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Biochar as an alternative to improve the *in vitro* environment for Pitaya (*Hylocereus undatus* Haw) and strawberry (*Fragaria x ananassa* Duch) growing

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In vitro **cultivation is widely used for micropropagation of several plant species; however, the increase in the gas concentration, such as ethylene gas, inside the growth vials can cause physiological changes. Thus, 4 experiments were carried out at the Federal University of Minas Gerais to evaluate the biochar use from sugarcane bagasse in vials with or without caps with porous membrane in the *in vitro* growing of pitaya (*Hylocereus undatus* Haw) (experiment 1) and strawberry (*Fragaria x ananassa* Duch) (experiment 2) and evaluating in combination of the use of biochar from sugarcane bagasse and $AgNO_3$ in the *in vitro* growing of pitaya (experiment 3) and strawberry (experiment 4). The types of caps did not affect the growth and number of pitaya and strawberry; however, using caps with porous membrane obtained more magnificent roots dry matter yield. The addition of 4 g L^{-1} of biochar, regardless of cap type, favored the pitaya and strawberry roots' growth and development. $AgNO_3$ alone contributed to the higher length and dry mass of pitaya roots and greater dry mass of strawberry roots. Adding biochar decreases the $AgNO_3$ effects *in vitro* culture. Biochar is an alternative to improve the *in vitro* environment of plant species.**

Key words: *Fragaria x ananassa* Duch, *Hylocereus undatus* Haw, Tissue culture, $AgNO_3$, gas exchange.

INTRODUCTION

In vitro micro propagation is a rapid technique used to produce large-scale seedlings of various plant species. The vails used are generally sealed, which interferes in gas exchange with the external environment, favors the increase of relative air humidity and concentration of gases such as ethylene, and reduces CO_2 and O_2 inside

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the vails. High gas concentrations inside the vails can alter some plant physiological processes, such as multiplication, growth, development, and survival (Naing et al., 2014; Waikhom and Louis, 2014). Gaseous exchange between the vials atmosphere and the outside environments favors the growth and development of plant tissues (explant) during *in vitro* growth. It improves the plant's adaptation to the acclimatization stage since the gas exchange is directly involved in the quantity and stomatal efficiency and plants' metabolite production (Iarema et al., 2012; Mohamed and Alsadon, 2010; Taiz et al., 2017; Vieira et al., 2019). Some techniques have been used to control gas concentrations and gas exchange *in vitro* growth, such as caps with a microporous membrane (Mohamed and Alsadon, 2011; Santana et al., 2011). The addition of activated charcoal or $AgNO_3$ is used to decrease the concentration of some undesirable gases as ethylene (Rodrigues et al., 2017). Ethylene gas is a plant hormone that can inhibit plant growth during *in vitro* growth (Di Lonardo et al., 2013). In addition to allowing gas exchange between the vials and the outside environments, the caps with microporous membranes favor increased CO_2 concentration and relative air humidity inside the vials and increase plant transpiration and uptake of water and nutrients (Kozai, 2010; Xiao et al., 2011). Due to these effects, there may be an increase in photosynthetic capacity, multiplication rate, and plants' survival in the acclimatization stage (Hoang et al., 2020). The activated charcoal addition in both liquid and semi-solid media for tissue culture aims to reduce ethylene's concentration inside the vials since activated charcoal particles have surface electric charges that immobilize ethylene and other compounds that can damage plants *in vitro* growing (George et al., 2008). Besides, the active charcoal can inhibit tissue browning and favor the embryogenesis, androgenesis, rooting, shoot, and root elongation (Thomas, 2008).

Some authors recommend adding $AgNO_3$ to the culture medium instead of activated charcoal. $AgNO_3$ reduces the harmful ethylene effects *in vitro* culture since silver ions inhibit this gas's action (Cardoso, 2019; Taiz et al., 2017). Another possible alternative to improve the microenvironments of culture vials is the use of biochars. Biochar are carbon-rich compounds obtained by pyrolysis (thermal decomposition in the absence or partial presence of O_2) of organic residues (Amoah-Antwi et al., 2020; Islam et al., 2021). Biochars have porous structures and surface functional groups with electric charges that can adsorb metals and gases (Butnan et al., 2015). Di Lonardo et al. (2013), working with *Populus alba* L. *in vitro*, found that the addition of biochar in the culture media reduced ethylene concentrations and favored plant development. The positive effects of activated charcoal and biochar on plant tissue culture are possibly related to the presence of some substances released by these materials that promote plant growth and development and the adsorption of some inhibitory compounds, such as ethylene (Di Lonardo et al., 2013; Thomas, 2008). Although there are few reports in the literature about the effects of biochar *in vitro* growth, this material is considered an adsorbent of organic and inorganic pollutants due to their properties (high aromaticity, surface charges, and porosity) (Bian et al., 2014; Wang et al., 2014). In general, there are significant increases in the number of thin root plants and plant yield in soils with biochar (Prendergast-Miller et al., 2014; Silva et al., 2017; Xiang et al., 2017). These biochar effects on the plant root system associated with their gas adsorption capacity are desirable for *in vitro* growth. Thus, the objective of this work is to evaluate the effects of biochar, the cap with or without porous membrane, activated charcoal, and the $AgNO_3$ on the internal micro-environment of vials used for the *in vitro* growth of pitaya and strawberry.

