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ORIGINAL RESEARCH PAPER

Biostimulants and bioactivator in strawberry production (*Fragaria vesca* L.): Post-harvest production parameters

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Abstract: Strawberries are classified as pseudofruits and are included in the genus *Fragaria*. There are several cultivars and hybrids of strawberries, with different lengths, diameters, colors and flavors. This study aimed to evaluate biostimulants and bioactivators in the development of morphological reproductive parameters in *Fragaria vesca* strawberry pseudofruits. The experiment was carried out in the field in pots, with two biostimulants (Stingray and ST Pro) and a bioactivator (Biozyme) inoculated into strawberry seeds. The evaluation parameters were length, diameter, fresh mass and dry mass of pseudofruits at the commercial maturity stage. The Stingray biostimulant proved to be an excellent biostimulant option with satisfactory results for pseudofruit length with an average of 3.87 cm, average diameter of 2.83 cm and fresh mass with an average of 13.49 g per fruit^{-1}. The biostimulants and bioactivator have positive effects on the strawberry crop (*Fragaria vesca*).

Keywords: *Fragaria* genus; strawberry pseudofruit diameter; ST Pro biostimulant; strawberry culture.

1. Introduction

The genomic composition of the octoploid (2n = 8x = 56) cultivated strawberry, *Fragaria x ananassa*, is one of the most complex in plant species of agricultural interest. The genus *Fragaria* is made up of 23 species with varied distribution throughout the Northern Hemisphere and also extends to Hawaii and south along the west coast of South America (Davis et al., 2007; Shulaev et al., 2011).

Several species of *Fragaria* and a large number of hybrids are cultivated in various parts of the world, including *F. chiloensis* in South America, and *F. moschata* and *F. vesca* in Europe (Darrow, 1966; Hancock, 1999; Doumett et al., 2011), however, The combined *Fragaria* strawberry crop is insignificant when compared to *Fragaria x ananassa*. The origin of strawberry cultivation dates back to the 1700s, when octaploid individuals of *F. chiloensis* and *F. virginiana* were brought from Europe to North and South America, where they were initially cultivated in European horticultural gardens.

The genus *Fragaria* belongs to the family Rosaceae and subfamily Rosoideae. *Fragaria* was initially represented in two molecular phylogenetic studies of the family Rosaceae by Morgan et al. (1994) and Eriksson et al. (1998), however, these large studies included only two species of *Fragaria*, where they did not provide information on the species' relationships with *Fragaria*. The monophyly of *Fragaria* is considered to be well-supported (Potter et al. 2000), but species relationships within *Fragaria* have not been adequately delineated. Molecular phylogenetic resolution within *Fragaria* has been limited, in part, by the low levels of variability detected in the nuclear ITS (internal transcribed spacer of rDNA) and chloroplast DNA (cpDNA) sequences that have been used for phylogenetic analysis by Harrison et al. (1997) and Potter et al. (2000).

According to Santos (2021) and Godinho & Beladeli (2022), strawberries are a pseudofruit that presents a greater quantity of aessorial tissue compared to other pseudofruits. Furthermore, it is highly appreciated throughout the world, being used fresh in the production of juices, sweets, jellies, confectionery and pectin production. Currently, the growing desire for culinary use and in sweets has led to this pseudo-fruit gaining attention from rural producers around the world. Today it is one of the most cultivated pseudofruits in Brazil, and the largest strawberry producing states are Minas Gerais (MG), Rio Grande do Sul (RS), Paraná (PR) and São Paulo (SP) (HFBrasil, 2020), however, this crop has also been cultivated in the Federal District (DF).

Brazil has great economic importance in the production of strawberries, being the second largest producer in Latin America (Carvalho et al., 2013). However, strawberry cultivation in Brazil presents a reduced number of scientific studies aimed at improving the production and development rates of these pseudofruits with greater mass, flavor and production (Franco et al., 2017). The trend towards a new agriculture through organic, semi-organic cultivation or in agroecological systems has been gaining strength on Brazilian soil, this is due to demanding consumers, stricter policies in maintaining the environment, reducing the use of pesticides that contaminate the environment soil and groundwater and encouraging the use of natural or synthetic biostimulants and bioactivator composed of micro and macronutrients, amino acids, plant hormones such as gibberellins (GA), auxin (AX) and cytokinins (CK), ethylene (Et), abscisic acid (ABA), brassinosteroids (BR), jasmonates (JA), salicylates (SA) and extracts from brown algae such as *Ascophyllum nodosum* capable of stimulating a higher rate of germination, development and increased agricultural production (Gonçalves et al., 2016; Taiz et al., 2017).

