin coloration of stipules               | Absent or very weak; weak; medium; strong; very strong          |  |  |  |
| Blistering of leaf                               | Absent or weak; medium; strong                                  |  |  |  |
|                                                  | Inflorescence                                                   |  |  |  |
| Number of flowers                                | Few; medium; many                                               |  |  |  |

Continued on the next page

#### Continued from Table 2

| Characteristics     | Description                                                                       |
|---------------------|-----------------------------------------------------------------------------------|
|                     | Fruit                                                                             |
| Size of fruit       | Very small; small; medium; large; very large                                      |
| Shape of fruit      | Reniform; conical; cordate; ovoid; cylindrical; rhomboid; obloid; globose; wedged |
| Color of fruit      | Whitish yellow; light orange; medium orange; orange red; medium red; dark red;    |
|                     | blackish red                                                                      |
| Glossiness of fruit | Weak; medium; strong                                                              |
| Position of achenes | Below surface; level with surface; above surface                                  |
| Color of flesh      | Whitish; light pink; orange red; light red; medium red; dark red                  |
| Color of core       | White; light red; medium red                                                      |
| Cavity of fruit     | Absent or small; medium; large                                                    |

#### Phenolic compounds of fruits

At the fruit maturation peak, in November (spring) of 2018 and 2019, of the ten harvests, samples of 100 g of fruits were used for the phenolic compounds extraction procedure (Revilla et al., 1998). Subsequently, the contents of total anthocyanins (TA), total flavonoids (TF), total polyphenols (TPO), and total antioxidant capacity (TAC) were quantified.

TA content was determined by the pH differential method (<u>Giusti & Wrolstad, 2001</u>; <u>Lee et al., 2005</u>) and results were expressed in milligrams of pelargonidin-3-*O*-glycoside equivalent per 100 g of fresh fruit (mg PE.100 g FF<sup>-1</sup>). TF content was quantified according to <u>Miliauskas et al.</u> (2004) and results were expressed as milligrams of rutin per 100 g of fresh fruit (mg rutin.100 g FF<sup>-1</sup>). TPO content was quantified by the Folin-Ciocalteu method (<u>Singleton et al., 1999</u>) and results were expressed in milligrams of gallic acid equivalent per 100 g of fresh fruit (mg GAE.100 g FF<sup>-1</sup>). TAC determination was obtained by the power of iron reducing activity (<u>Zhu et al., 2002</u>) and results were expressed in mig GAE.100 g FF<sup>-1</sup>.

#### **Statistical analysis**

To analyze the morphological descriptors, we performed a multivariate analysis of the information collected in our study, using the Genes<sup>®</sup> software (<u>Cruz, 2016</u>). To illustrate the relationship among the seven strawberry cultivars regarding the 18 descriptors evaluated, using dendrogram, we used cluster analysis by the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) calculated by the Euclidean distance (D). Subsequently, the dendrogram that obtained the highest grouping consistency for the cultivars was constructed, using the cophenetic correlation coefficient (CCC), distortion and stress as criteria for validation. Furthermore, we investigated the relative contribution of morphological descriptors to divergence among cultivars, by the <u>Singh (1981)</u> method, and by principal components.

To classify the strawberry cultivars in groups according to the descriptors, we used the principal component analysis (PCA), performed after the standardization of the attributes, in which each one had a mean of 0 and variance of 1. We performed the PCA to condense the amount of information (Hair et al., 2005) of the 18 morphological attributes investigated in our study. We disregarded eigenvalues below 1 from the analysis, as we did not have relevant information (Kaiser, 1958).

Data regarding the production and quality of strawberries were submitted to analysis of variance (Anova) and the differences among means were compared by the Scott-Knott test at 5% error probability, with the aid of the Sisvar<sup>®</sup> program (Ferreira, 2019).

#### **Results**

#### Strawberry morphological characterization

Of the 18 morphological characters present in the descriptors of the species under study (<u>Table 2</u>), six of them (leaf stem color, edge of the terminal leaflet, number of flowers, fruit brightness, achene position, and fruit cavity) were similar among the strawberry cultivars studied, in two consecutive cycles (<u>Table 3</u>). Only 'Camino Real' showed yellowish green stem color, achene position at the same height as the surface and large fruit cavity. 'Monterey' differed from the other cultivars by presenting a serrated to crenate terminal leaflet edge. 'Camarosa' produced a high number of flowers, while 'Aromas' produced fruits with medium brightness (<u>Table 3</u>).

