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Role of sanitizers and biostimulants on root and shoot growth and enzyme activity of arracacha propagules

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ABSTRACT

Biostimulants are chemical or biological components adopted to improve nutrient uptake/efficiency and tolerance to abiotic stresses in crops. We studied three biostimulants (Stimulate®, tryptophol and *Bacillus subtilis* C-3102) associated to two sanitizers (sodium hypochlorite or thymol), on initial growth of propagules of *Arracacia xanthorrhiza*. Sodium hypochlorite associated to *B. subtilis* improve the leaf gas exchange, furthermore this treatment showed greater root volume. The interaction among sodium hypochlorite and tryptophol improves the plant branching; in addition this association showed better results for root dry mass. Different biostimulants improve differently the arracacha organs development, hence it is necessary to evaluate the plant morphophysiological competence to apply the correct biostimulant and sanitizer.

Keywords: *Arracacia xanthorrhiza*, phenolic compound, NaClO, bacteria, plant growth, plant metabolism.

RESUMO

Papel de sanitizantes e bioestimulantes sobre o crescimento de raízes e parte aérea e atividade enzimática de propágulos de batata baroa

Bioestimulantes são produtos químicos ou biológicos que melhoram a absorção ou eficiência dos nutrientes e a tolerância contra estresses abióticos em cultivos. Foram estudados três bioestimulantes (Stimulate®, tryptofol e *Bacillus subtilis* C-3102) e dois sanitizantes (hipocloclorito de sódio ou timol), sobre o crescimento inicial de *Arracacia xanthorrhiza*. Hipoclorito de sódio associado ao *B. subtilis* melhoram as trocas gasosas e induzem maior volume de raiz. A interação entre hipoclorito de sódio e triptofol aprimorou a ramificação das hastes e a biomassa seca das raízes. Diferentes bioestimulantes agem diferentemente sobre o desenvolvimento dos órgãos de batata baroa; portanto é necessário avaliar a competência morfofisiológica da planta para aplicar o bioestimulante e sanitizante correto.

Palavras-chave: *Arracacia xanthorrhiza*, compostos fenólicos, NaClO, bactéria, crescimento vegetal, metabolismo vegetal.

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The arracacha plant (*Arracacia xanthorrhiza*) is originally from the Andean region, belonging to the Apiaceae family, grown from Mexico to South America. Its roots are rich on added value products, as carbohydrates, ascorbic acid, vitamin A and minerals (Hermann, 1997). Nonetheless, it is considered an underexplored Andean root.

Agronomically, the main way to propagate the arracacha is vegetatively, being the cormel used traditionally, and exclusively, as the propagule. Therefore, it is important to obtain arracacha propagules with high sanity and genetic vigor, because root productivity depends greatly on the preparation of the propagule (Hermann, 1997). However, the preparation of these propagules reduces significantly its reserve structure, where the plant branches (80 to 120 cm long) are shortened to small shoots (3 to 6 cm long), with 3.0 to 6.0 g of mass, also called cormels (Hermann, 1997). Thus, this procedure causes several damages, leading the plant to redirect its reserves towards protective constituents, as well as, favoring the infection by phytopathogens into the segmented tissue (Reghin *et al.*, 2000). That's why, it is common to apply sanitizers as sodium hypochlorite to prevent contamination by pathogens, or thymol, a phenolic compound described as a sustainable tool for seed treatment (Lima *et al.*, 2019), and/or biostimulants, to reduce possible losses and damage during the preparation of propagules (Reghin *et al*., 2000).

Recognized biostimulants promote plant nutritional efficiency, and/or tolerance to biotic and abiotic stresses, and may even improve the expression of a characteristic of crops (Jardin, 2015). Additionally, bioactive compounds, isolated from macro or microorganisms, have proven to be excellent biostimulants, as tryptophol, another phenolic compound, with recognized effect on bean plants growth (Nascimento *et al.*, 2016).

Although, the biostimulants definition has been discussed in the last years, it is difficult to issue a definitive concept, probably due to the multicomponent composition of some biostimulants and the several modes and mechanisms of action, since the functionality of biostimulants is often no limited to only one compound, but to interactions between compounds (Jardin, 2015; Arnao & Hernández-Ruiz, 2019).