Ascophyllum nodosum (Linnaeus) Le Jolis belongs to the family Fucaceae of the order Fucales, brown algae (Phylum Ochrophyta, Class Phaeophyceae). The group has several representatives in colder waters of the southern hemisphere, *Ascophyllum* being the only one from the northern. A near relative is the South African *Axillariella constricta* (Kützing) Gruber (Baardseth, 1970; Guiry; Guiry, 2020; Pereira et al., 2020). *A. nodosum* has polyphenols and polysaccharide complexes in its natural chemical composition, with antioxidant effects and which can stimulate stress tolerance in plants, in addition to plant hormones that help in plant development (Araújo, 2016; Vieira et al., 2023).

Species *Brassica napus*, *Solanum lycopersicum*, *Daucus carota*, *Cucumis sativus*, *Glycine max*, *Spinacia oleracea*, and *Hordeum vulgare* were all stimulated by agricultural biostimulants (De Saeger et al., 2020; Kumari et al., 2023). According by Arioli et al. (2020) and Hussain et al. (2021), the seaweed extracts have the capacity to sustainably increase the quality and yield of crops with different soil types and farming systems; for example, foliar and soil applications of seaweed extract enhanced the yield and post-harvest qualities of strawberries, tomatoes, vegetables, and sugarcane.

The use of biostimulants is an innovative tool capable of producing satisfactory results in the development of various crops, including strawberries. Briefly, biostimulants are natural or synthetic products composed of a mixture of nutrients and microorganisms that, when applied via seed inoculation, furrow or foliar route, increase nutritional efficiency and tolerance to abiotic and biotic stresses, improving the quality of the crop, with a direct impact. and potential on productivity and post-harvest quality (Campos, 2020).

Izidório et al. (2015) discussed advances in the current market of natural products capable of biostimulation, significantly increasing crop productivity. However, Brazil has difficulties for companies to produce these products on a large scale, as there are no specific laws for this class of plant stimulants, being registered in the "Plant Growth Regulator" class by MAPA – *Instrumentação Normativa Conjunta* "Joint Normative Instrument" nº 32, of October 26, 2005 (Brazil, 2005; Campos et al., 2020).

According to Du Jardin (2015) and Campos et al. (2020) the registration of biostimulants is carried out only by the rates of macro and micronutrient content and the existence of contaminating residues, similar to that carried out for pesticides and conventional fertilizers, as there are no defined parameters for analyzing this category of products in the germination process, growth, development, and productivity gains of crops.

Therefore, observing the few studies that evaluate the use of biostimulants through seed inoculation for strawberry cultivation and their mass gain attributes and morphological parameters in the reproductive phase, this study aimed to evaluate three biostimulants and bioactivator via strawberry seed inoculation (*Fragaria vesca*) (Figure 1) on morphological effects in post-harvest.

Figure 1. Strawberry pseudofruits (*Fragaria vesca*). Source: Authors, 2024.

2. Material and Methods

2.1. Experimental location

The experiment was conducted in an open area in full sun in the research area Menezes Agricultural Research, Antônio Menezes & Filhos Farm, located in the municipality of Rio Verde, State of Goiás, Brazil, at geographic coordinates (17º43’06.7”S and 50º53’07.0”W) between the months of September to December 2023.

The experiment site is located at an altitude of 748 m above sea level, with an AW (tropical rainy) climate according to the Köppen-Geiger classification, with an average of 25.6 ºC and two well-defined seasons, one rainy (October to May) and dry (May to October. Rainfall between 750 and 2000 mm/year (Cardoso et al., 2014).

2.2. Strawberry cultivar

The strawberry cultivar used was *F. vesca* (Sementes Isla, Brazil). Characteristics: naked seeds, red skin color, red pulp color, greater sweetness, conical shape, perennial annual cycle, summer cycle 80, winter cycle 110, pseudofruit diameter 2-4 cm. Seed requirement for planting: germination days 7-14 days, spacing between rows 20 cm, spacing between plants 20 cm, approximate number of seeds per gram 2853 g. Germination rate of 80% producer. Our study obtained a germination rate of 91%.

2.3. Planting soil analysis

Soil parameters were determined in a layer between 0-20 cm deep with the following results: Ca = 2.13, Mg = 1.43, K = 0.30, P = 3.0, S = 9.0, Na = 1.0, organic matter (OM) = 61.1 and pH = 4.9. Clay = 30.3, Silt = 25.2 and sand = 44.4.

