

## Effect of a fungal glycoprotein on scab control caused by *Cladosporium herbarum* in passion fruit plants

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**Abstract**-Phytosanitary problems drastically affect passion fruit cultivation around the world. Scab, a fungal disease that attacks the aerial part of plants, especially the younger leaves, impairs development and reduces plant productivity. The objective of this work was to evaluate the effect of treatment with a fungal cell wall glycoprotein, named pGM from peptidogalactomanann, in the control scab caused by *Cladosporium hebarum* infection. Under greenhouse conditions, morphological parameters or plant development were evaluated in two passion fruit genotypes, 'H09-110/111' and 'FB300'. pGM treatment was able to mitigate the damage caused to plant development in parameters such as height, the number of leaves, stem diameter, leaf area and biomass in the 'H09-110/111' genotype compared to the control. However, in the genotype 'FB300', no significant differences were observed concerning the control. Passion fruit scab disease incidence and severity were also reduced by pGM treatment. Therefore, this study suggests that the use of pGM can lead to control and attenuation of the damage caused by this fungus in the early stages of passion fruit plants 'H09-110/111' when the plants are more susceptible to biotic stresses.

**Index terms:** Peptidogalactomanann; *Passiflora edulis*; scab, elicitor, *Cladosporium herbarum*.

## Efeito de uma glicoproteína fúngica no controle da verrugose causada por *Cladosporium herbarum* em maracujazeiro

**Resumo** -Problemas fitossanitários atingem drasticamente a cultura do maracujazeiro em todo o mundo. Entre elas, a verrugose, doença fúngica que ataca a parte aérea das plantas, principalmente as folhas mais jovens, prejudica o desenvolvimento e reduz a produtividade das plantas. O objetivo deste trabalho foi avaliar o efeito de uma glicoproteína de parede celular fúngica chamada pGM de peptidogalactomanana, no controle da infecção por *Cladosporium hebarum*, causadora da verrugose. Parâmetros morfológicos e de desenvolvimento da planta foram avaliados em dois genótipos de maracujá, 'H09-110/111' e 'FB300', sob condições de casa de vegetação. O tratamento com pGM foi capaz de mitigar os danos causados ao desenvolvimento das plantas em parâmetros como: altura, número de folhas, diâmetro do caule, área foliar e biomassa no genótipo 'H09-110/111'; quando comparado com o controle. Porém, no genótipo 'FB300', não foram observadas diferenças significativas em relação ao controle. A incidência e a severidade da doença também foram reduzidas pelo tratamento com pGM. Portanto, este estudo sugere que o uso da pGM pode levar ao controle e à atenuação dos danos causados por este fungo nas fases iniciais do maracujá 'H09-110/111', quando as plantas são mais suscetíveis a estresses bióticos.

**Termos para indexação:** Peptideogalactomanana, *Passiflora edulis*, verrugose, elicitor, *Cladosporium herbarum*.

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## Introduction

Passion fruit (*Passiflora edulis* Sims) is one of the most important fruits grown in Brazil. Although Brazilian production is quite significant to other producing countries (IBGE, 2020), the volume produced is insufficient to meet the domestic demand for fresh fruits, and concentrated juice. Productivity is impaired by several diseases that can compromise plant development and fruit quality. Diseases that affect this crop in the initial stages of plant growth are a significant problem, as they cause physiological distress resulting in plant development delay. Among the fungal diseases affecting passion fruit plants, the scab is relevant in the fresh fruit market due to the visual appearance of the fruit, which presents rough lesions with a cork appearance, which does not favor the choice of the product by consumers. The causal agent of scab is *Cladosporium herbarum*, a ubiquitous species of the family *Cladosporiaceae* of the recently proposed order *Cladosporiales* in the *Dothideomycetes* (ABDOLLAHZADEH et al., 2020). In addition to causing lesions on the fruits, this fungus also affects the aerial part of the young passion fruit plant, causing lesions on the branches, leaves and flowers (JOY et al., 2016). Fungal diseases of the aerial part have been basically controlled by the applying of fungicides, imposing the risks of overspraying, and increasing production costs. Therefore, it is important to address new alternatives for controlling these diseases by strengthening measures based on good agricultural practices and eco-friendly products, which help to mitigate damage caused by them. Fungal infection by *C. herbarum* is favored under conditions of mild temperatures between 15 and 25 °C and high humidity. The spread of the pathogen occurs mainly through contaminated seeds and seedlings and by wind. In addition, when scabs occur in passion fruit seedlings, they can cause symptoms on the stems and leaves, which can cause the seedlings to die (JOY et al., 2016).