Through multivariate analysis, we observed that there was heterogeneity among the cultivars studied. The dissimilarity among the strawberry cultivars, in relation to the descriptors, was illustrated by the dendrogram generated by the UPGMA method (Figure 2), whose adjustment to the Euclidean distance matrix, calculated by the CCC, was 93%, indicating model adequacy. Thus, there was the formation of two groups (Figure 2), evidencing the dissimilarity among the cultivars regarding the attributes investigated.

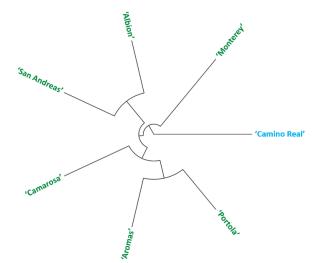
Group 1 (green color) gathered six strawberry cultivars, of which one ('Camarosa') is classified as short-day (SD) and five ('Albion', 'Aromas', 'Monterey', 'Portola', and 'San Andreas') as neutral day (ND) for flowering (Figure 2). This group of cultivars presented similar performance in relation to the descriptors referring to stem color, achene position and fruit cavity (Table 3). Group 2 (blue color) was composed of the cultivar 'Camino Real' (SD) (Figure 2). This cultivar showed particular characteristics regarding the anthocyanin pigmentation of the stipules and the shape of the fruits (Table 3).

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**Table 3.** Characterization of strawberry cultivars in two cultivation cycles, according to the morphological descriptors of the culture.

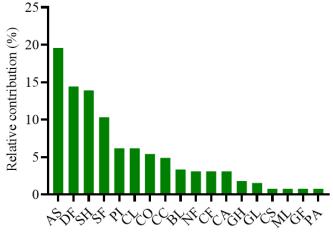
|                                                  | Morphological description <sup>1</sup> |                    |                        |                       |                    |                    |                    |  |  |
|--------------------------------------------------|----------------------------------------|--------------------|------------------------|-----------------------|--------------------|--------------------|--------------------|--|--|
| Characteristics                                  | 'Albion'                               | 'Aromas'           | 'Camarosa'             | 'Camino<br>Real'      | 'Monterey'         | 'Portola'          | 'San<br>Andreas'   |  |  |
|                                                  |                                        |                    | Plant                  |                       |                    |                    |                    |  |  |
| Growth habit                                     | Semi-<br>upright                       | Semi-<br>upright   | Spreading              | Semi-<br>upright      | Upright            | Semi-<br>upright   | Semi-<br>upright   |  |  |
| Density of foliage                               | Sparse                                 | Medium             | Dense                  | Medium                | Sparse             | Medium             | Dense              |  |  |
| Position of inflorescence in relation to foliage | Above                                  | Beneath            | Beneath                | Above                 | Beneath            | Beneath            | Above              |  |  |
|                                                  |                                        |                    | Leaf                   |                       |                    |                    |                    |  |  |
| Color of stem                                    | Light green                            | Light green        | Light green            | Yellow<br>green       | Light green        | Light green        | Light green        |  |  |
| Color of leaf                                    | Dark green                             | Light green        | Light green            | Dark green            | Dark green         | Light green        | Dark green         |  |  |
| Glossiness of leaf                               | Absent or<br>weak                      | Absent or<br>weak  | Absent or<br>weak      | Medium                | Medium             | Medium             | Absent or<br>weak  |  |  |
| Margin of terminal leaflet                       | Crenate                                | Crenate            | Crenate                | Crenate               | Serrate to crenate | Crenate            | Crenate            |  |  |
| Anthocyanin coloration of stipules               | Weak                                   | Weak               | Absent or<br>very weak | Medium                | Strong             | Weak               | Weak               |  |  |
| Blistering of leaf                               | Absent or<br>weak                      | Strong             | Strong                 | Strong                | Strong             | Strong             | Medium             |  |  |
|                                                  |                                        |                    | Inflorescence          |                       |                    |                    |                    |  |  |
| Number of flowers                                | Medium                                 | Medium             | Many                   | Medium                | Medium             | Medium             | Medium             |  |  |
|                                                  |                                        |                    | Fruit                  |                       |                    |                    |                    |  |  |
| Size of fruit                                    | Medium                                 | Medium             | Large                  | Large                 | Medium             | Medium             | Small              |  |  |
| Shape of fruit                                   | Conical                                | Cordate            | Cordate                | Rhomboid              | Conical            | Conical            | Cylindrical        |  |  |
| Color of fruit                                   | Dark red                               | Medium<br>red      | Medium<br>red          | Dark red              | Dark red           | Orange red         | Medium<br>red      |  |  |
| Glossiness of fruit                              | Strong                                 | Medium             | Strong                 | Strong                | Strong             | Strong             | Strong             |  |  |
| Position of achenes                              | Below<br>surface                       | Below<br>surface   | Below<br>surface       | Level with<br>surface | Below<br>surface   | Below<br>surface   | Below<br>surface   |  |  |
| Color of flesh                                   | Medium<br>red                          | Light red          | Dark red               | Dark red              | Dark red           | Light red          | Light red          |  |  |
| Color of core                                    | Medium<br>red                          | White              | Light red              | White                 | White              | White              | Medium<br>red      |  |  |
| Cavity of fruit                                  | Absent or<br>small                     | Absent or<br>small | Absent or<br>small     | Large                 | Absent or<br>small | Absent or<br>small | Absent or<br>small |  |  |