2 g of lime was applied per pot 10 days before the application of nutrient solution via soil (Forth Frutas) NKP+9 nutrients. Nitrogen (N) 12%, Phosphorus (P_20_5) 5%, Potassium (K_2O) 15%, Calcium (Ca) 1%, Magnesium (Mg) 1%, Sulfur (S) 13%, Boron (B) 0.06% , Copper (Cu) 0.05%, Iron (Fe) 0.22%, Manganese (Mn) 0.10%, Molybdenum (Mo) 0.005% and Zinc (Zn) 0.20%. The solution consists of 75 g of product for 100 L of water. For each repetition, 150 mL of solution was applied.

2.4. Bioestimulants and bioactivator

Biostimulant ST Pro (Grap)® mixed mineral fertilizers with Cobalt (Co) 1% (12.8 g/L), and Mn 7.0% (89.6 g/L), density 1.28 g mL^{-1}, electrical conductivity (EC) = 51.53 mS cm^{-1}, saline index 19.56%, physical nature fluid in solution and *Ascophyllum nodosum* algae extract. Stingray (Koppert)® composed of K_2O 5.3% (61.46 g/L), Total organic carbon (C) 6% (69.60 g/L), pH = 8.0, density 1.16 g/mL^{-1} at 20 ºC, saline index 18% and extract of *Ascophyllum nodosum* algae 100%, and biostimulator Biozyme (Arysta LyfeScience)® composed of Nitrogen (N_2) 1% (18 g/L), Potassium oxide (K_2O) 5% (60 g/L), B 0.08% (0.96 g/L), Fe 0.40% (4.8 g/L), Mn 1% (12 g/L), Zn 2% (24 g/L) and total C 3.50% (42 g/L).

2.5. Experimental design, planting and cultural treatments

The experimental design adopted was a randomized block design (RBD) 3x10+1, where three was the number of biostimulants and bioactivator, ten was the number of replications + one control.

The planting system used was in “tunnels” with 75% white shade applied throughout the experiment period. The experiment was carried out in 5 L black High Density Polyethylene pots. Each representation received 4.5 kg of soil. For seed inoculation, 1.5 mL of biostimulants and bioactivator were used per kg of seeds.

After inoculation, the seeds remained in the biostimulator solution until planting (~15 min). 15 seeds were sown per replication at a depth of 0.5 cm. Daily watering was always carried out early in the morning (7 am) and early in the evening (6 pm) with natural lagoon water. 30 days after germination, only 4 plants were maintained per replication and after 65 days, 2 plants were maintained per replication.

Cultural treatments were carried out weekly and biological control was used preventively through the application of insecticide to control mites (*Neoseiulus californicus*) and fungicide to control *Beauveria bassiana*. In the natural environment, pollinating insects composed of bees *Tetragonisca angustula* and *Apis mellifera* were used naturally in the experiment to increase the pollination efficiency of flowers.

2.6. Post-harvest analyzes

For post-harvest evaluation, 5 strawberries were randomly collected per repetition, in the state of commercial maturity (i.e. 100% of the surface with red color) (~100 days). Fruit length (FL) and fruit diameter (FD) were analyzed using a digital caliper with results expressed in centimeters (cm).

The fresh (FMPs) and dry mass (DMPs) of the pseudofruits were obtained using a digital analytical balance (Shimadzu, Modl AY220, Japan) and the results are expressed in (g). For dry mass, the pseudofruits were placed in metal trays and transferred to an oven with forced air circulation (Thoth, Mod. Th-510-480, Brazil) with a temperature set at 65 ºC for 24 h.

2.7. Statistical analysis

Based on the average values obtained in the post-harvest parameters, analysis of variance (ANOVA) was carried out and when a significant variation was found, the *Tukey* test was adopted with 5% significance using SISVAR software (Ferreira, 2019).

3. Results

3.1. Post-harvest parameters

The results demonstrated that for the length and diameter of pseudofruits, the Stingray biostimulant demonstrated a significant difference between the other biostimulants, bioactivator and control ($p < 0.05$). The Stingray biostimulant showed superior results for pseudofruit fresh mass with an average of 13 g ($p < 0.05$) compared to the other products and control.

For dry mass of pseudofruits, two groups were observed (a) ST Pro and Stingray and (b) Control and Biozyme with ($p > 0.05$) showing no statistical difference between the products and control within the two groups, however, the group (a) demonstrated results with averages between 0.9 and 1.0 g higher than group (b) (Table 1).

Table 1. Post-harvest parameters for strawberry pseudofruits (*Fragaria vesca*) using biostimulants and bioactivator.

