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Isolation and Screening of *Fusarium* Species for Gibberellic Acid (Ga_3) Production

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ABSTRACT
The essentiality of agriculture in the national economy underscores the pursuit of alternative solutions to amplify the production of food and raw materials. In this context, plant hormones are regarded as a key factor to enhance the technical proficiency and financial viability of agricultural production processes. Gibberellic acid (GA_3), a vital plant hormone that influences growth and development in plants, is commonly used in various agricultural and horticultural fields. Many bacteria and fungi are known to produce GA_3 as their secondary metabolite, along with plants. Fungi are known to produce more GA_3 than other microbes. For that attempt, Fusarium species were isolated from the various soil samples collected from different places of Ahmedabad, Gujarat. Fifteen isolates were evaluated for GA_3 production through spectrophotometric methods and HPLC. It was found that two isolates, designated JF4 and JF6, produced 11.32 mg/L and 6.66 mg/L respectively which was higher amount of GA_3 compared to the other fungi isolated.
Key words: *Gibberellic acid (GA_3), Isolation, Screening, Fusarium spp., HPLC*

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INTRODUCTION

Agriculture is an indispensable sector of the economy that provides food and raw materials to sustain life while contributing significantly to the growth and development of a nation. The growing demand for agricultural products requires finding alternative solutions to enhance production. In this context, plant hormones are emerging as a key factor to increase technical proficiency and financial viability in agricultural production processes [1]. Plant growth hormones, also known as phytohormones, are naturally occurring organic compounds that regulate plant growth and development. Plant hormones play a vital role during the various stages of growth and development. In that, Gibberellic acid has worldwide consideration due to its valuable applicability in agricultural and brewing industry. Gibberellic acid (GA_3) is a well-known plant growth regulator that has been used in agriculture for many years to increase yields and improve quality. GA_3 is involved in various physiological processes, such as cell elongation, seed germination, flowering, and fruit development [2,3]. The production of GA_3 using traditional chemical methods was expensive and often involves toxic substances. Now, the bulk production of this natural plant hormone is fulfilled by the fermentation technology using *Fusarium moniliforme*. This fungus was earlier known as *Gibberella fujikuroi* [4]. *Fusarium* spp. are potential source for GA_3 production. The amount of GA_3 produced varies depending on culture conditions. For example, some studies have shown that *Fusarium moniliforme* can produce high levels of GA_3 than others [1]. Furthermore, culture conditions such as pH, temperature, and nutrient availability can significantly affect GA_3 production in *Fusarium* species. The aim of this study to isolate and screen *Fusarium* spp. from soil samples for their ability to produce GA_3.

MATERIALS AND METHODS

Collection of soil sample

The soil sample was taken from the different geographical area of Ahmedabad (Gujarat, India) having the plants with symptoms of wilt and abnormal growth. Firstly, the upper part of the soil and other debris were cleared. Then the soil was excavated to depth near to 2 cm from where the soil samples were collected.

Isolation of fungi

For the purpose of isolating fungi from soil, the serial dilution method was used. one gram of soil sample was serially transferred into 9 ml of sterile distilled water, and dilutions up to 10^{-6} were prepared. Then, 0.1 mL sample was spreaded on Potato dextrose agar (PDA) plates containing streptomycin (0.1 g % w/v) used as the isolation medium. The plates were incubated at 28±1 °C for 6 days. Typical fungal isolates were taken then analyzed by macroscopic characterization and microscopic examination using lactophenol cotton blue mount. The cultures were primarily identified according to the similar characteristics described in literature [5]. Fungal strains were subcultured onto fresh PDA plates until pure cultures were obtained. They were maintained as purified on PDA slants and stored at 4°C.

Screening of fungi for GA_3 production

Isolated cultures were grown on PDA slant at 28±1 °C for 7 days to obtain spores. After 7 days using sterile Tween 80 water (0.1 % v/v), spores were collected and transferred into separate sterile flask. This spore suspension was used for inoculation into Czapek-Dox (CD) broth with the concentration of 10^6 spores/mL [6]. The pH of medium was 5.5. All the cultures were screened for GA_3 producing capacity using same fermentation broth at 28±1 °C for 7 days of fermentation in incubator shaker (150 rpm). Then selecting a higher producing strain for further production processes.