Products developed from natural or biological sources have a high potential to control plant diseases. Bioelicitors act by activating plant resistance mechanisms, thus generating a cascade of signals that culminate in activating of the host plant's defense genes. This phenomenon is known as resistance induction. It is a natural phenomenon of plant response to pathogen attack, activated as soon as the interaction between host and pathogen occurs (PASCHOLATI et al., 2011). The success in plant infection is due to the pathogen's ability to overcome the protective barriers imposed by the plant, whether structural or biochemical, pre- or postformed. In addition, much of this success is linked to the speed of the plant's response at the time of interaction with the virulent microorganism; that is, the faster the plant's response is, the lower the probability of occurrence of the disease (MELO et al., 2017). Due to the demands of organic

farming and pesticide-free fruit in the consumer market, there is growing interest in developing safer and more effective alternative compounds to control plant diseases. Exploring of natural products in the control of fungi is of great importance in the alternative control of passion fruit seedlings. This study aimed to evaluate the effect of a fungal cell wall peptidogalactomanann named pGM in controlling scab disease and mitigating the damage caused by the fungus *C. herbarum* in two genotypes of passion fruit seedlings under greenhouse conditions.

## Materials and methods

Experiments were carried out in a greenhouse in Embrapa Agrobiologia (Empresa Brasileira de Pesquisa Agropecuária), located in the municipality of Seropédica, Rio de Janeiro State. The geographical coordinates are 22° 48' 00" south latitude and 43° 41' 00" west longitude. Two genotypes of *Passiflora edulis*, the hybrid 'H09-110/111' and the cultivar 'FB300', were grown in tubes containing 1/3 vermiculite, 1/3 earthworm humus and 1/3 washed sand and maintained in greenhouse conditions under tropical area natural light and temperature conditions. Two months later, the seedlings were transplanted to plastic pots of 40 liters containing coconut fiber substrate supplemented with 100 g of phosphorus and 800 g of organic compound (Organosolo). One month after, 800 g of earthworm humus was added to each pot.

For the peptidogalactomannan (pGM) extraction, *Cladosporium herbarum* fungus was grown in potato dextrose broth medium (PDB) for 7 days, and the fungal mass was obtained using 3MM paper filtration. Glycoprotein extraction was performed according to Haido et al. (1998). The precipitate was resuspended in water, dialyzed, and freeze-dried to obtain crude pGM.

Plants were naturally infected by scab. Plants of 'HB09-110/111' of 'FB300' showing strong symptoms of the fungus were selected. The experimental design was randomized for each genotype and each treatment, and all the plants had the same level of severity (number of spots) and development (height and number of leaves) before starting foliar spray.

Twelve plants from the hybrid 'H09-110/111' and twelve from the cultivar 'FB300' were treated with 100 µg.ml of pGM, and the same number of plants were treated with water. Treated and control-plants were evaluated for 9 weeks for the presence of scab symptoms in the new leaves growing after treatment.

Treatments were performed in plants with 2 to 3 true leaves, with a foliar spray of 100 µg.mL<sup>-1</sup> pGM, performed with a costal manual sprayer (Jacto - XP), using a conical nozzle with a working pressure of 30 PSI. Control plants were treated with foliar spray of tap water.

Disease incidence (DI%) was determined based on the presence of symptoms in at least one of the three youngest leaves of the diseased plants. The proportion of diseased plants was estimated by  $DI = (n/N) \times 100$  (DI = incidence; n = number of diseased plants; N = total number of plants assessed) (KONE et al. 2017). The severity of “scab” disease was evaluated until 9 weeks after treatment starting, with biweekly intervals, in the first three youngest leaves of the plant, according to a scale of score: 1= 0 - 3 spots; 2= 3 - 6 spots; 3= 6 - 12 spots; 4= 12 - 25 spots; 5= 25 - 50 spots, described by Negreiros et al. (2004).