<sup>1</sup> Morphological descriptors presented in UPOV (2012).



Cophenetic correlation index: 0.93. Distortion and stress (%): 0.34 and 5.82, respectively. **Figure 2.** Dendrogram of dissimilarity among strawberry cultivars obtained by the UPGMA method based on the Euclidian distance matrix (D). The morphological descriptor that most contributed to the divergence among cultivars (Figure 2) corresponded to AS, which explained 19.59% of the variability among the cultivars studied (Figure 3). Added to this, the descriptors with the highest relative contribution to dissimilarity were DF, SH, and SF, with 14.43, 13.92, and 10.31%, respectively. These attributes represented 58.25% of the total dissimilarity among cultivars (Figure 3).

The PCA results showed that the first five PC can be used to study the relationship among the attributes evaluated in our study, since the eigenvalues ( $\lambda$ i) were greater than 1 (Table 4). The first two PC had a cumulative variance of 55.74% (Table 4). The first PC explained 32.40% of the total variance and the second component explained 23.34% (Figure 4). In addition, we used the PC technique to select the descriptors that most contributed to variation within each component. The descriptors CA, PA, CS, AS, and CF contributed with 52.56% of the variation of CP1 (Table 4). For the second PC the descriptors GH, ML, DF, SH, and SF

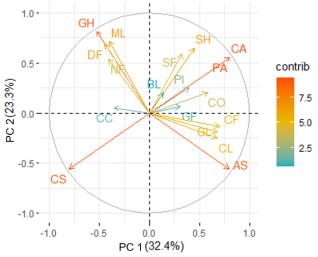


AS - Anthocyanin coloration of stipules; DF - Density of foliage; SH - Shape of fruit; SF - Size of fruit; PI - Position of inflorescence in relation to foliage; CL - Color of leaf; CO - Color of flesh; CC - Color of core; BL - Blistering of leaf; NF - Number of flowers; CF - Color of fruit; CA - Cavity of fruit; GH - Growth habit; GL - Glossiness of leaf; CS - Color of stem; ML - Margin of terminal leaflet; GF - Glossiness of fruit; PA - Position of achenes.

**Figure 3.** Relative contribution, according to <u>Singh (1981)</u>, of the characters to dissimilarity among strawberry cultivars.

contributed with 57.32%, reinforcing the importance of these descriptors for studies of genetic diversity. In PC 3 the descriptors BL, CC, and PI retained 62.02% of the total variation. In the other components (PC 4, PC 5, PC 6, and PC 7) it is suggested to discard the descriptors CO, GF, DF, and BL (Table 4).

The AS descriptor showed a negative association with GH (-0.91) and ML (-0.79) (Figure 4). The results showed that NF positively correlated with GH (0.76) (Figure 4).



GH - Growth habit; DF - Density of foliage; PI - Position of inflorescence in relation to foliage; CS - Color of stem; CL - Color of leaf; GL - Glossiness of leaf; ML - Margin of terminal leaflet; AS - Anthocyanin coloration of stipules; BL - Blistering of leaf; NF - Number of flowers; SF - Size of fruit; SH - Shape of fruit; CF - Color of fruit; GF - Glossiness of fruit; PA - Position of achenes; CO - Color of flesh; CC - Color of core; CA - Cavity of fruit.

**Figure 4.** Principal component analysis with the percentage contribution of attributes referring to the morphological descriptors analyzed in the study.

#### **Fruit production**

In the first cycle (2018/2019), fruit production for all cultivars started in September 2018 and ended in March 2019. In the second cycle (2019/2020), fruit production for all cultivars started in July 2019 and ended in January from 2020.