MATERIAL AND METHODS

The experiment was carried out at the Federal University of Minas Gerais, in the city of Montes Claros, MG, Brazil (16°40'57.50" S; 43°50'26.07" W; 650 m). In this study, four experiments were carried out. In the experiment 1 and 2, vials sealed with caps with or without porous membrane that allowed gas exchange (orifice sealed with microporous membrane) were used for *in vitro* growth of pitaya and strawberry, respectively. Explants of pitaya (1 dm in length) and strawberry (2 cm in length) were previously produced *in vitro* from seeds of *Hylocereus undatus* Haw and propagules of *Fragaria x ananassa* Duch, respectively. The experiments were carried out in a completely randomized design, in a 5x2+2 factorial scheme, with 6 replications and 5 explants per vial. The treatments consisted of 5 doses of biochar (0, 1, 2, 3, 4 g L^{-1}), 2 types of caps (with and without microporous membrane), and 2 additional treatments with 2 g L^{-1} of activated charcoal in vials with and without caps with microporous membrane (Figure 1). Experiments 3 and 4 were carried out in a completely randomized design, 5x2 factorial schemes, with 6 replications and 5 explants per vial of pitaya and strawberry, respectively. The treatments consisted of five $AgNO_3$ concentrations (0, 5, 10, 20, 40 µM L^{-1}), without and with 4g L^{-1} of biochar from sugarcane bagasse (Figure 1). Each experimental unit consisted of a 250 cm^3 vials containing 40 mL of on solid MS (Murashige and Skoog, 1962) gelled (0.7% agar, w/v) medium supplemented with 30 g L^{-1} of sucrose, 7 g L^{-1} of agar, 0.49 µM of Indolbutyric Acid (IBA), at pH 5.5. The media were sterilized by autoclaving at 121°C and 108 kPa for 20 min. The experimental units were maintained in a growth chamber at 25 ± 2ºC, 36 µmol mol m^{-2} s^{-1} of irradiance, supplied by 20 W LED lamps, and 16 h photoperiod. On 60 days after the experiments set up, the seedlings were removed from the culture medium and separated into shoots and roots, which were submitted to distilled water asepsis to remove the excess of medium adhered to them. At the end of the experimental period, the length, number, and dry mass of roots were evaluated. For dry mass evaluation, the roots were dried in a forced-air oven at 70°C for 72 h (Figure 1). The biochar was produced from sugarcane bagasse after the mechanical extraction of the juice. The temperature was elevated at a rate of approximately 5°C min^{-1} until 450°C (temperature was controlled by a thermocouple inserted in the center of the carbonized mass) with a residence time of 30 min. The biochar was ground and passed through a 0.5 mm mesh sieve for the experiment and analysis. Biochar yield was calculated as the pyrolyzed biochar (dry wt.) ratio to the dry mass of the unpyrolyzed feedstock (dry wt.) (biochar yield