Plant development was evaluated by estimations of the height, the number of leaves, the stem diameter, the leaf area of the youngest fifth leaf (count from above to below) and the dry biomass of aerial parts and roots. Height was measured with the aid of a measuring tape. The leaves were manually counted; the diameter of the stem was measured with the aid of a digital caliper (MTX-316119); the leaf area was measured by means of a leaf area scanner (LI-Cor Biosciences, model LI-3100c). Biomass was determined using an accuracy electronic scale (Bioprecisa – JA3003N). For biomass evaluation, the plant shoots and root system were cut, placed in individual paper bags and dried at 60 °C for 72 h, and the dry weight of the aerial part and root system was obtained.

The external environment’s temperature and humidity data were taken from the INMET website data. Data obtained for development parameters and disease incidence were analyzed using a paired Student’s t-test at a significance level of  $p < 0.05$ ; for disease severity, a nonparametric Wilcoxon rank sum test was used ( $p < 0.05$ ). GraphPad Prism software version 5.00 for Windows was used for statistical analysis.

## Results and Discussion

Passion fruit seedlings growing in a greenhouse and naturally infected by *C. herbarum* were evaluated for scab disease and showed a disease index of 100% for all plants from the two passion fruit genotypes. Infected plants were treated with pGM or water and the incidence of scab in newly developed leaves evaluated. In ‘H09-110/111’, the incidence of the disease was considerably lower in the leaves that grew after treatment in treated plants compared with water-treated plants. At 5 weeks after treatment (wat), the DI of these new young leaves was 70% lower in pGM-treated plants compared to water-treated plants (Table 1a) (Disease incidence, 5 wat:  $F_{1,21} = 8.65$ ,  $p < 0.05$ ). At 7 and 9 wat, the new developed leaves did not showed scab symptoms. The newly developed leaves of water-treated plants showed a lower DI at the end of the experiment than its beginning, probably because the relative humidity started to fall during these weeks (INMET, 2021), and the fungal conditions were more favorable with high moisture. Compared to water-treated plants, a lower number of ‘FB300’ pGM-treated plants showed disease symptoms in the new developed leaves. However, significative differences weren’t observed on the DI of the leaves of ‘FB300’ plants developed after the treatment. New experiments using a bigger sample number are necessary to confirm the impact of the treatment in ‘FB300’ plants.

**Table 1.** Effect of pGM treatment on the scab disease incidence (DI%) in passion fruit plants (a) ‘H09-110/111’ and (b) ‘FB300’ 0 at 9 weeks after treatment (wat).

*Temperature (°C)	21.3	19.2	19.8	18.9	23.2
*Relative humidity (%)	76	74	69.9	66.5	61.5
<b>a. ‘H09110/111’</b>					
	Disease incidence (DI%)				
Treatments	0 wat	2 wat	5 wat	7 wat	9 wat
Water treatment	100 ± 0a	100 ± 0a	83.3 ± 38.9a	25 ± 45.0a	41.6 ± 51.4a
pGM treatment	100 ± 0a	100 ± 0a	25.0 ± 45.2b	0.0 ± 0.0a	0.0 ± 0.0b
<b>b. ‘FB300’</b>					
	Disease incidence (DI%)				
Treatments	0 wat	2 wat	5 wat	7 wat	9 wat
Water treatment	100 ± 0a	100 ± 0a	83.3 ± 38.9a	8.3 ± 28.8a	41.6 ± 51.4a
pGM treatment	100 ± 0a	100 ± 0a	58.3 ± 51.4a	0.0 ± 0a	16.6 ± 38.9a

Each value is the means (± standard deviation) of an independent experiment using 12 plants per treatment. Different letters in columns indicate significant differences between treatments according to t-test ( $p > 0.05$ ).

Corresponding to t-test, (a) H09-110/111, 0 wat:  $t=1.0$ ,  $df=11$ ,  $p > 0.05$ ; 2 wat:  $t=1.0$ ,  $df=11$ ,  $p > 0.05$ ; 5 wat:  $t=3.0$ ,  $df=11$ ,  $p < 0.05$ ; 7 wat:  $t=1.9$ ,  $df=11$ ,  $p > 0.05$ ; 9 wat:  $t=2.8$ ,  $df=11$ ,  $p < 0.05$ . (b) FB300, 0 wat:  $t=1.0$ ,  $df=11$ ,  $p > 0.05$ ; 2 wat:  $t=1.0$ ,  $df=11$ ,  $p > 0.05$ ; 5 wat:  $t=1.3$ ,  $df=11$ ,  $p > 0.05$ ; 7 wat:  $t=1.0$ ,  $df=11$ ,  $p > 0.05$ ; 9 wat:  $t=1.9$ ,  $df=11$ ,  $p > 0.05$ .