Since this is a new and sustainable research, the comprehension of several biostimulants in plants still need to be better understood, mainly to characterize their application and effect on crops like arracacha. Therefore, it is essential to improve techniques and technologies that promote efficient sanitization and aid the establishment and initial development of the propagules. The aim of this research is to evaluate the effect of two sanitizers (thymol and sodium hypochlorite) and three biostimulants (tryptophol, stimulate® and *Bacillus subtilis* C-3102) on initial development of arracacha plants.

MATERIAL AND METHODS

The research was conducted in a greenhouse, the temperature ranging from 11 to 39°C. The arracacia propagules were grown in plastic pots, 8.5 dm^3 capacity, containing red oxisol, and the following chemical characteristics: P (resin) = 64 mg/dm^3; S-SO_4= 6 mg/dm^3; K= 0.3 cmolc/dm^3; Ca= 4.9 cmolc/dm^3; Mg= 0.8 cmolc/dm^3; M.O.= 35 g/dm^3 and pH= 5.5 ($CaCl_2$). Soil fertilization was carried out in accordance with the crop recommendations: 4 g of N-P-K (4-14-8) applied in each pot.

Propagules from 'Amarela de Senador Amaral' cultivar were used in this research; first, roots and leaves were removed from the selected plants, and the remaining material was partitioned in seedlings. We evaluated two sanitizers, where the propagules were sanitized in thymol ($C_{10}H_{14}O$, Sigma-Aldrich) solution, containing 1 mg/L of thymol solution (m/v) (Lima *et al.*, 2019), or sanitized in sodium hypochlorite solution, containing 5% of NaClO (v/v). This procedure was considered a protocol pattern of propagule sanitization. For both treatments, propagules were immersed for 5 minutes in the respective solutions, and then washed under running tap water, and dried indoor without climatic control.

After sanitization procedures, the biostimulants were applied to propagules, as follows: tryptophol ($C_{10}H_{11}NO$, Sigma Aldrich) = 0.4 mg/L (Nascimento *et al.*, 2016); Stimulate® (0.09 g/L of kinetin, 0.05 g/L of gibberellic acid, and 0.05 g/L of indol butyric acid) = 7 mL/L (Reghin *et al.*, 2000), and 0.5 g of *B. subtilis* C-3102 (5 x 10^9 UFC/g), per dm^3 of soil. Cormels were planted on pots, on January 29, 2021, 4 cm deep. The irrigation system was manual, according to water plant requirement. Twenty-eight days after planting (DAP), plants were fertilized with 4 g of N-P-K (4-14-8), and at thirty-eight DAP nearly 200 g of brachiaria straw was added to the soil surface in the pots. We adopted an organic management to control pests and pathogens.

The CO_2 assimilation rate (μmol/m^2/s) and foliar transpiration (mmol of H_2O/m^2/s) were obtained at 42 DAP, through a portable photosynthesis system (LI-6400XT; Li-Cor Biosciences, USA) equipped with a modulated fluorometer under saturating photosynthetic active radiation (*Q*) of 900 μmol/m^2/s. The plant shoot biometry was analyzed from 7 to 42 DAP, by measuring the length of the principal and lateral shoots (cm). At the end of research (72 DAP), volume and root area were analyzed by SAFIRA® software and simultaneously the root dry matter was taken. Mature leaves were collected to analyze: total soluble protein content (TSP) (Bradford, 1976). Ascorbate peroxidase (APX) and catalase (CAT), were also evaluated in UV-Vis spectrophotometer (Sperotto, 2014).

The research was set up in a completely randomized design, in 2 x 4 factorial arrangement (2 sanitizers associated to 3 biostimulants or not (control treatment), each treatment was performed in 4 replications. The data were subjected to normality, homoscedasticity, and additivity tests; subsequently analysis of variance and means were compared by the SNK test, in Spreadsheet Program SpeedStat (version. 2.5). For shoot biometric variables, descriptive analyzes were conducted. The enzymatic metabolism was investigated by Principal Components Analysis (PCA) and the figure plotted using the PAST (Paleontological Statistics, version 4.02).

