GIBBERELLIN PRODUCTION BY ANTAGONISTIC FUNGUS STRAINS (*Trichoderma harzianum*)

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Abstract:

Trichoderma is one of the most important fungi in agriculture. It has demonstrated many capabilities to be used as biofertilizers as well as bio-fungicides. It has also shown its sustainability and various mechanisms of providing the crop with nutrients. It was reported to improve the plant growth of many crops. Six strains of *Trichoderma harzianum* were isolated and identified in the laboratory of biopesticides in the National Institute of Agronomic Research of Algeria (INRAA). All the obtained strains were tested for their capacity to produce gibberellin. The results revealed that all the isolated strains have the ability to produce GA3 whereas the 1st strain showed the production of GA3 with no difference between days. Unlike, the other strains showed very highly significant production of GA3 between days. The production of GA3 reached the highest value after 14 days, unlike the 7 and 21 days after. On the other hand, S2 presented the highest average for filtrate content of GA3 with 83 mg/100mL, followed by S with 76 mg/100mL, and S5 with 68 mg/100mL, while other isolates have recorded the lowest average of 64, 75, and 48 mg/100mL.

Keywords: *Trichoderma*, filtrate, gibberellin, plant growth, bio-stimulant,

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Introduction

A sustainable agricultural industry is required to feed the ever-increasing population of the earth. Huge quantities of chemical products are utilized by the agriculture industry for crop growth and higher yields where the application of these products cause huge damage to the environment and are hazardous to human health. Biological products may be a very good alternative as they are eco-friendly and also need fewer financial inputs. Meanwhile, fungal endophytes are well known to contribute plant fitness benefits, enabling the adaptation of the plant host to biotic and abiotic stresses (Rodriguez and Redman 2008; Aly et al. 2011; Franken 2012; Johnson et al. 2013; Johnson et al. 2014; Card et al. 2016), where they present an important source of natural bioactive components with their potential applications in agriculture, medicine, and food industry (Tan and Zou, 2001). These organic substances are able to modify the plant's physiological functions and growth, even at low concentrations. Owen and Hundley (2004) have proposed that endophytes were «chemical synthesisers in plants». These fungi can provide several benefits to plants such as protection against disease (Redman et al., 1999, 2001), production of secondary metabolites effective against host pathogens (Liu et al., 2001), protection against insect pests (Azevedo et al., 2000; Anke and Sterner, 2002) and resistance to herbivores (Latch, 1993).

Plant hormones are involved in several stages of plant growth and development, it is essential for plant defense (Costacurta and Vanderleyden, 1995). The promotion of plant growth by endophytic fungi is partly due to their production of phytohormones such as auxins (AIA), cytokinins, gibberellins, and other growth substances. Gibberellins (AGs) are involved in various processes of plant growth and development, such as the induction of hydrolytic enzyme activity during seed germination, improvement of crop yield, overcoming dwarfism, elimination of dormancy, sex expression, leaf expansion, stem elongation, increased number and size of flowers (Rangaswamy 2012; Rios-Iribe, et al., 2010; Bruckner and Blechschmidt 1991; Kumar and Lonsane 1989). Several works confirmed the importance of AG_3 in improving the synergy and crop performance under salinity conditions. This enhancing effect of gibberellic acid can affect several parameters including growth and photosynthesis. Also, it interacts with other hormones to regulate various plant processes. *Trichoderma* spp. are well-known mycoparasites of several fungi, able to secrete cell wall degrading enzymes (CWDE), such as chitinases and glucanases, to aid in parasitism (Gruber et al. 2012, Gajera et al. 2013). Also, it has been reported to promote plant growth in various ways where farmers have used it as a biofertilizer, especially in India, because of its ability to stimulate the plant growth of many crops. It comes as an alternative to chemical fertilizer or as an amendment to improve crop production. Many attributes qualified it to be used as an alternative or amendment to improve fertilization sustainably. The main reservoirs for *Trichoderma* communities are soil, rhizosphere, and plant microbiomes. For this, the present work came to evaluate the phytohormone production (gibberellins) of *Trichoderma* spp. isolated from different plants categories in the south-eastern region of Algeria (Touggourt).