\*Temperature (°C) and relative humidity (%) data obtained from the INMET (Instituto Nacional de Meteorologia) database, 2021.

Regarding the disease severity caused by the fungus, it was observed that the newly developed leaves of 'H09-110/111', showed a decrease in the DS 5 wat in pGM-treated plants compared to the control. These differences, however, were not observed at 7 wat as the disease severity in the leaves of the water-treated control plants also decreased; moreover, at 9 wat significant differences were observed between pGM and water-treated plants (Table 2a) (Wilcoxon rank sum test, at 5 wat:  $W=33$ ;  $p<0.05$ ; at 7 wat:  $W=3$ ;  $p>0.05$ ; at 9 wat:

$W=26$ ;  $p<0.05$ ). In 'FB300', differences between water- and pGM-treated plant responses were observed since 2 wat. The severity of the control also decreased in the following weeks, without significant differences between pGM and water-treated plants. However, at 9 wat, the severity increased in the water-treated plants, leading to significant differences between treatments (Table 2b) (Wilcoxon rank sum test, at 2 weeks:  $W=57$ ;  $p<0.05$ ; at 9 weeks:  $W=21$ ;  $p<0.05$ ).

**Table 2.** Effect of pGM treatment on the scab disease severity (DS) in passion fruit plants (a) 'H09-110/111' and (b) 'FB300' along 9 weeks (wat).

*Temperature (°C)	21.3	19.2	19.8	18.9	23.2
*Relative humidity (%)	76	74	69.9	66.5	61.5
<b>a. 'H09-110/111'</b>					
	Disease severity (DS%)				
Treatments	0 wat	2 wat	5 wat	7 wat	9 wat
Water treatment	2.08 ± 0.76a	1.56 ± 0.58a	1.45 ± 0.40a	1.02 ± 0.9a	1.2 ± 0.26a
pGM treatment	2.16 ± 0.78a	1.66 ± 0.65a	1.05 ± 0.12b	1.0 ± 0.0a	1.0 ± 0.0b
<b>b. 'FB300'</b>					
	Disease severity (DS%)				
Treatments	0 wat	2 wat	5 wat	7 wat	9 wat
Water treatment	2.25 ± 0.58a	1.80 ± 0.47a	1.22 ± 0.32a	1.08 ± 0.2a	1.38 ± 0.50a
pGM treatment	2.33 ± 0.61a	1.29 ± 0.45b	1.22 ± 0.32a	1.0 ± 0.0a	1.02 ± 0.09b

Each value is the means ( $\pm$  standard deviation) of an independent experiment using 12 plants per treatment. Different letters in columns indicate significant differences between treatments according to non-parametric Wilcoxon rank sum test ( $p < 0.05$ ).