We observed an interactive effect between cultivars and production cycles in all attributes referring to productive potential (<u>Table 5</u>).

**Table 4.** Attributes, principal components (PC), estimates of variances (eigenvalue  $\lambda$ j), percentage of variance explained by components (importance %) and accumulated variance (% accumulated) of strawberry cultivars.

| Attributes    | PC 1   | PC 2   | PC 3   | PC 4    | PC 5    | PC 6    | PC 7    |
|---------------|--------|--------|--------|---------|---------|---------|---------|
| GH            | 4.754  | 15.857 | 0.014  | 2.023   | 0.207   | 1.133   | 0.239   |
| DF            | 3.387  | 11.251 | 0.004  | 1.009   | 14.029  | 17.259* | 0.315   |
| PI            | 2.678  | 1.495  | 20.147 | 0.550   | 0.008   | 0.632   | 9.651   |
| CS            | 11.086 | 7.347  | 0.004  | 1.637   | 0.356   | 0.571   | 8.305   |
| CL            | 7.858  | 1.514  | 11.218 | 0.798   | 1.192   | 2.568   | 6.190   |
| GL            | 7.904  | 0.842  | 6.382  | 0.916   | 12.729  | 11.264  | 9.749   |
| ML            | 2.723  | 12.037 | 2.595  | 5.343   | 2.001   | 11.494  | 9.830   |
| AS            | 10.904 | 7.391  | 0.542  | 0.205   | 0.362   | 2.866   | 3.745   |
| BL            | 0.333  | 0.973  | 21.417 | 1.463   | 2.519   | 7.905   | 16.085* |
| NF            | 2.824  | 6.829  | 2.053  | 20.106  | 0.517   | 3.112   | 6.982   |
| SF            | 1.844  | 8.215  | 6.583  | 8.300   | 6.444   | 5.063   | 0.621   |
| SH            | 3.438  | 9.966  | 2.328  | 5.737   | 2.074   | 16.897  | 0.406   |
| CF            | 8.405  | 0.418  | 2.971  | 8.135   | 13.841  | 5.132   | 0.065   |
| GF            | 1.639  | 0.112  | 1.975  | 11.301  | 42.133* | 11.227  | 4.775   |
| PA            | 11.086 | 7.347  | 0.004  | 1.637   | 0.356   | 0.571   | 5.868   |
| CO            | 5.876  | 0.993  | 1.278  | 25.341* | 0.131   | 1.567   | 11.125  |
| CC            | 2.175  | 0.067  | 20.480 | 3.863   | 0.745   | 0.167   | 0.510   |
| CA            | 11.086 | 7.347  | 0.004  | 1.637   | 0.356   | 0.571   | 5.538   |
| λj            | 5.83   | 4.20   | 3.79   | 2.18    | 1.15    | 0.85    | 0.00    |
| nportance (%) | 32.40  | 23.34  | 21.06  | 12.11   | 6.37    | 4.73    | 0.00    |
| cumulated (%) | 32.40  | 55.74  | 76.80  | 88.90   | 95.27   | 100.00  | 100.00  |

\*According to the criterion of Jolliffe (1973). GH - Growth habit; DF - Density of foliage; PI - Position of inflorescence in relation to foliage; CS - Color of stem; CL - Color of leaf; GL - Glossiness of leaf; ML - Margin of terminal leaflet; AS - Anthocyanin coloration of stipules; BL - Blistering of leaf; NF - Number of flowers; SF - Size of fruit; SH - Shape of fruit; CF - Color of fruit; GF - Glossiness of fruit; PA - Position of achenes; CO - Color of flesh; CC - Color of core; CA - Cavity of fruit.