Extraction of GA_3

The extraction procedure was conducted with minor adaptations to the protocol outlined in literature [7]. Specifically, on the 7th day of the fermentation experiment, samples were extracted and subjected to centrifugation at 6708 g (10,000 rpm) for a duration of 15 minutes. Subsequently, 5 mL of the supernatant was mixed with an equal volume of ethyl acetate in separating funnel and vigorously agitated for 1 minute to facilitate liquid-liquid extraction. The mixture was allowed to settle for 5 minutes to ensure proper phase separation, following which the upper ethyl acetate phase was transferred to a separate flask. This procedure was repeated thrice to maximize the extraction yield. The cumulative ethyl acetate fraction was then collected and subjected to evaporation. The resulting residue was subsequently utilized for the estimation of GA_3.

Estimation of GA_3

GA_3 estimation was done by HPLC method [8]. The residues were dissolved by adding 2 mL mobile phase. C_{18} column (Phenomenex® Luna 5µ C18) used as stationary phase and methanol: water (80:20) was selected as mobile phase. Ortho phosphoric acid was added in mobile phase with the concentration 20 µL per 100 mL. HPLC system was SPD-20A Shimadzu, Japan. The wavelength and flow rate were 206 nm, 0.6 mL/min respectively. The sample volume injected into HPLC system was 20 µL. Total run time of HPLC was 10 minutes.

Calculation

Calculation of GA_3 concentration using HPLC was done as described in Bhalla et al., [8] as below.

$\gamma = \alpha \times c \times v/\beta$

Where γ is the concentration of unknown; α is the respective peak area of unknown sample; β is the peak area of standard GA_3; c is the concentration of standard GA_3 solution (500 µg/ mL); v is the volume made for sample extract.

Size measurement

Size measurement of macroconidia and length between two septa was measured using microscope (Lawrence & mayo LM-52-3002) calibrated using stage micrometer.

RESULTS AND DISCUSSION

Isolation of fungi

The production of GA_3 was found to be predominantly associated with *Fusarium* spp., prompting to focus on isolating these species. Through soil sampling, total 15 distinct isolates were obtained. These isolates were strains of *Fusarium*, which were tentatively confirmed as such via examination of their macro and micro-conidial structures under a microscope. These isolates were assigned isolate codes ranging from JF1 to JF15,

Screening for GA_3 production

After screening of all 15 *Fusarium* spp. isolates, only six exhibited promising potential for the production of GA_3. Subsequent analysis of these six cultures under optimal conditions revealed that cultures JF4 and JF6 displayed the highest levels of GA3 production at 5.66 mg/L and 3.33 mg/L respectively, after a 7-day incubation period in CD broth at 28±1 °C. The results of this screening are presented in Figure 1. HPLC

system was utilized to confirm GA_3 production in which partial purified sample from of the production broth was carried out. The retention time for standard GA_3 determined to be 4.995 minutes at a concentration of 500 µg/mL, as shown in Figure 2. In the analysis of sample from culture JF4, a peak was observed at 5.029 minutes. That indicating the presence of GA_3, as shown in Figure 3.

Figure 1 Screening of GA_3 production

Figure 2 HPLC analysis of standard GA_3 solution

Figure 3 HPLC analysis of sample (culture no. JF4)

Characterization of JF4 culture

The characteristics of JF4, both morphological and microscopic analyses were conducted. Microscopic examination revealed the presence of a mycelium with a size between two septa measuring 23.65 µm, as well as mono phialide and micro conidia. Macro conidia were also observed, with an average size of 24 µm. Additionally, JF4 was found to display a distinct pinkish white colony morphology when cultured on CDA plates, and the presence of chlamydospores was also noted.

Figure 4 Microscopy of JF4 culture: A- wet mount; B- lectophenol staining; C- Macroconidia, 40x lens, total magnification 400 x)