Material and methods

Isolation of antagonistic fungi

Several crops (tomato, wheat, date palm, …etc) from different exploitations, in the region of Touggourt (southeastern Algeria), were investigated for their association with antagonistic fungi. Leaf, stem, root, and soil of these crops were collected and carried to the biopesticide laboratory, in the National Institute of Agronomic Research of Algeria (INRAA). Then surface sterilization was done using ethanol (70%) for 5 minutes, NaCl (Sodium Hypochlorite) (3%) for 5 minutes, and again (70%) ethanol for 5 minutes. Leaf, stem, and root pieces were cut into small discs (3mm) and then placed on an LBM medium containing and kept at room temperature for 5 days (Hallmann et al., 2007). After the emergence, obtained fungi were inoculated on PDA (potato dextrose agar) medium for purification. For GAs collection, the Czapek-Dox broth medium was used (Khan et al., 2009).

Gibberellin production capacity

The isolated fungi were prepared and cultured in 250 ml glass bottles containing 100 ml of two discs with a diameter of 5 mm from colonies growing on a Czapek-Dox broth sterile medium for 7 days at a temperature of 28±2° C in a stirrer with 150 rmp/min in dark. Then, the nutrient medium was filtered, using a fine filter (40 and 20 milliliters), by taking 5 ml of the filtrate and putting it in a separating funnel of 100 ml and completing the volume to 10 ml by adding sterile distilled water, then adjusting the pH to 2.5 using N HCl. 20 ml of Ethyl acetate were added to the solution and shaken to separate the layers, then, the lower aqueous phase was transferred to the separation again. The same procedure was repeated with Ethyl acetate three times. Gibberellic acid was re-extracted from the collected phases of Ethyl acetate using 20, 15, and 10 ml of phosphate buffer solution (pH: 7.4), successively, where the solution was shaken for one minute and the extracted quantities were collected in a 50 ml beaker. Complete the content of the beaker to 50 ml by adding a phosphate buffer solution (Altaie and Alwan, 2018). 20 ml of the extracted solution was taken and transferred to two 100 ml flasks, then, 10 ml of absolute alcohol was added to each of them. The volume of one of the beakers was completed to 100 ml by adding HCL acid (35%) where the absorbance of the samples was measured after 80 minutes at 20° C using a Spectrophotometer UV at 254 nm. As for the second flask, 35 ml of diluted HCL acid (5%) was added, then, the volume was filled with water. Immediately after that, the absorbance was measured using a Spectrophotometer at 254 nm (Bruckner and Blechschmidt, 1991). The experiment was carried out with three replications for each fungus. The same process was repeated after 14 and 21 days.

Using a spectrophotometer UV, at 254 nm, gibberellic acid was measured by drawing curves for the production of fungal isolates of auxin and compared with the standard curve of gibberellic acid as well to determine the IC_{50} (Holbrook et al., 1961).

Statistical analysis

All the obtained results were statistically experimented with using SPSS software (V.20.0). The difference between the production of GA3 from the different strains, as well as the difference between days, was performed using Least Significant Difference (LSD).

Results

Isolation of endophytic fungi

Six strains of *Trichoderma harzianum* were isolated and identified in the laboratory of biopesticides in the INRAA, Touggourt station.

Gibberellin production

All the obtained strains of *T. harzianum* were tested for their capacity to produce gibberellin. The standard curve of gibberellic acid (GA3) was drawn in Figure 1.