|                               | TNF (numb           | er per plant) <sup>1</sup> | TP (gram       | ns per plant) | AFFM (grams) |            |  |
|-------------------------------|---------------------|----------------------------|----------------|---------------|--------------|------------|--|
| Cultivars                     | Cycles <sup>2</sup> |                            | C              | ycles         | Cycles       |            |  |
|                               | First               | Second                     | First          | Second        | First        | Second     |  |
| 'Albion'                      | 48.1±6.4 Ac         | 40.8±6.2 Ac                | 681.1±87.5 Ab  | 385.9±44.2 Bb | 14.0±1.5 Ab  | 9.5±0.7 Ba |  |
| 'Aromas'                      | 95.5±9.4 Aa         | 105.0±9.9 Aa               | 1065.4±99.4 Aa | 902.6±90.6 Aa | 11.2±1.1 Ad  | 8.6±0.6 Ba |  |
| 'Camarosa'                    | 65.7±7.8 Bb         | 99.2±8.8 Aa                | 776.8±90.1 Ab  | 746.8±88.4 Aa | 12.1±1.2 Ac  | 7.5±0.5 Bb |  |
| 'Camino Real'                 | 45.0±6.7 Bc         | 82.0±7.5 Ab                | 676.3±85.9 Ab  | 747.9±83.1 Aa | 14.9±1.1 Aa  | 9.3±0.7 Ba |  |
| 'Monterey'                    | 63.2±7.7 Bb         | 79.4±6.3 Ab                | 878.6±91.4 Ab  | 715.3±89.2 Aa | 13.8±1.3 Ab  | 9.0±0.7 Ba |  |
| 'Portola'                     | 87.4±9.1 Aa         | 83.9±7.8 Ab                | 1069.7±99.3 Aa | 789.8±85.6 Ba | 12.2±1.1 Ac  | 9.5±0.7 Ba |  |
| 'San Andreas'                 | 64.5±7.5 Bb         | 79.6±7.1 Ab                | 791.2±89.9 Ab  | 746.7±82.9 Aa | 12.2±1.2 Ac  | 9.3±0.7 Ba |  |
| CV <sup>3</sup> Cultivars (%) | 1                   | 8.49                       | 1              | .8.42         | 6            | .81        |  |
| CV Cycles (%)                 | 1                   | 4.74                       | 1              | 9.89          | 7            | .66        |  |

Data were presented as mean ± standard deviation. Means followed by the same capital letter in the row and lowercase in the column do not differ significantly by F and Scott-Knott test (p ≥ 0.05), respectively.

<sup>1</sup> TNF - Total number of fruits; TP - Total production; AFFM - Average fresh fruit mass.

<sup>2</sup> First cycle: from September 2018 to March 2019; second cycle: from July 2019 to January 2020.

<sup>3</sup> CV - Coefficient of variation.

The total number of fruits produced in the second cycle was higher, compared to the first cycle, for 'Camarosa', 'Camino Real', 'Monterey', and 'San Andreas'. In the first cycle, 'Aromas' and 'Portola' stood out for the highest number of fruits produced per plant. In the second cycle, 'Aromas' maintained its performance, along with 'Camarosa'. 'Albion' produced the lowest number of fruits in both production cycles (Table 5).

The average mass of fruits produced by all cultivars was higher in the first cycle. In the first cycle, 'Camino Real' produced fruits with greater mass, followed by 'Albion' and 'Monterey', that is, cultivars that presented the lowest number of fruits per plant. On the other hand, 'Aromas', which stood out for the greater number of fruits, produced strawberries with lower average mass. In the second cycle, 'Camarosa', which together with 'Aromas' had produced more fruits, had the lowest average mass (Table 5). These results revealed the effect of greater or lesser competition for photoassimilates and reserves among the fruits.

Reflecting the combination between the number of fruits and their average fresh mass, the production of fruits per plant was lower in the second cycle, compared to the first cycle, only for 'Albion' and 'Portola'. In the first cycle, 'Aromas' and 'Portola' were the most productive, as a result of the greater number of fruits. In the second cycle, 'Albion' presented the lowest production due to the lower number of fruits (Table 5).

#### Phenolic compounds of strawberries

The concentration of the anthocyanins, flavonoids and total polyphenols and the total antioxidant capacity determined in the fruits showed no significant interaction between cultivars x production cycles. However, independently, the content of biomolecules in the berries differed between the two cycles (Table 6) and among the cultivars (Figure 5), except for the content of total anthocyanins among cultivars.

In the second cycle, the total concentrations of anthocyanins, flavonoids and polyphenols and the total antioxidant capacity were higher by 3.8, 4.0, 3.9, and 4.7%, respectively, compared to the first cycle (<u>Table 6</u>).

'Aromas', 'Camarosa', 'Camino Real', and 'Monterey' showed higher levels of total flavonoids in relation to the other cultivars (Figure 5A). 'Camarosa' and 'San Andreas' produced strawberries with total polyphenol contents 36% higher than the fruits produced by the other five cultivars (Figure 5B). Finally, 'Aromas' and 'Camarosa' produced berries with 18% higher total antioxidant capacity than the strawberries produced by the other cultivars (Figure 5C).