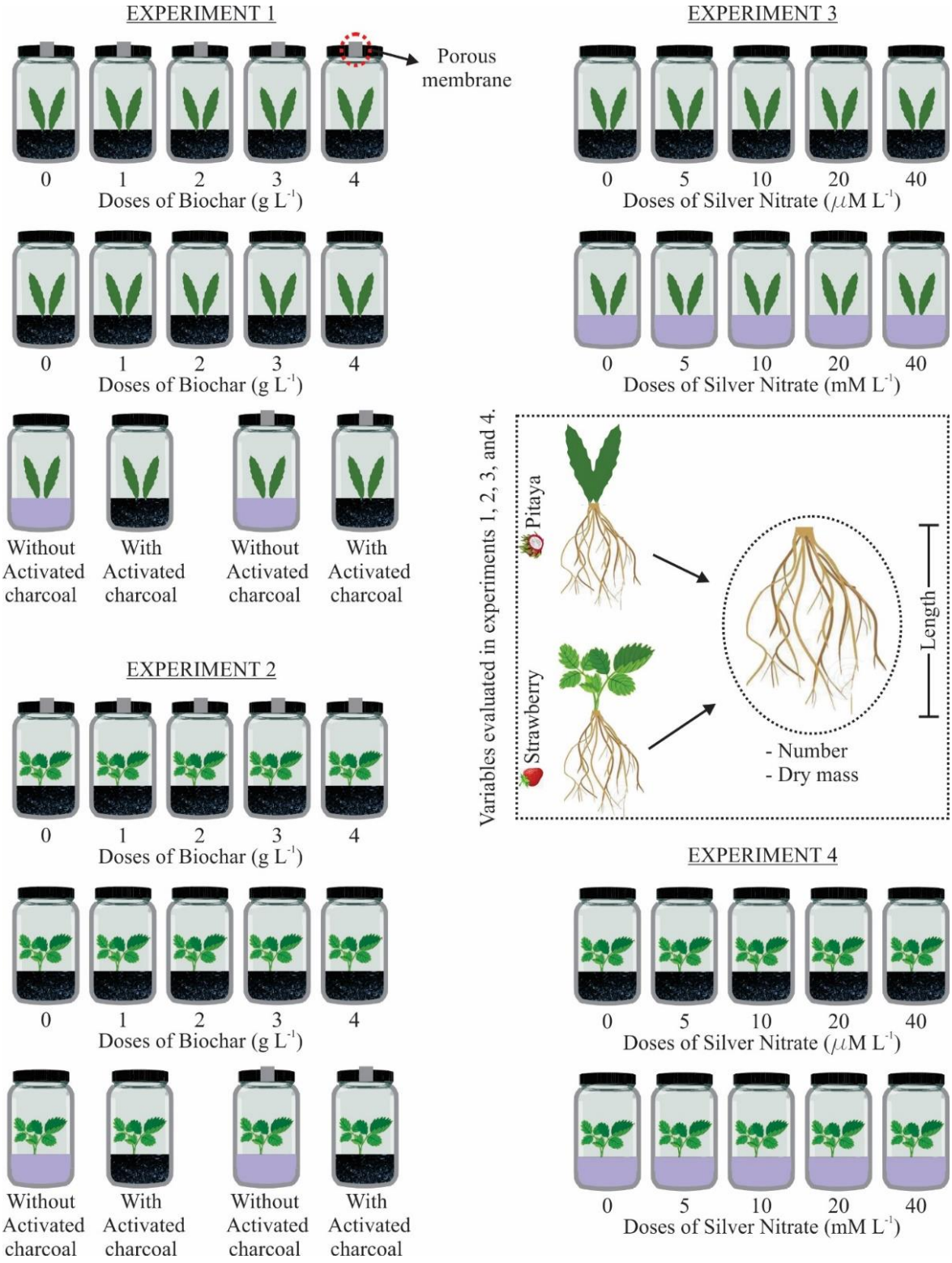

Figure 1. Schematic representation of the methodology. Source: Author

(%) = (biochar dry mass/feedstock dry mass) × 100) (Table 1).

For the biochars characterization, pH and EC in water (1:10 v/v) were determined following (Rajkovich et al., 2012), and ash was measured according to ASTM D1762-84. Total nutrients and trace elements were determined via ICP-MS/MS (Agilent 8800 triple quadrupole), after microwave digestion (MARS 6 - Microwave

Table 1. Biochar characterization.

Property	Value
Yield (%)	29 ± 1.8
Maximum temperature (^{o}C)	350 ± 0.0
Carbonization time (h)	2.0 ± 0.0
pH	6.5 ± 0.3
Electric conductivity (mS cm^{-1})	99.3 ± 3.4
Density (kg m^{-3})	680 ± 10.5
Ashes (%)	30 ± 1,5
Total carbon (g kg^{-1})	283.5 ± 11.5
Total nitrogen (g kg^{-1})	12.3 ± 0.9
C/N ratio	23/1
P (g kg^{-1})	23.5 ± 1.8
K (g kg^{-1})	4.3 ± 0.4
Ca (g kg^{-1})	18.2 ± 1.1
Mg (g kg^{-1})	5.6 ± 0.7
S (g kg^{-1})	12.5 ± 0.6
Cu (mg kg^{-1})	45 ± 1.5
Mn (mg kg^{-1})	184 ± 14.7
Zn (mg kg^{-1})	326 ± 14.6
Fe (mg kg^{-1})	23.9 ± 7.4
B (mg kg^{-1})	37.1 ± 1.3