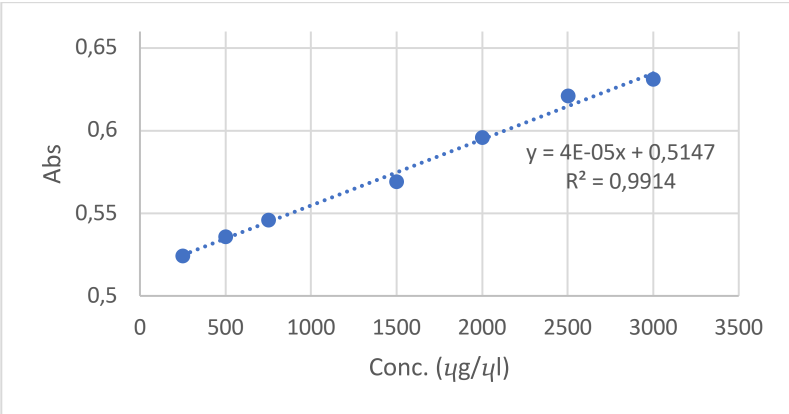

Fig. 01 - Standard curve of gibberellic acid (GA3)

In terms of strains, all the tested ones showed a significant difference in the production of geberrillin (GA3) between days, unless, the 1st strain has shown the production of GA3 with no difference between days (Fig. 2A). Unlike, the other strains showed a very high significance in the production of GA3 between days. The 2nd and the 5th stains presented a very highly significant between days (p = 0.000) (Fig. 2B, 2E), whereas the 3rd, 4th and 6th strains showed a highly significant difference in the production of GA3 between days (Fig. 2C, 2D, 2F).

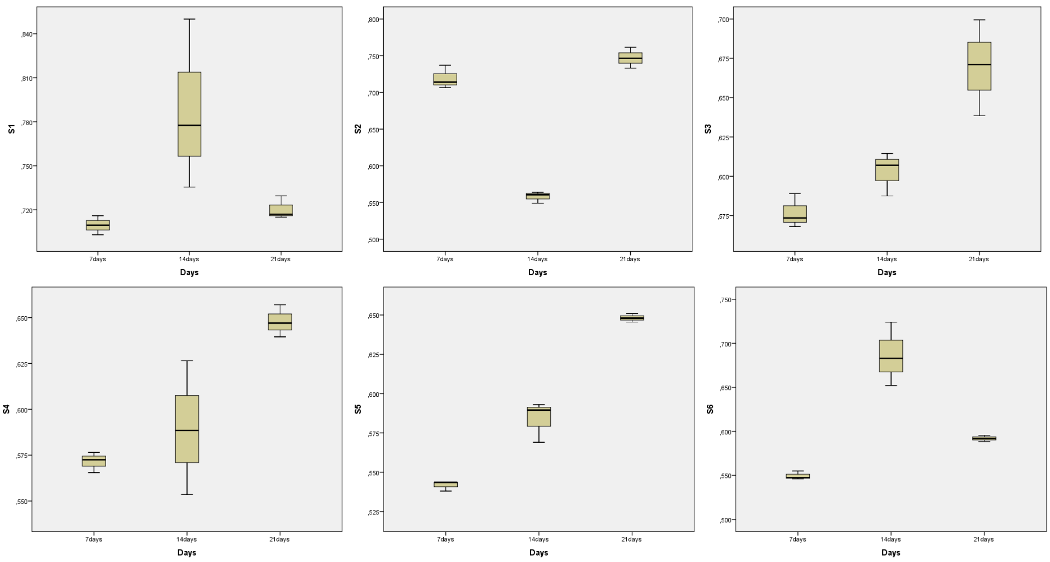

Fig. 02 - Gibberellin production by the strains of *T. harzianum*

According to the obtained results, the highest strain that produced GA3 after 7 days was S2 with 5.11 ug/ml followed by S1 with 4.86 ug/ml (Fig. 2B, 2A). Unlike, after 14 days S1 showed the highest concentration with 6.81 ug/ml, followed by the last strain with 3.87 ug/ml (Fig. 2A, 2F). After 21 days, the 2nd (5.78 ug/ml) and the 1st strains (5.03 ug/ml) have performed the highest production of GA3 (Fig. 2B, 2A).