## Discussion

The seven strawberry cultivars differed in terms of morphological characters, yield components and phenolic compounds of fruits. The multivariate analysis formed two heterogeneous groups regarding the strawberry

| Table 6. Total levels of phenolic compounds and antioxidant capacity of strawberry cultivars in two production cycles. |
|------------------------------------------------------------------------------------------------------------------------|
|------------------------------------------------------------------------------------------------------------------------|

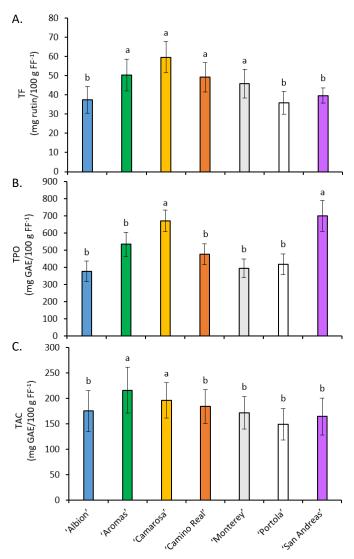
| Cueles              | ТА                                           | TF                                 | ТРО                              | TAC                              |
|---------------------|----------------------------------------------|------------------------------------|----------------------------------|----------------------------------|
| Cycles <sup>1</sup> | (mg PE.100 g FF <sup>-1</sup> ) <sup>2</sup> | (mg rutin.100 g FF <sup>-1</sup> ) | (mg GAE.100 g FF <sup>-1</sup> ) | (mg GAE.100 g FF <sup>-1</sup> ) |
| First               | 805.29±90.5 b                                | 44.46±7.8 b                        | 508.90±77.8 b                    | 175.11±10.4 b                    |
| Second              | 837.79±93.6 a                                | 46.29±8.1 a                        | 529.58±79.3 a                    | 183.76±12.3 a                    |
| CV <sup>3</sup> (%) | 1.25                                         | 1.35                               | 1.16                             | 3.06                             |

Data were presented as mean ± standard deviation. Means followed by the same letter in the column do not differ between each other by F test (p ≥ 0.05).

<sup>1</sup> First cycle: from September 2018 to March 2019; second cycle: from July 2019 to January 2020.

<sup>2</sup> TA - Total anthocyanins; TF - Total flavonoids; TPO - Total polyphenols; TAC - Total antioxidant capacity.

<sup>3</sup> CV - Coefficient of variation.



Means followed by the same capital letter do not differ significantly by Scott-Knott test (p  $\geq$  0.05).

**Figure 5.** Total contents of flavonoids (A) and polyphenols (B) and total antioxidant capacity (C) of fruits of strawberry cultivars in two production cycles.

morphological descriptors. In the first production cycle 'Albion', 'Camino Real', and 'Monterey' had superior fruit production, with the highest average fresh masses. 'Aromas' had productive potential for cultivation for two consecutive cycles. The phenolic compounds of fruits were better in the second cycle, with emphasis on 'Camarosa', which produced fruits with the highest phenolic compounds contents.

The morphological characterization of the seven cultivars, based on the descriptors of this vegetable crop, demonstrated the variability among the materials, regardless of the photoperiod classification as to flowering. The anthocyanin color of the stipules was the morphological descriptor that most contributed to the divergence among cultivars (Figure 3), which reinforces its importance for studies of genetic diversity. Thus, this character can work as a morphological marker of strawberry and support genetic linkage maps applied to plant breeding.

The literature indicates that the external morphoanatomy of the strawberry is contrasting among cultivars (Costa et al., 2019) and can be influenced by environmental conditions, such as temperature and photoperiod (Costa et al., 2021), and by cultural practices, such as nutrient supply and daughter plant transplant times (Trentin et al., 2022). Due to edaphoclimatic, biogeographical, and ecophysiological effects, our results regarding strawberry morphology differ from the literature. 'Camarosa' is an example of a cultivar that presents phenotypic plasticity and, therefore, is quite variable in its morphology (Kamangar et al., 2014; Costa et al., 2021). The domestication of the strawberry caused the reduction of its morphological and genetic diversity. Thus, it is important to study the characteristics available in the genetic resources of this horticultural crop in order to improve the cultivars to ensure biodiversity and to meet the demand for fruit production.

With the growing appreciation of strawberries by the consumer market, producers need to adopt strategies to supply more berries to distribution centers. Among the phytotechnical tools that can be used is the management of plants after their transplant, still in the first production cycle. Commonly, strawberries produced in the first production cycle are larger and, apparently, more attractive (Antunes et al., 2014; Zeist et al., 2019), as verified in our study (Table 5). This is mainly due to the greater quantity and size of leaves originated in the first crop, symbolizing the source and drain dynamics in the relationships between growth characteristics and those of production (Ahn et al., 2021).