Average of 5 replicates and ± confidence interval.
Source: Author

Digestion System, CEM Mars Corporation) with concentrated nitric acid using method described in USEPA 3051; and full C and N content by dry combustion method using a LECO CN-2000 elemental analyzer (Leco Corp., St. Joseph, MI, USA). Data were assessed for normality and heterogeneity of variance. In experiments 1 and 2, data were submitted to analysis of variance. When significant, the F ($P < 0.05$) test compared the type of caps, and each dose of biochar was compared individually with the control treatment (activated charcoal) by Dunnett's test ($P < 0.05$). For the biochar doses, regression equations were adjusted. For the variable number of roots, the data were transformed to $(x + 1)^{0.5}$. In experiments 3 and 4, the data were submitted to analysis of variance, and, when significant, $AgNO_3$ and biochar were compared by the F test ($P < 0.05$). For $AgNO_3$ doses, regression equations were adjusted. All static analyses were performed using the R Studio software (R Development Core Team, 2017).

RESULTS AND DISCUSSION

In vitro micropropagation is a technique used to produce seedlings of Pitaya and Strawberry. However, the presence of biochar and AgNO3 in the culture media and porous membrane in caps causes changes in the in vitro culture environment, which promotes changes in the growth and development of the cultivated seedlings. Figure 2 illustrates a cap with a membrane that allows gas exchange (A) and *in vitro* strawberry growth in vials with caps with membrane and addition of 0 (B) and 4 g L^{-1} (C) of biochar and 2 g L^{-1} of activated charcoal (D). Cap without a membrane that allows gas exchange (E) and *in vitro* strawberry growth in vials with caps without membrane and addition of 0 (F) and 4 L^{-1} (G) of biochar and 2 g L^{-1} of activated charcoal (H).. *In vitro* growth of pitaya with biochar and doses of $AgNO_3$: 0 (I), 5 (J), (K), and 40 µM L^{-1} (L). *In vitro* growth of pitaya without addition of biochar and doses of $AgNO_3$: 0 (M), 5 (N), 20 (O), and 40 µM L^{-1} (P). The type of vials caps, regardless of the addition of biochar or activated charcoal to the culture medium, did no effect on the length, number, and dry mass of roots of pitaya and strawberry, except in doses 0 and 2 g L^{-1} of biochar, where the dry mass of the strawberry root was more significant when using caps with microporous membrane (Table 2).

Using microporous membrane caps in vials for *in vitro* growth allows gas exchange between the inner microenvironmental (inside the vial) and the outer environment (out of the vial). Although we did not obtain significant differences between the types of caps in this study, less formation of water droplets was observed on the vials' inner wall with a porous membrane on the caps, demonstrating the influence of the type of cap the internal microenvironment of the vials. By allowing gas exchange, porous membrane caps had similar effects to activated charcoal in reducing ethylene gas concentrations inside the vials and favored *Annona glabra* L. root growth *in vitro* culture (Santana et al., 2011). High concentrations of ethylene inside the vials can have deleterious effects, such as mortality and abscission of plants (Gupta and Pandey, 2019). *In vitro* culture of *Capsicum annuum* L. (Mohamed and Alsadon, 2011), *Thymus vulgaris* L. (Bandeira et al., 2007), and *Hancornia speciosa* Gomes (Sá et al., 2012), when growing in vials with the caps' porous membrane, showed better growth and development due to the gas exchange. The number and length of roots are essential characteristics of plant adaptability during the acclimatization stage, after in *vitro* growth. Corroborating the results of this study, other authors also detected significant increases in root length with the addition of biochar *in vitro* cultivation of lettuce (*Lactuca sativa* L.) and arabidopsis (*Arabidopsis thaliana* L.) (Viger et al., 2015).