RESULTS AND DISCUSSION

Regarding the analysis of CO_2 assimilation rate, in arracacha leaves, highest averages of its variable were recorded when propagules were subjected to NaClO associated to *B. subtilis* C-3102 treatment, statistically significant when compared to treatment NaClO associated to tryptophol. Treatments of thymol isolated or associated to biostimulants had not affected CO_2 assimilation rate (Figure 1A). For transpiration, no differences were observed among treatments (Figure 1B). According to data obtained in CO_2 assimilation (Figure 1A) we observed a positive interaction among NaClO sanitizer and soil bacteria *Bacillus subtilis* C-3102. The microbial diversity (eg. *Pseudomonas*, *Mesorhizobium* and *Bacillus*) present in the soil has proven to be very efficient in production of plant hormones and solubilization of inorganic elements present in the soil environment (Ahmad *et al.*, 2008). The presence of bacteria in the soil can contribute to gains in CO_2 assimilation rate (Efthimiadou *et al.*, 2020). Furthermore, the leaf gas exchange in arracacha cultivars is severely affected by variations in edafoclimatic conditions, as soil, nutrients, water and light conditions (Jaimez *et al.*, 2008). So, the use of biological biostimulant (*Bacillus subtilis* C-3102) is a sustainable alternative to improve the photosynthesis.

A uniform growth of the shoots, compared to the principal shoot was observed in the course of time (Figure 1C), whereas, the lateral sprout was impaired by thymol as sanitizer, or by the use of NaClO associated to *Bacillus subtilis* C-3102 (Figure 1D). A prominent main stem elongation was observed until 14 days after planting (Figure 1C), in plants treated with NaClO + *Bacillus subtilis* C-3102, also a major lateral shoot growth was observed in plants treated with NaClO + tryptophol. As describe by Ahmad *et al*. (2008), the microorganisms present in the soil stimulate plant growth by diverse pathways, and a natural biostimulant found in microorganisms improve the biological process (Nascimento *et al.*, 2016).

The analysis of root growth showed a positive effect of NaClO associated to *B. subtilis* C-3102 on root volume (Figure 1E), and tryptophol associated to NaClO and thymol on root dry matter (Figure 1F). However, potential phytotoxic effect was recorded for root volume subjected to NaClO sanitizer in combination with Stimulate®, and root dry matter subjected to NaClO alone, or associated to Stimulate and *B. subtilis* C-3102.

Attention is drawn to the fact that bacteria improved the root volume. According to the research with rice, *B. subtilis* increased the endogenous IAA production and improved nitrogen and phosphorus nutrition (Jamily *et al.*, 2019). We considered that this increase is linked to the availability of phosphorus in the soil and auxin signaling from microorganisms. The effect of tryptophol on the root system is directly related to the indole metabolism of plants (Nascimento *et al.*, 2016). In soybean plants, gains were also observed in the root system, through the radial expansion of the main root (Macedo *et al.*, 2018). The use of thymol associated to biostimulants showed a very similar response, but without significant interaction.