In the growing regions of the Brazilian subtropics, after transplanting bare-root daughter plant, produced in environments with quantity and quality of cold, the plant directs its photoassimilates to emit leaves as a survival strategy (Trentin et al., 2022). Especially in ND cultivars, this occurs because their flowering takes place by the autonomous route, mediated by the emission of a certain number of leaves that precedes the differentiation of flowering buds (Costa et al., 2021). Thus, this greater amount of vegetative canopy characterizes a greater source of photoassimilates in plants, which are converted, through photomorphogenesis, into fruits. As the leaves were removed throughout the crop cycle and at the time of plant renewal (transition between the first and second cycles), aiming at the phytosanitary quality of the materials being cultivated, fruit size reduced in the second cycle (Table 5). Partial leaf removal compromises strawberry yield components because the removal of energy source organs reduces the production of photoassimilates (Sønsteby et al., 2021).

The literature reports different productive performances of strawberry cultivars. <u>Chiomento et al. (2021)</u>, in a study conducted in traditional cultivation (soil planting) and in a greenhouse, found that the production of marketable fruits (grams per plant) was 295, 217, 434, 369, 356, 453, 420, 327, and 316 for 'Albion', 'Aromas', 'Camarosa', 'Camino Real', 'Fronteras', 'Merced', 'Monterey', 'Portola', and 'San Andreas', respectively. However, in substrate and greenhouse cultivation, for example, the production (grams per plant) was 265, 303, 253, 318, 325, 288, and 234 for 'Albion', 'Aromas', 'Camino Real', 'Fronteras', 'Monterey', 'Portola', and 'San Andreas', respectively (<u>Chiomento et al., 2023</u>). These values were lower than those obtained in our study (<u>Table 5</u>), regardless of the cultivation cycle.

In the first production cycle, 'Albion', 'Camino Real', and 'Monterey' produced the largest berries, which contributed to these cultivars having the lowest number of fruits. Plants with many fruits do not have adequate distribution of photoassimilates among these drains, which compromises the size and quality of strawberries. This is due to the insufficient amount of leaf area to adequately supply the demand of photoassimilates for fruit development. In strawberry, genes involved in photosynthesis (gene32260v1.0-hybrid, gene02096-v1.0-hybrid, gene20355-v1.0hybrid, gene19575-v1.0-hybrid, gene27842v1.0-hybrid) increase their level of expression under high concentration of carbon dioxide (CO<sub>2</sub>), which improves the ability to transport photoassimilates (Li et al., 2020). Thus, high CO<sub>2</sub> accelerates fruit growth, improves berry quality, and increases crop yield (Trentin et al., 2022).

In the second cycle, 'Camarosa', 'Camino Real', 'Monterey', and 'San Andreas' produced the greatest amount of fruit. These results contradict those obtained by <u>Zeist et al. (2019)</u>, who observed a reduction in the number of strawberries in the second cycle for 'Albion', 'Camino Real', 'Monterey', and 'San Andreas'. This evidences that the cultivation agroecosystem, the production system and the soil and climate conditions interfere in the productive potential of strawberry cultivars maintained for more than one cycle. Furthermore, an interactive set of microclimatic factors in greenhouses, such as photoperiod, light intensity and air and soil temperature (<u>Costa et al., 2021</u>), affect strawberry productivity (<u>Ahn et al., 2021</u>).

Fruit production per plant in the second cycle, compared to the first, was lower only for 'Albion' and 'Portola' (Table 5). The genetic characteristics of these cultivars may have influenced their productive performance in the second cycle, in the adopted cropping system. Therefore, both cultivars cannot be used as an alternative for producers who aim to keep the same plants for more than one consecutive cycle. 'Aromas', in turn, had the best production performance in both production cycles (Table 5). Thus, this cultivar has the potential to be cultivated for two consecutive seasons. The literature reports that 'Aromas' stands out for its productive performance and phenolic compounds of fruits (Chiomento et al., 2021), important attributes in the strawberry crop.

The antioxidant activity and the levels of anthocyanins, flavonoids and polyphenols determined in the fruits showed no interaction among cultivars and production cycles. However, independently, they differed between cycles and cultivars. In the second cycle, the concentrations of anthocyanins, flavonoids, total polyphenols, and the total antioxidant capacity in the berries were higher than in the first cycle (Table 6). These results differ from those reported by <u>Antunes et al. (2014)</u>, in which fruits produced in the first cycle had better physicochemical and sensory characteristics. Again, we emphasize that biogeographic, ecophysiological, and edaphoclimatic conditions can interfere with the production of biomolecules in berries (<u>Chiomento et al.</u>, 2021).