According to Xiang et al., (2017), roots are the interfaces between biochar particles and plants. Xiang et al (2017) found that, in 32 and 52% of the studies, biochar's application increased the biomass and length of roots, respectively and concluded that the application of biochar favors root morphological development to increase water and nutrient uptake. According to Viger et al. (2015), biochars alter plant metabolism, such as auxin biosynthesis-related to root system development. The length and dry mass of pitaya and strawberry roots grown with activated charcoal (control treatment, TC) were generally similar to those obtained with the biochar, regardless of the caps types (Table 2), except the dry mass of strawberry roots in the absence of biochar was higher than that obtained with activated charcoal. On the

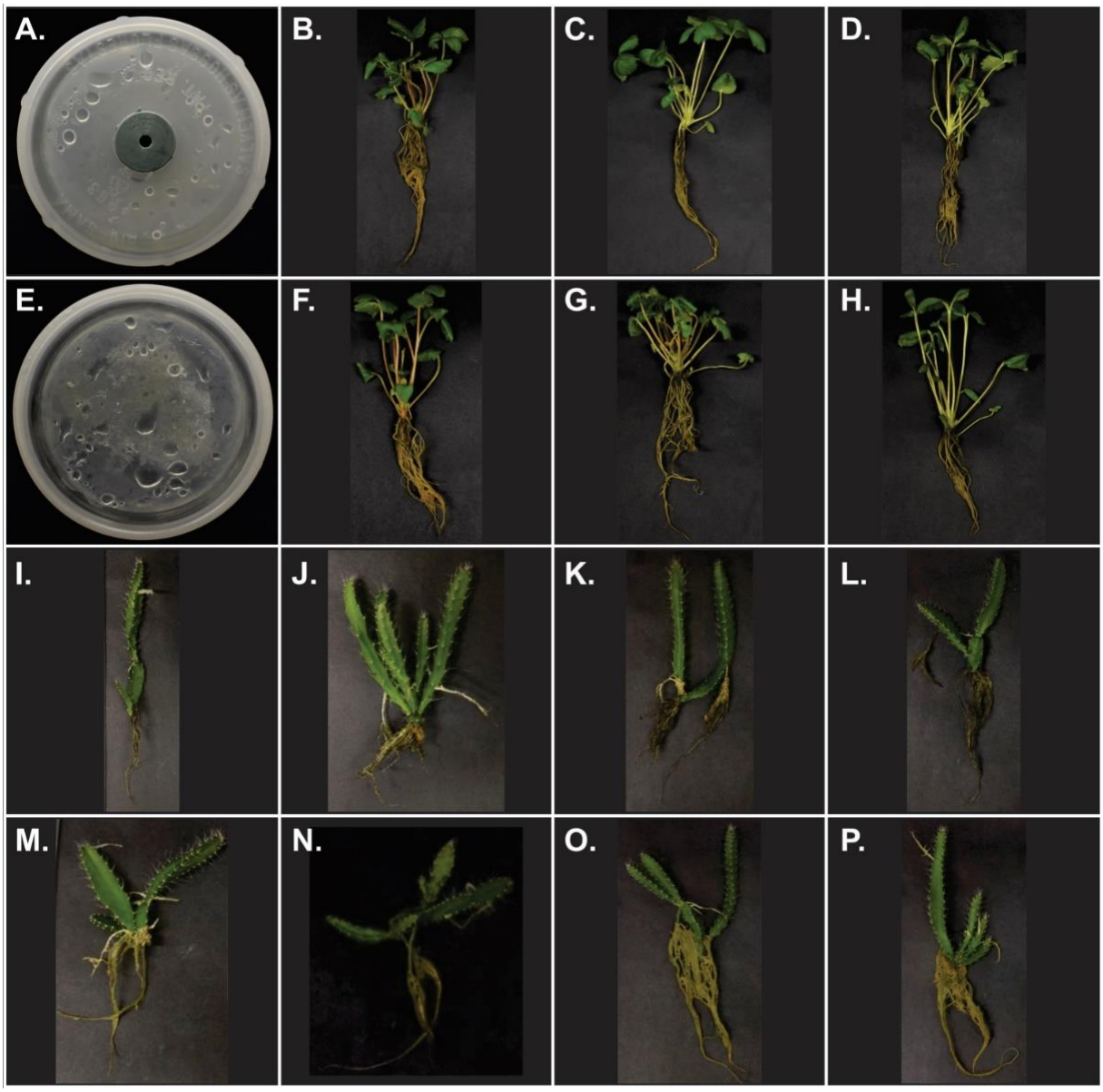

Figure 2. Cap with membrane that allows gas exchange (A), strawberry growth in vials with cap that allow gas exchange with addition of 0 (B), and 4g L^{-1} (C) of biochar and 2 g L^{-1} of activated charcoal (D). Cap that does not allow gas exchange (E), of strawberry growth in vials with caps that do not allow gas exchange, with adduction of 0 (F) and 4 L^{-1} (G) of biochar and 2 g L^{-1} of activated charcoal (H). *In vitro* growth of pitaya with biochar and doses of $AgNO_3$: 0 (I), 5 (J), 20 (K), and 40 µM L^{-1} (L). *In vitro* growth of pitaya without addition of biochar and doses of $AgNO_3$: 0 (M), 5 (N), 20 (O), and 40 µM L^{-1} (P).
Source: Author