Bio and Act	FL (cm)	FD (cm)	FMPs (g)	DMPs (g)
Control	2.96 c	2.28 c	6.91 c	0.52 b
ST Pro	3.43 b	2.61 ab	10.64 ab	0.98 a
Stingray	3.87 a	2.83 a	13.49 a	1.05 a
Biozyme	3.40 b	2.43 bc	9.79 bc	0.65 b
CV (%)	10.00	9.00	25.46	29.04

Note: Bio = Biostimulants; Act = Activator. Means followed by the same letters statistically equal by the *Tukey* test at 5% significance.

4. Discussion

The biostimulatory and promoting action in obtaining results of superior morphological parameters for strawberry fruits varies between the concentrations of the biostimulants. Researchers such as Buchanan et al. (2001) and Santos et al. (2017) discuss the effects of biostimulants on seeds. Abiotic factors must be highlighted, as the action of biostimulants on the seeds and the application of biostimulants on the surface of the seeds do not guarantee their absorption, even in their entirety. Still in these studies, the amount of biostimulant absorbed depends on the seed contact area and the amount of water and concentration of the solution, containing biostimulants, absorbed by the seeds, where the lower absorption of biostimulants may have compromised some of our results.

Some studies show the positive effect of applying biostimulants in strawberry cultivation on reproductive parameters. However, vegetative parameters are controversial or non-existent, and this was observed by Godinho & Beladeli (2022) where they found no significant difference for the height of strawberry plants, however, for the diameter of the pseudofruits with an average value of 4.48 cm, it was observed significant difference ($p < 0.05$) when biostimulant based on coffee grounds syrup was applied.

Encouraging results were also described by Oliveira (2020) where a biostimulant based on cytokinin, auxin and gibberellin was applied to the strawberry cultivar 'San Andres' via foliar with a larger diameter for the pseudofruits as well as for total commercial production; Dias et al. (2015) found that biofertilizers applied to strawberry cultivation in the State of Ceará, Brazil, obtained satisfactory results. In a study carried out in Portugal by Silva et al. (2014) using biostimulants at a dose of 4.5 L/ha^{-1} applied every 15 days demonstrated an increase in strawberry productivity of up to 20%; positive effects are also described by Vignolo et al (2011) when applying natural fertilizers pre-planting strawberries with results superior to the control throughout the crop cycle, and finally, Alam et al. (2013) obtained an increase in productivity between 30-40% and a greater number of strawberry pseudofruits per plant for the cultivars 'Camarosa', 'Chandler' and 'Festival' with doses between 1-2 g/L of the biostimulant powder based on of *A. nodosum*.

In our results, we verified that the ST Pro and Biozyme stimulant showed no effect when applying a biostimulant with less than 100% extract and the bioactivator without *A. nodosum* extract. In contrast, the biostimulant Stingray and *F. vesca* showed an interaction, possibly due to the concentration of the *A. nodosum* algae extract being 100%, which enabled superior and significant effects ($p < 0.05$). Similar results were obtained by Alam et al. (2013) where the strawberry cultivar 'Albion' did not demonstrate sensitivity to the application of the biostimulant, offering no response in terms of productivity parameters. In this sense, we highlight the comments reported by Buchanan et al. (2001) and Santos et al. (2017) at the beginning of this discussion.

As noted, the use of biostimulants for strawberries or any other crop presents satisfactory results, however, the doses must be respected, and when we do not know the correct dose, this must be elucidated through experiments with dose variations. In this sense, Oliveira et al. (2014) report in a study that doses of biostimulants higher than recommended can interfere with the development of plants of agricultural interest, as it leads to a nutritional imbalance in the soil and, at the same time, a reduction in productivity.

5. Conclusion

The use of biostimulants and bioactivators for the morphological parameters of *Fragaria vesca* strawberry pseudofruits presented encouraging results regarding the use of products based on brown marine algae *Ascophyllum nodosum*, however, the concentration of this extract must be 100% for real biostimulatory activity in evaluated production parameters.

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Author's Contributions

Lorraine Boa Ventura de Jesus: project writing, field study development, analysis, article writing and publication. *Antonio Carlos Pereira de Menezes Filho*: writing the study, monitoring the field experiment, writing and translation. *Porshia Sharma*: translation, grammatical corrections and complementing ideas. *Aurélio Ferreira Melo*: text corrections, laboratory analyses, discussion of results. *Matheus Vinícius Abadia Ventura*: statistical analysis, post-evaluation corrections and publication.

Ethics

No applicable.