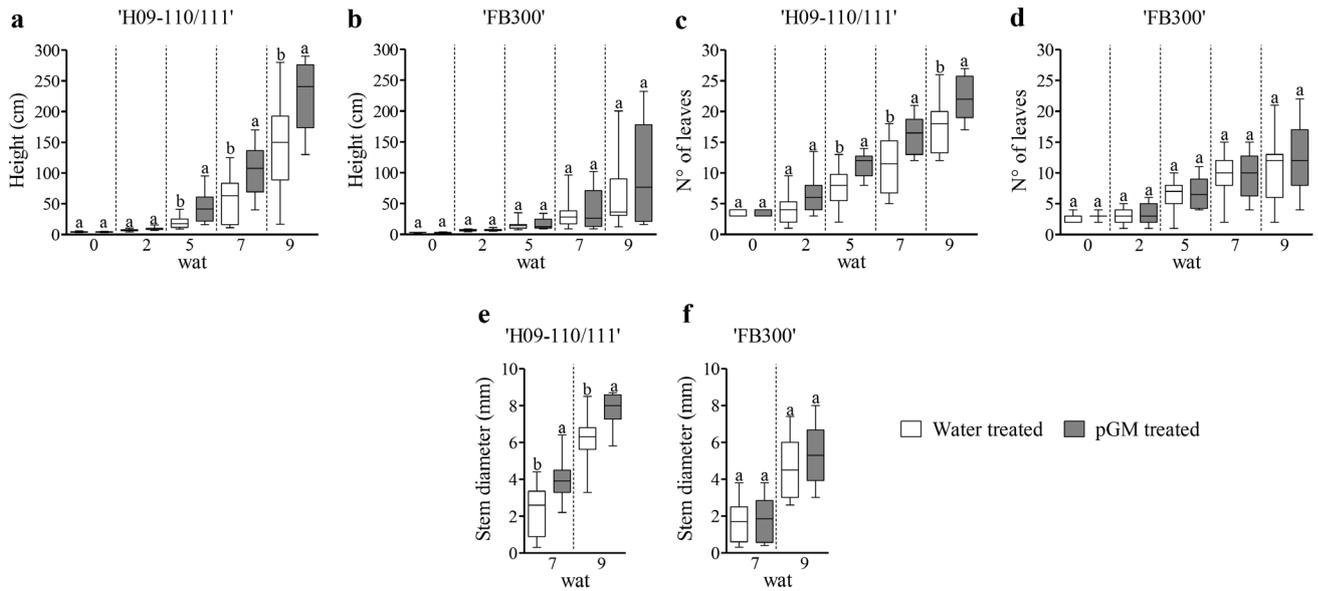
\*Temperature (°C) and relative humidity (%) data obtained from the INMET (Instituto Nacional de Meteorología) database, 2021.

Other studies showed that the concentration of *Cladosporium* spp. conidia in the air showed a high correlation with relative humidity of approximately 80%, and temperatures between 23 - 29 °C (SADYŚ et al., 2015). So, we can speculate that the decrease of disease severity observed in the new developed leaves of water-treated plants may be associated to a small decrease in the relative humidity since the 5 wat.

The results obtained in this study showed that pGM treatment of *C. herbarum* infected plants may decrease scab severity on the new developed young leaves of passion fruit seedlings growing under greenhouse conditions. To date, few studies have reported the control of scab disease. Willingham et al. (2002) observed that in fields with favorable climatic conditions for fungal diseases, scab incidence was significantly reduced in passion fruit plants with the use of chemical fungicides such as azoxystrobin, acibenzolar and trifloxystrobin. These same authors observed that chemical control with acibenzolar could induce protection of passion fruit seedlings decreasing the severity caused by scab. Campo-Arana et al. (2019)

observed that the application of Mancozeb alternating with potassium phosphite and Mancozeb alternating with azoxystrobin showed outstanding control of anthracnose (*Colletotrichum gloesporioides*) by significantly reducing the levels of severity in yellow passion fruit. In the same way, Mengal et al. (2020), observed that Iprovalicarb + Propineb, Neem extract and *Neurospora* sp. can be used for *Cladosporium* control in grapes. However, Pérez and Lannacone (2006) reported that the application of fungicides is not a fully efficient alternative to control fungal diseases caused by *Colletotrichum*, for example.