other hand, the number of roots obtained in the activated charcoal treatments was lower than in the higher doses of biochar. Besides, the roots in biochar treatments were thinner than in activated charcoal treatments. Di Lonardo et al., (2013), working *in vitro* cultivation of two clones of *Populus alba* L. found that the addition of biochar or activated charcoal contributed positively to the growth of the shoot and number of roots. According to these authors, activated carbon and biochar significantly reduce the ethylene concentration inside the vials by adsorbing this gas in their particles. High ethylene concentrations inside vials for *in vitro* growth may inhibit organogenesis, cause leaf collapse and abscission, while low ethylene concentrations may contribute to plant growth (Taiz et al., 2017). (Firoozabady et al., 2006) also identified the positive effects of activated charcoal to the culture medium for *in vitro* growth. These authors observed significant increases in rooting and sprouting of pineapple

Table 2. Length, number, and dry weight of pitaya and strawberry roots growing in vials sealed with caps with or without porous membrane as a function of biochar doses or activated carbon.

Variable	Cap	Biochar doses					Activated charcoal	y	R^2
		0	1	2	3	4			
Pitaya									
Root length	With orifice	5.65^{aB}	4.30^{aA}	4.46^{aA}	4.85^{aA}	5.23^{aA}	4.28^{A}	4.89^{ns}	
	Without orifice	5.99^{aB}	6.26^{aB}	5.81^{aB}	4.85^{aA}	5.08^{aA}	4.25^{A}	5.58^{ns}	
Root number	With orifice	12.07^{aA}	19.13^{aB}	21.50^{aB}	23.65^{aB}	31.27^{aB}	16.70^{A}	12.94+4.297**x	0.98
	Without orifice	8.63^{aB}	20.07^{aB}	22.02^{aB}	24.15^{aB}	32.92^{aB}	14.93^{A}	11.03+5.265**x	0.91
Root dry mass	With orifice	9.12^{aA}	7.18^{aA}	7.19^{aA}	6.34^{aA}	6.28^{aA}	8.14^{A}	$y=7.60^{ns}$	
	Without orifice	10.41^{aA}	6.21^{aA}	6.25^{aA}	6.28^{aA}	6.32^{aA}	9.41^{A}	$9.93-3.23**x+0.61**x^2$	0.88
Strawberry									
Root length	With orifice	6.08^{aB}	6.61^{aA}	7.90^{aA}	7.94^{aA}	8.42^{aA}	7.79^{A}	6.18+0.0601**x	0.92
	Without orifice	6.43^{aB}	5.57aB	7.35 a	6.30aB	7.00aB	9.46^{A}	6.31^{ns}	
Root number	With orifice	17.17^{aA}	20.89^{aA}	21.63^{aA}	29.63^{aB}	34.38^{a}B	18.71^{A}	$16.11+4.318**xR^2=0.94$	
	Without orifice	19.64^{aA}	21.85^{aA}	21.57^{aA}	31.30^{aB}	34.26^{aB}	14.53^{A}	17.99+3.869**x	0.86
Root dry mass	With orifice	124.23^{aB}	27.13^{aA}	40.42^{aB}	6.09^{bB}	15.38^{aA}	22.42^{A}	$114.00-71.40**x+11.90**x^2$	0.95
	Without orifice	53.00^{bB}	30.00^{aA}	13.00^{bA}	12.37^{aA}	13.16^{aA}	20.15^{A}	$53.40 - 28.30**x + 4.70**x^2$	0.99

ns - not-significant. Lower case letters in the columns compare treatments with or without porous membrane cap. The means followed by the same lowercase letter do not differ by the F test ($P < 0.05$). Capital letters on the lines compare each dose of biochar with the control treatment (activated charcoal). The means followed by the same capital letter do not differ by Dunnett's test ($P < 0.05$). ** Significant by t-test ($P < 0.05$).
Source: Author

in cultivate *in vitro* with activated charcoal. There is little information available on the effects of biochar as a plant hormone adsorbent (Di Lonardo et al., 2013). However, several studies show biochars' ability to adsorb pesticide molecules (Song et al., 2012; Sopeña et al., 2012) and immobilize organic and inorganic pollutants (Bian et al., 2014). On the other hand, some organic compounds can be produced during the pyrolysis process, including ethylene (Spokas et al., 2010). Thus, for the use of biochar *in vitro* growth, the presence and quantity of toxic compounds must be considered. Besides, depending on the pyrolysis conditions and the raw material, biochar may have high toxic compound concentrations, such as dioxins and furans (Free et al., 2010).