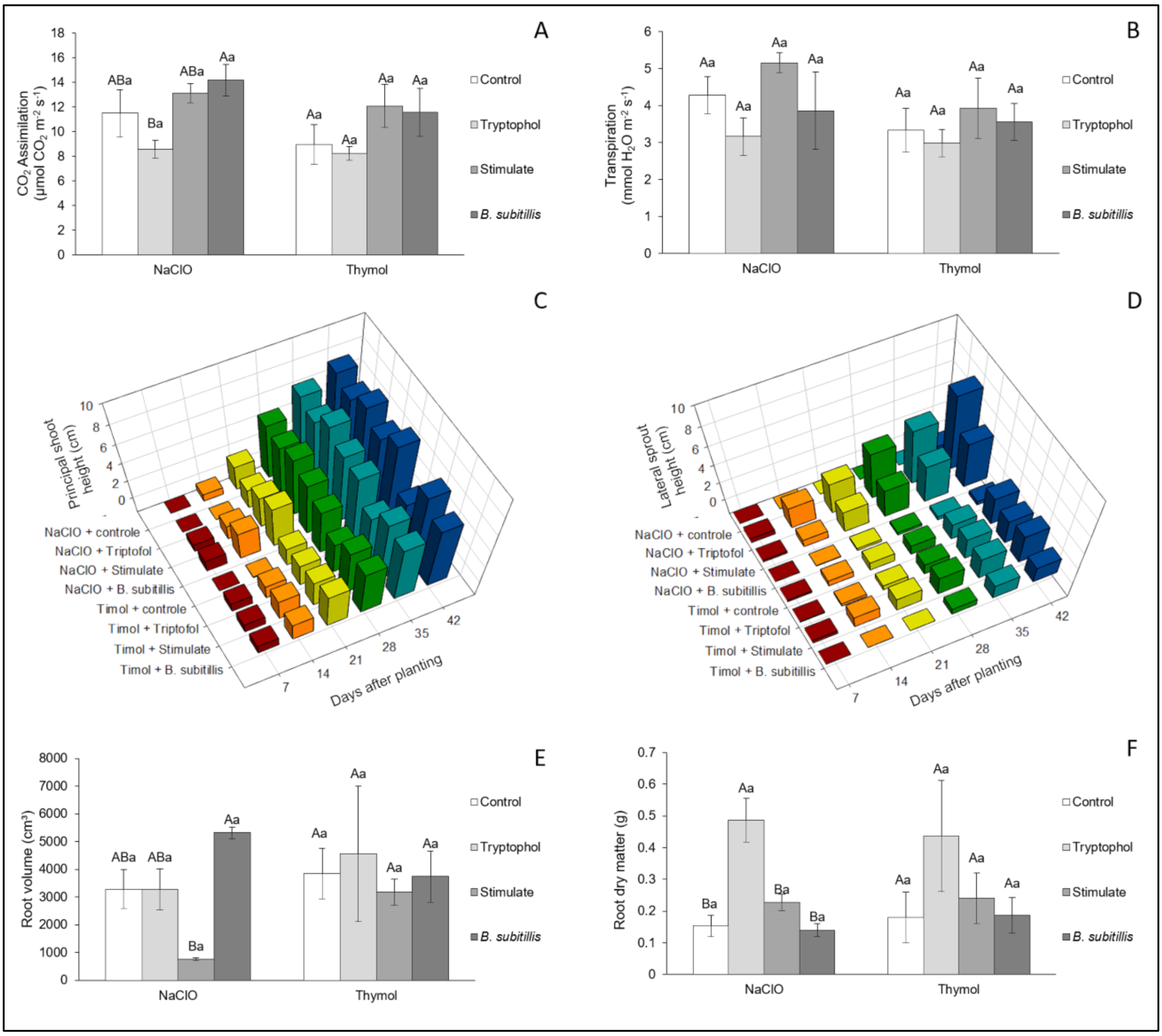

Figure 1. CO_2 assimilation (A), foliar transpiration (B), principal shoot height (C), lateral sprout height (D), root volume (E) and root dry matter (F) of *Arracacia xanthorrhiza* plants, submitted to 2 sanitizers (NaClO and thymol) and 4 biostimulants (control, tryptophol, Stimulate® and *B. subtilis* C-3102). Means with the same capital letters are not significantly different for biostimulants levels, and identical lowercase letters do not differ from each other for sanitizer levels (p≤0,10). Rio Paranaíba, UFV, 2021.

The multifactorial analysis of the antioxidant metabolism of arracacha plants submitted to sanitizers and biostimulants, showed that the principal components (PC1 and PC2) corresponded to 82.5% of the total variance (Figure 2). These data demonstrate superior enzymatic activity (APX and CAT) for propagules sanitized only with thymol and for the treatment with thymol in combination with tryptophol. It was observed that the position of TPS and of the CAT and APX enzymes are opposite, indicating an expected response pattern in the defense mechanism, with soluble protein deviation (growth metabolism). For the protection metabolism (Figure 1E), we consider this phenomenon a plant protection response to the use of this sanitizer, which exceptionally may have signalled an increase in reactive oxygen species (ROS). The antioxidant enzymes APX and CAT are related to the protection of plant tissues against superoxide and hydroxyl radical, highly reactive and unstable compounds that in high concentrations cause damage to the plant cell structure (Mittler *et al.*, 2022)

The antioxidant enzymes (APX and CAT) are related to the protection of plant tissues against ROS, with emphasis on superoxide and the hydroxyl radical, which are highly reactive and unstable and in high concentrations can damage the plant cell structure (Waszczak *et al.*, 2018). Our results showed that thymol sanitizer applied isolated or associated to tryptophol induce plant signal for APX and CAT activities, possibly to defend plant tissue against pathogens, considering that both molecules are originated from phenols (Lima *et al.*, 2019; Palmieri & Petrini, 2019).