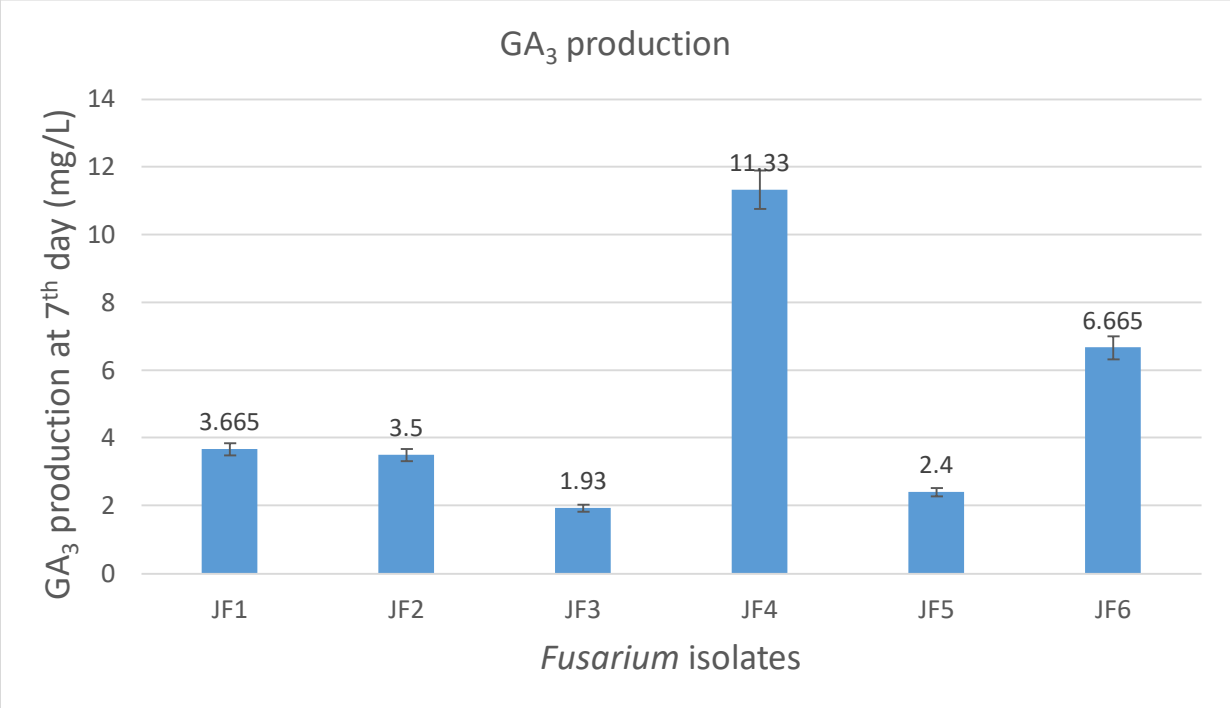

Figure 5 Screening of GA_3 production

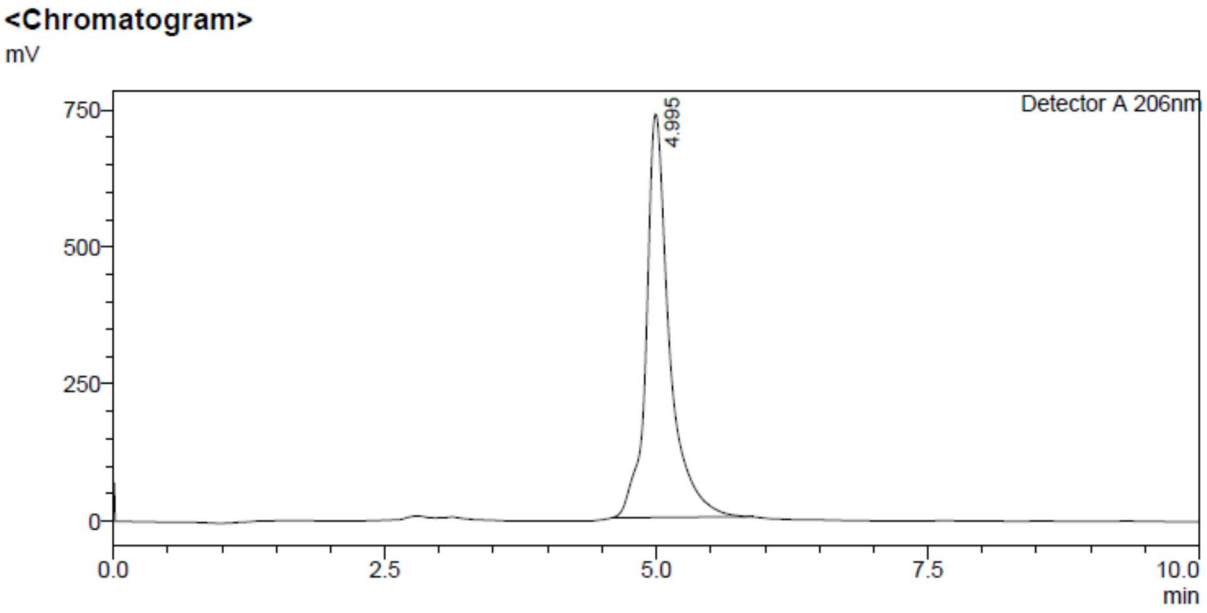

Figure 6 HPLC analysis of standard GA_3 solution

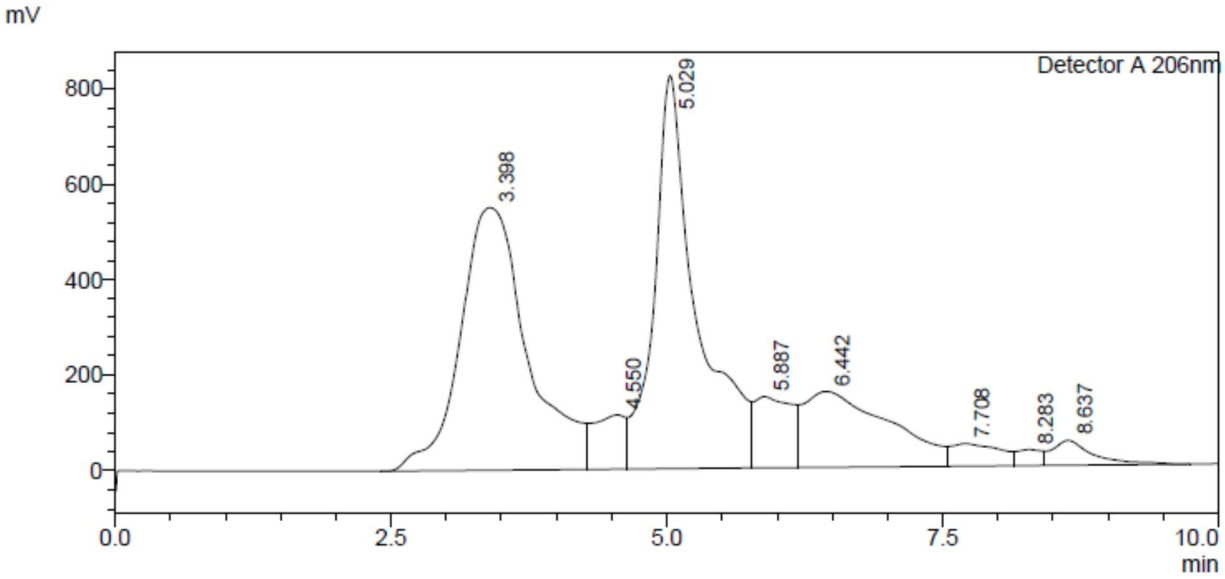

Figure 7 HPLC analysis of sample (culture no. JF4)

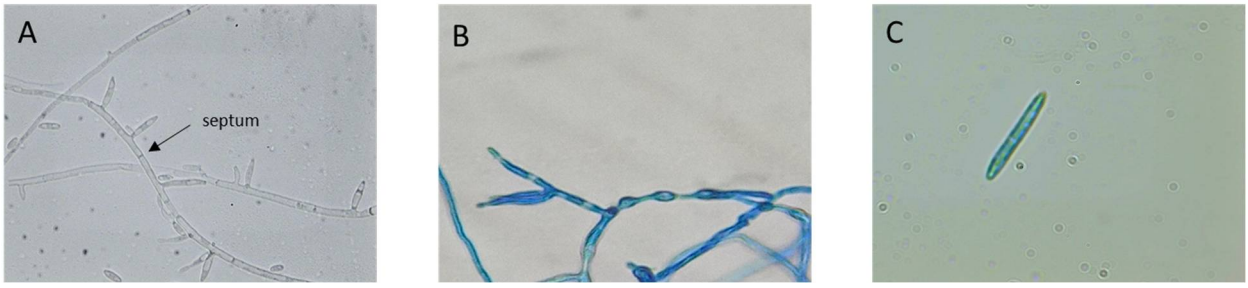

Figure 8 Microscopy of JF4 culture: A- wet mount; B- lectophenol staining; C- Macroconidia, 40x lens, total magnification 400 x)

CONCLUSION

From the above experimentation, the results of this study provide valuable insight into the potential of *Fusarium* spp. as a source of GA_3 production. Through screening of 15 distinct *Fusarium* isolates, six cultures that exhibited promising potential for GA_3 production that GA_3 production was estimated using HPLC system. JF4 culture was further characterized for morphological studies. These primary studies will be beneficial for screening purpose of GA_3 for any researcher that willing to work on related to this topic.

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COMPETING INTERESTS

The authors have declared that no competing interest exists.

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