According to some studies, plants can uptake carbon nanoparticles from biochar, which can inhibit the expression of some genes related to secondary metabolism, including ethylene production. The uptake of biochar nanoparticles may also negatively affect the expression of genes responsible for the jasmonic and salicylic acid pathways that participate in ethylene signaling, plant defense against insect attack and pathogens, and stress control (Viger et al., 2015). Therefore, in addition to ethylene adsorption, the biochar effects on the metabolism of plants grown *in vitro* should be considered. In this sense, many authors have verified the positive impact of biochars on root development and morphology, improving water and nutrient uptake (Prendergast-Miller et al., 2014) and crop yield (Xiang et al., 2017).

From the equations adjusted for the biochar doses, regardless of the type of the cap, no significant effect of biochar on pitaya root length was identified. In contrast, the strawberry root

length increased linearly with biochar doses in treatments with porous membranes in the caps. Comparing the doses 0 and 4g of biochar, the strawberry root length at the highest dose of biochar was 1.4 times longer than at zero. From the equations adjusted for the biochar doses, regardless of the type of the cap, no significant effect of biochar on pitaya root length was identified. In contrast, the strawberry root length increased linearly with biochar doses (Table 2) in treatments with porous membranes in the caps. Comparing the doses 0 (6.18 cm) and 4g L^{-1} (8.59 cm) of biochar, the strawberry root length at the highest dose of biochar was 1.4 times longer than at zero (Table 2). The number of pitaya and strawberry roots increased linearly with the biochar doses, regardless of the type of caps (Table 2). For the pitaya grown in vials with the porous membrane in the caps, the number of roots increased by 2.32 times from 0 dose (12.94) to the dose 4 g L^{-1} of biochar (30,11), while in the vials without membrane in the caps, the increase from 0 dose (11.03) to 4 g L^{-1} of biochar (32, 04) was 2.90 times higher (Table 2). For strawberry (Figure 2D), the increase in the number of roots from 0 dose (16.11) to 4 g L^{-1} of biochar (33.38) was 2.07 times higher in the treatments with the membrane in the caps, and 1.86 times higher (17.9 roots at 0 dose and 33.46 roots at 4g L-1 of biochar) for caps without membrane (Table 2). For the dry mass of pitaya roots, it was verified that there were no significant differences for biochar doses, regardless of the type of the cap. In contrast, for strawberry, increasing doses of biochar, regardless of caps type, decreased root dry mass production (Table 2). Thus, for the strawberry, it is observed that, although there was an increase in the number of roots, the roots were thin, reflecting in the lower production of dry mass. Silva et al. (2017) also found many thin roots in common bean plants when biochar was added to the soil. Biochar may probably affect plant metabolism, and thinner roots may be an ecological advantage for plant adaptation in low fertility soils (Viger et al., 2015). In the experiments with biochar and $AgNO_3$, the addition of biochar in the culture medium did not influence the length and number of pitaya and strawberry roots. On the other hand, the dry mass yield of pitaya and strawberry roots decreased in biochar treatments. The length, number, and dry mass of pitaya and strawberry roots were adjusted to a quadratic model in the function of $AgNO_3$ doses, except for the number of strawberry roots in treatments with biochar (linear model) and length of pitaya roots the treatments without biochar (there were no differences between $AgNO_3$ doses) (Table 3). According to the results, for both the pitaya and strawberry, there was a reduction in root dry mass production in treatments with biochar (Table 3). The purpose of $AgNO_3$ addition of *in vitro* culture medium is to inhibit ethylene's action on plants. In this study, biochar may have probably retained ethylene molecules in the surface charges of particles, thereby reducing the concentrations of this gas inside the vials, as discussed earlier. However, the lower root dry mass yield can be attributed to the immobilization of silver ions (Ag^+) by biochar. In general, due to ash content, rich in alkali metal oxides and hydroxides (De Almeida et al., 2020), biochars raise the medium's pH, and, consequently, precipitation of cationic metal ions can occur (Rajapaksha et al., 2016; Rizwan et al., 2016). Besides, biochars are very efficient adsorbents of cationic metal ions due to the high surface area, particle porosity, and functional groups that exhibit negative electric charges (Bian et al., 2014; Lu et al., 2014; Tang et al., 2013). The biochar functional groups O-H, C-H, C=O, and C-O, for example, when deprotonated, show negative charges on the particle surface, and can hold Ag^+ ions. When making the relationship between the length and the number of roots with the production of dry mass, the results showed that the treatments with biochar provided thinner roots. In treatments without biochar, root dry mass production was 2.1 and 1.7 times higher than in the treatment with biochar, for pitaya and strawberry, respectively (Table 3). The use of microporous membrane caps, activated charcoal, and, or $AgNO_3$ to the culture medium to reduce ethylene concentrations inside vials for *in vitro* growth has been explored in the literature (Cardoso, 2019; Galdiano Junior et al., 2012; Santana et al., 2011). However, according to our results, the addition of biochar to the culture medium inhibits the effects of $AgNO_3$. Sugarcane bagasse biochar favors the growth and development of pitaya and strawberry roots, regardless of the use of vials with caps with or without porous membranes for gas exchange. There is no difference between vial cap types in the presence of biochar or activated charcoal in the culture medium. Thus, porous membrane caps, sugarcane bagasse biochar, and $AgNO_3$ are viable alternatives to improve the conditions for *in vitro* growth of pitaya and strawberry. However, it is not recommended to add $AgNO_3$ and biochar together to the same culture medium. Using biochar for *in vitro* micropropagation of plants proved to be a promising alternative in different conditions (presence and absence of activated charcoal or porous membranes in cap). However, there are still issues to address in future research as: to whether the type of raw material, temperature, and pyrolysis time used to generate the biochar influence the growth and development of plants *in vitro*.