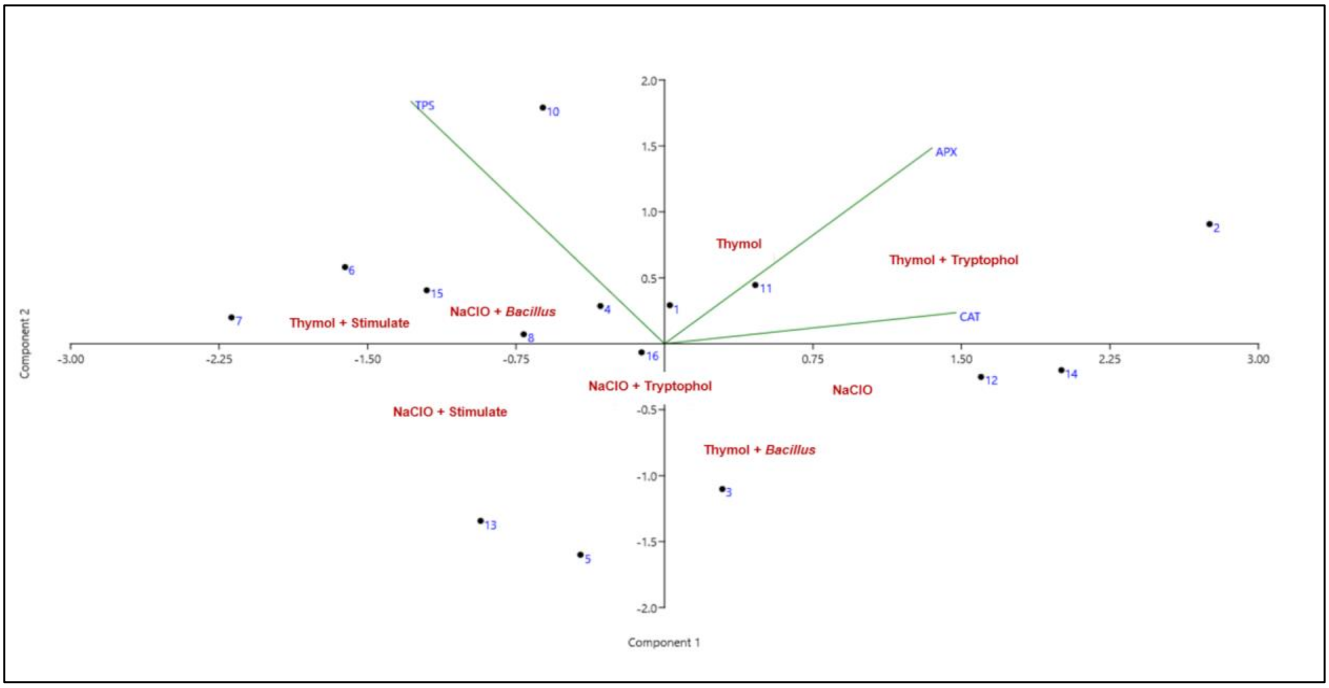

Figure 2. Antioxidant metabolism in *Arracacia xanthorrhiza* plants, submitted to 2 sanitizers (NaClO and thymol) and 4 biostimulants (control, tryptophol, Stimulate® and *B. subtilis* C-3102). Rio Paranaíba, UFV, 2021.

The analysis of the total soluble proteins presented in the leaf tissues, showed superior results for the treatments involving NaClO, with higher concentration observed in the treatment with NaClO associated to Stimulate®. This result is probably related to a better plant regulation, which resulted in a lower activity of proteolytic enzymes causing an increase in the protein content in the leaves.

In conclusion, the treatment with NaClO and thymol allowed the establishment of healthy plants, the NaClO had less impact on the initial development in arracacha seedlings, when compared to the use of thymol. The interactions between sanitizers and biostimulants showed distinct responses in arracacha, where uses of Stimulate® and *B. subtilis* C-3102 reflected in the adequate development of the plant shoot, while tryptophol expressed better responses in roots. It was also observed that the use of thymol induced oxidative protection mechanisms in plant tissues, due to the greater expression of CAT and APX enzymes.

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