Meanwhile, S2 presented the highest average for filtrate content of GA3 with 83 mg/100mL, followed by S1 with 76 mg/100mL, and S5 with 68 mg/100mL, while other isolates have recorded the lowest average 64, 75, and 48 mg/100m

Discussion

The establishment of microbial symbioses to promote plant growth and nutrient acquisition by beneficial microbes has been correlated to the biosynthesis of plant growth regulators and phytohormones (Boivin et al. 2016, Egamberdieva et al. 2017). Among these later, gibberellic acid (GA) that were evaluated in the current study from different strains of *Trichoderma*.

The present experiment was continued according to the results obtained by Lakhdari et al. (2020a, b) evaluating the bio-stimulant potential of *Trichoderma* strains on different crops such as *Carthamus tinctorius* L. and *Abelmoschus esculentus* L. in south-eastern Algeria. These studies revealed that the contribution of this fungus in the enhancement of these examined plant growth especially their production (yield) allowed us to investigate their capacity in the production of this phytohormone (gibberellins).

According to the obtained results, all the strains showed that the production of GA3 reached the highest value after 14 days, unlike the 7 and 21 days after. The same ascertainment was mentioned by Altaie and Alwan (2018), where *Trichoderma hamatum* recorded the highest production of GA3 after 15 days with 113.3mg/100ml. In comparison to other productive fungi, the same authors found that the production of GA3 hormone identify through fungal filtrate content in both shaking 120 r/min and static incubation for 7, 14 and 21 days respectively at 28±2° C. *Aspergillus fumigatus* has the highest average of filtrate content of GA3 hormone with 2.018 mg/L, followed by *A. niger* with an average 0.742 mg/L, while *Trichoderma hamatum* has the lowest average 0.698 mg/L after 21 days in shaking incubation conditions. In static incubation conditions, isolate *A. fumigatus* has the highest average filtrate content of GA3 hormone at 2.433 mg/L, followed by isolate *A. niger* at 0.477 mg/L, while *T. hamatum* has the lowest average of 0.443 mg/L. Bader et al. (2020) reported that the presence of *Trichoderma* increases the production of some growth hormones such as indole-3-acetic acid (IAA) and gibberellic acid. Whereas these two hormones are important in promoting plant growth, they are responsible for plant elongation (Singh et al. 2019). On the other hand, Illescas et al. (2021), declared that *T. harzianum* T115 was the strain in which the levels of GA_1 and ABA production in PDB were significantly the highest. Whereas, *T. virens* T49 and *T. harzianum* T115 were the only strains that produced GA4 and GA1, respectively, in a medium not supplemented with Tryptophane. On the other hand, Rubio et al. (2014, 2017) mentioned that the application of *T. parareesei* T6 or *T. harzianum* T34 to tomato seeds improved the tolerance of plants to salt stress and enhanced the growth when plants grew under this adverse condition. Also, improved drought tolerance was observed in rice genotypes inoculated with *T. harzianum* Th-56, in which the antioxidant machinery was activated in a dose-dependent manner (Pandey et al. 2016).

Jaroszuk-Sciseł et al. (2019) mentioned the presence of a very strong positive correlation between GA concentration and dry weight of mycelium as well as a negative correlation with incubation temperature was demonstrated. At a temperature of 20 and 28° C, GA concentration reached about 9.0 µg/mL, and at 12° C it was almost 20-times lower (about 0.5 µg/mL)

Conclusion

The current study reports valuable information on the GAs production capacity of the tested strains of *Trichoderma harzianum.* Also, it confirmed the importance of this fungus and its role in promoting the growth of plants, especially yields. However, the presence of these microorganisms in the soil contributes to the protection and enhancement of plants growth. It is therefore an option for farmers to use for sustainable cropping and increase yields and quality of the produce.

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