Conclusion

The use of porous membrane caps contributed only to increase the roots dry matter yield of strawberries grown *in vitro* with culture media with 0, 1, and 2 g L^{-1} of biochar. The use of porous membrane caps did not influence the growth and development of pitaya roots grown *in vitro*. The addition of 4 g L^{-1} of biochar in the culture media favored the growth and development of the pitaya and

Table 3. Length, number, and dry weight of pitaya and strawberry roots as a function of $AgNO_3$ doses, with or without biochar.

Variable	With biochar				Without biochar			
	Equation	R^2	MV	MD	Equation	R^2	MV	MD
Pitaya								
Length (cm)	$y = 5.29 + 0.19^{**}x - 0.004^{**}x^2$	0.80	7.4	22.6	$y = 7.28^{ns}$	-	7.2	-
Number	$y = 12.95 + 1.19^{**}x - 0.03^{**}x^2$	0.99	36.3	22.3	$y = 15.14 + 0.69^{**}x - 0.019^{**}x^2$	0.93	21.2	18.4
Dry mass (g)	$y = 5.67 + 2.01^{**}x - 0.047^{**}x^2$	0.99	27.2	21.4	$y = 19.95 + 3.67^{**}x - 0.092^{**}x^2$	0.95	57.3	20.1
Strawberry								
Length (cm)	$y = 15.1 + 0.17^{**}x - 0.008^{**}x^2$	0.70	16.1	11.2	$y = 14.07 + 0.64^{**}x - 0.017^{**}x^2$	0.79	20.4	18.3
Number	$y = 14.48 + 0.39^{**}x$	0.87	30.1	40.0	$y = 20.71 + 0.84^{**}x - 0.014^{**}x^2$	0.93	33.2	30.4
Dry mass (g)	$y = 9.34 + 1.39^{**}x - 0.035^{**}x^2$	0.95	23.1	20.2	$y = 58.34 - 1.92^{**}x + 0.045^{**}x^2$	0.89	38.5	21.3

The maximum values were estimated from the adjusted equations for $AgNO_3$ doses.MV - Maximum value of length, number, or dry mass of roots; MD - Maximum doses of $AgNO_3$; ns - not-significant; ** Significant by t test (P<0.05).
Source: Author

strawberry roots, regardless of the use or not of porous membrane caps in vials of the *in vitro* culture. The $AgNO_3$ alone contributed to the greater length and dry mass of pitaya roots and higher dry mass of strawberry roots. The addition of biochar in the culture medium decreases the $AgNO_3$ effects *in vitro* growth.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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