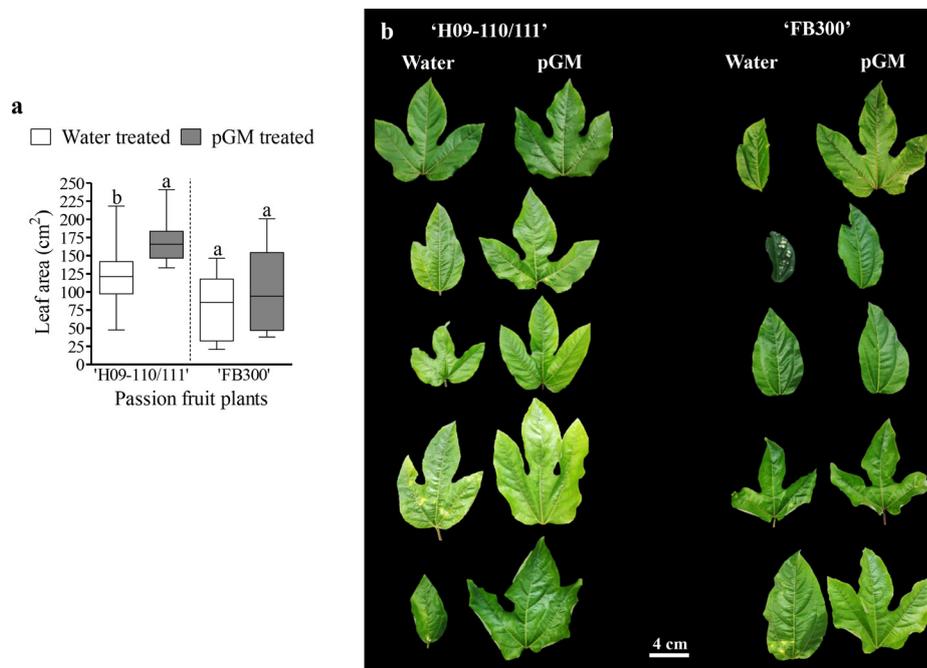
Development parameters were evaluated throughout the experiment. The plant height showed a significant increase in 'H09-110/111' plants since 5 weeks after pGM treatment, compared to water-treated plants (Figure 1a) (Height, at 5 wat:  $t=3.85$ ,  $df=11$ ,  $p < 0.05$ ; at 7 wat:  $t=3.81$ ,  $df=11$ ,  $p < 0.05$ ; at 9 wat:  $t=3.17$ ,  $df=11$ ,  $p < 0.05$ ). Concerning leaf emission, it was observed that there was a greater number of leaves in 'H09-110/111' pGM-treated plants along the evaluation time (Figure 1c) (Number of leaves at 5 wat:  $t=3.39$ ,  $df=11$ ,  $p < 0.05$ ; at 7 wat:  $t=3.73$ ,  $df=11$ ,  $p < 0.05$ ; at 9 wat:  $t=3.11$ ,  $df=11$ ,  $p < 0.05$ ). Another developmental parameter evaluated was the stem diameter. Significant differences were observed in pGM-treated plants at 7 and 9 wat in 'H09-110/111' compared to water-treated plants (Figure 1e) (Stem diameter, at 7 wats:  $t=3.71$ ,  $df=11$ ,  $p < 0.05$ ; at 9 wats:  $t=4.46$ ,  $df=11$ ,  $p < 0.05$ ).



**Figure 1.** Effect of pGM on plant development parameters in passion fruit infected with scab disease. Height of ‘H09-110/111’ (a) and ‘FB300’ (b); number of leaves of ‘H09-110/111’ (c) and ‘FB300’ (d); stem diameter of ‘H09-110/111’ (e) and ‘FB300’ (f). Averages correspond to independent experiments using 12 plants per treatment in greenhouse conditions through the time after treatment. Bars represent the averages of the plants per treatment each, and different letters denote groups between which there is a significant difference at  $p < 0.05$ . The lower and upper edges of boxes represent the first and third quartiles, with the horizontal line inside representing the average value. Whiskers extend to the highest and lowest data points in the interquartile range.

The size of the leaf area of the youngest fifth leaf at 9 wat was also significant bigger in ‘H09-110/111’ in pGM treatment compared with water-treated control plants (Figure 2) (Leaf area, ‘H09-110/111’ at 9 wat:

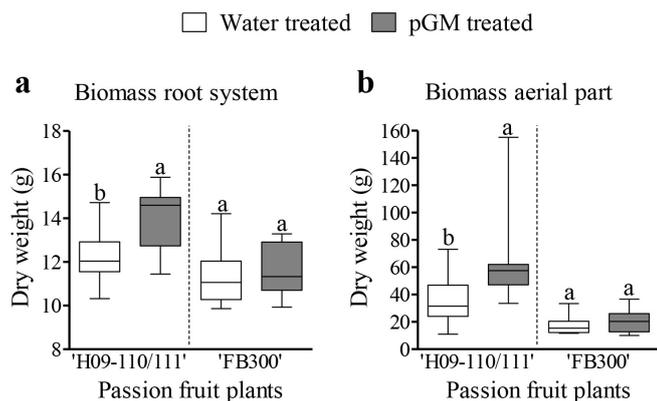
$t=2.80$ ,  $df=11$ ,  $p < 0.05$ ). Differences in plant biomass parameter were observed at 9 wat in ‘H09-110/111’ pGM-treated plants.