The main factors that influence the phenolic compounds of strawberries are the fruit harvest period, the soil and climate conditions of cultivation and the production systems adopted. However, in our study, the variability in the phenolic compounds of the berries can not be explained by the mentioned factors because all the cultivars were inserted in the same agroecosystem and in the same growth medium (Table 1). Therefore, we emphasize that the differences in the phenolic compounds are attributed to the genetic characteristics of the cultivars studied, which has already been demonstrated by <u>Chiomento et al. (2021</u>). The effect of genotype on strawberry quality is stronger than the effect of growing conditions (<u>Zhang et al., 2022</u>).

Anthocyanins are responsible for the red color of strawberries and have numerous health benefits. The main anthocyanin found in this berry is pelargonidin-3-Oglycoside, with cyanidin-3-O-glycoside and pelargonidin-3-O-rutinoside present as minor components. In strawberries, for example, flavonoids act as an antioxidant and are important in the prevention of cardiovascular diseases. On a global scale it has been observed that repeat purchases by consumers depend on the taste and quality of the berries. So, the challenge is to encourage consumers to pay more for locally produced fruits with higher quality and to instruct producers to pay more attention to the maturity and flavor of the harvested strawberries (Taghavi et al., 2019). Among the seven cultivars studied, 'Camarosa' produced fruits with the highest levels of biomolecules (Figure 5), which was also reported by Chiomento et al. (2021). Therefore, we recommend the cultivation of 'Camarosa', in a staggering system of cultivars, so that producers can meet an increasingly demanding consumer market.

By studying the morphology, phenolic compounds, and yield components, we were able to know and understand the performance of seven strawberry cultivars kept in cultivation for two consecutive cycles. Our results suggest that different cultivars can be used for different purposes (production or phenolic compounds). Thus, producers must choose materials with different aptitudes (production and fruit quality) according to the demands of the consumer market. For example, 'Aromas' can be indicated to producers who are looking for a material that remains in high production for a second production cycle. This makes it possible to positively impact producers' income and enhance the strawberry production chain. Furthermore, 'Aromas' and 'Camarosa' could participate as progenitors in strawberry breeding programs aimed at obtaining new materials with greater productive and qualitative potential.

Knowledge of this horticultural variability can be useful for the selection of more productive materials and/or rich in

bioactive compounds (<u>Chiomento et al., 2021</u>). For example, phenolic compounds are considered a hereditary trait, which can be enhanced through plant breeding programs, which aim to produce cultivars with better nutritional quality combined with high production efficiency (<u>Zhang et al.,</u> <u>2022</u>). We emphasize that future studies should focus on: (1) investigating pruning times and intensities of cultivars at the end of their productive cycle; (2) understand the interaction among agroecosystems, geoedaphoclimatic aspects and strawberry cultivars, in order to select the best combinations; (3) to verify the efficiency of maintaining strawberry cultivars under conditions of multiple stressors.

## Conclusion

Strawberry cultivars grown for two consecutive cycles in a greenhouse show variations in their horticultural potential.

The multivariate analysis forms heterogeneous groups regarding the morphological descriptors of the strawberry.

'Aromas' has productive potential for cultivation for two consecutive cycles. In the first production cycle 'Albion', 'Camino Real', and 'Monterey' stand out for their fruit production, with the highest average fresh masses.

Phenolic compounds of fruits are better in the second cycle, indicating that the maintenance of the same plants for the next harvest can be a strategy to increase the content of these biomolecules in the berries. 'Camarosa' produces fruits with the highest phenolic compounds content.

Producers who want to stagger their crops should choose 'Aromas' to obtain greater productive potential and 'Camarosa' to meet the demanding consumer market in fruit quality.

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## **Compliance with Ethical Standards**

**Author contributions:** Conceptualization: MDC, EOC, JLTC; Data curation: MDC, TST, FSN; Formal analysis: MDC, DCS; Investigation: MDC, EOC, JLTC; Methodology: MDC, TST, FSN; Project administration: MDC, JLTC; Resources: MDC, JLTC; Supervision: MDC, JLTC; Validation: MDC, EOC, JLTC; Visualization: MDC; Writing - original draft: MDC, JLTC; Writing - review & editing: JLTC.

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