**Figure 2.** pGM effect in the leaf area on the fifth youngest leaf of passion fruit plants ‘H09-110/111’ and ‘FB300’ (a) of the independent experiment using 12 leaves per treatment in greenhouse conditions at 9 weeks after treatment (wat). Visual representation of some leaves collected for leaf area reading (b). Bars represent the averages of the plants per treatment each, and different letters denote groups between which there is a significant difference at  $p < 0.05$ . The lower and upper edges of boxes represent the first and third quartiles, with the horizontal line inside representing the average value. Whiskers extend to the highest and lowest data points in the interquartile range.

Significantly bigger dry weights of the root system and aboveground aerial part were observed (Figure 3a) (Dry weight of root system, 'H09-110/111' at 9 wat:  $t=4.08$ ,  $df=11$ ,  $p < 0.05$ ) and (Figure 3b) (Dry weight of

the aerial part, H09-110/111 at 9 wat:  $t=2.91$ ,  $df=11$ ,  $p < 0.05$ ). Figure 3c shows the general appearance of the roots after the treatment (Figure 3c).



**Figure 3. Evaluation of biomass in passion fruit under greenhouse conditions.** Dry weight of the root system (a), aerial part (b), and appearance of root (c) in passion fruit 'H09-110/111' and 'FB300' of the independent experiment using 12 plants per treatment in greenhouse conditions at 9 weeks after treatment (wat). Bars represent the averages of the plants per treatment each, and different letters denote groups between which there is a significant difference at  $p < 0.05$ . The lower and upper edges of boxes represent the first and third quartiles, with the horizontal line inside representing the average value. Whiskers extend to the highest and lowest data points in the interquartile range.

For 'FB300' genotype, however, no significant differences in the morphological and developmental parameters were observed after pGM treatment when compared to the control (Figure 1b, 1d, 1f; figure 2 and Figure 3) ( $p > 0.05$ ).

Application of control agent before germination and establishment of fungal diseases is generally more effective than applications after pathogen establishment (AGRIOS, 2005). However, for virus infection, some reports already shown the effectiveness of biostimulants as curative treatments after disease establishment (YAN et al., 2018; BERNARDINO et al., 2020). Here, we demonstrate the effect of pGM in the scab control after a natural infection with the causal disease agent, leading to reduced severity and mitigating the damage caused by the

fungus in the development of passion fruit 'H09-110/111' seedlings in terms of height, the number of leaves, the diameter of the stem, biomass and leaf area (Figures 1, 2, and 3). pGM pre-treated passion fruit plants induced an important reduction on CABMV symptoms after virus infection associated with the induction of defense-related genes (SANTOS-JIMÉNEZ et al., 2022a). Here, we observed that pGM-treated passion fruit plants looked better than the control-treated plants at 9 wat, being more developed. This is especially evident for 'H09-110/111' genotype. The increase in plant development parameters associated with the use of pGM after scab infection in a greenhouse can be attributed to the ability of this bioelicitor to delay or harm the fungal infection process in passion fruit seedlings. Studies carried out in the pretreatment of

passion fruit for the biocontrol of *Fusarium* wilt using *Trichoderma harzianum* led to a decrease in the severity of this disease and an increase in plant height and dry weight (WASIKE, 2014). Possible explanations for the increase in plant development parameters using defense bioinducers may be a direct consequence of pathogen control, allowing the plant to achieve a stronger growth, soil nutrient uptake and growth hormone production (DEWEN et al., 2017). However, a recent published paper of the group showed that pGM also works as a developmental biostimulant in addition to inducing defense responses (SANTOS-JIMÉNEZ et al., 2022b), so the effects of pGM on the developmental parameters observed here can be attributed to the sum of both activities of the molecule.

This is the first report on scab in passion fruit plants treated with a bioelicitor after infection.

## Conclusions

Fungal glycoprotein (pGM) may be used in passion fruit plants 'H09-110/111' infected with *Cladosporium herbarum* to reduce scab disease progression.

pGM treatment led to decreased in fungal severity and an increased in plant development parameters compared to the control in 'H09-110/111' genotype.

Results of pGM treatment observed for the 'H09-110/111' genotype may be explained by a faster activation of the defense response induced by pGM in this genotype.

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