Journal of Applied Biology & Biotechnology Vol. 13(1), pp. 235-242, Jan-Feb, 2025
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2024.209502

In vitro production of gibberellic acid and fusaric acid by *Fusarium* spp. and their role in bakanae disease development

Asmaul Husna[1,2], Md. Asaduzzaman Miah[2], Latiffah Zakaria[1], Nik Mohd Izham Mohamed Nor[1]*

[1]School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia.
[2]Faculty of Agriculture, Patuakhali Science and Technology University, Patuakhali, Bangladesh.

ARTICLE INFO

Article history:
Received on: July 11, 2024
Accepted on: September 04, 2024
Available Online: November 15, 2024

Key words:
Bakanae, elongation, *Fusarium*, gibberellic acid, and ultra-performance liquid chromatography

ABSTRACT

Gibberellic acid (GA_3) and fusaric acid (FA) are directly correlated with the symptom development of bakanae disease in rice plants. The role of GA_3 and FA, produced *in vitro* by several *Fusarium* species associated with bakanae disease, was studied to understand the disease's etiology and its impact on symptom development. In total, 121 *Fusarium* strains were obtained from bakanae-infected rice plants collected from various rice cultivation regions in Bangladesh. Finally, 18 highly virulent *Fusarium* strains were selected based on virulence assay and further evaluated for GA_3 and FA production through ultra-performance liquid chromatography analysis. Among the *Fusarium* strains, *Fusarium fujikuroi* strains produced a high amount of GA_3 and a low amount of FA. In contrast, *Fusarium proliferatum* and *Fusarium verticillioides* strains produced a high amount of FA and a low amount of GA_3. In exception, the *Fusarium* strain BD117R of F. *fujikuroi* produced no GA_3 but a high amount of FA. Interestingly, *Fusarium commune* produced only FA in high concentration. In bakanae disease development, GA_3 production was positively related to elongation symptoms whereas FA contributes to stunting symptoms. This is the first record of the production of GA_3 and FA by *Fusarium* species causing bakanae disease of rice in Bangladesh.

1. INTRODUCTION

Gibberellic acid (GA_3) is a well-recognized plant growth-promoting hormone preferably produced by *Fusarium* species. Among the *Fusarium* species, *Fusarium fujikuroi* is the most frequently used in the production of GA_3 biotechnologically [1]. GA_3 was first isolated from *F. fujikuroi*, a predominant causal pathogen of bakane disease of rice. Previously, it was thought that *F. fujikuroi* was the only species able to produce GA_3 [2]. But later, it was reported that *Fusarium proliferatum* and *Fusarium verticillioides* also produced GA_3 [3,4]. Therefore, it has been considered that *F. proliferatum, F. fujikuroi*, and *F. verticillioides* produce GA_3 that is related to bakanae disease development. The most important thing, the pathogen's virulence is associated with GA_3 and the virulence depends on excessive secretion of GA_3. GA_3 is not necessary for the growth and development of fungi but it contributes to the virulence mechanism of fungi [5]. Fusaric acid (FA) is a secondary metabolite (SM) as well as a mycotoxin produced by *Fusarium* species, and was first recovered from *Fusarium heterosporum*. FA has also been produced by different types of *Fusarium* species including *F. proliferatum*, *F. verticillioides, F. fujikuroi, Fusarium subglutinans*, *Fusarium sambucinum, Fusarium crookwellense*, *Fusarium oxysporum*, *F. heterosporum,* and *Fusarium solani* [6]. Among the FA-producing *Fusarium* species, *F. fujikuroi, F. verticillioides, F. proliferatum*, and *F. subglutinans* were found to be involved with rice bakanae disease [7].

Different *Fusarium* species have been produced GA_3 and FA, the most prevalent and oldest known SMs [6,8]. A number of *Fusarium* species have already been reported for causing bakanae disease of rice [9] and some of them were studied for GA_3 and FA production. The other *Fusarium* species causing bakanae disease of rice need to be investigated for GA_3 and FA production. *Fusarium* species have species-specific SM profiles [2,10]. Therefore, it was essential to find out which strain of *Fusarium* species produced GA_3 and FA in bakanae-infected plants.

The role of GA_3 and FA in bakanae symptoms development has been reported previously. GA_3 promotes plant elongation [3] and FA has an important role in stunting [4]. FA was also reported for *Fusarium* wilt in tomato [11]. The exact function of GA_3 and FA in the development of disease symptoms is not yet verified, as the specific symptoms caused by the pathogen depend on the production of GA_3 and FA, amount of inoculums level, and environmental factors [12,13]. In fact, the SMs profiles of *Fusarium* species depended on the geographical locations [14,15]. The GA_3 and FA-producing *Fusarium* species causing rice bakanae disease have been reported in several countries including Malaysia [2,4] and India [3]. To the best of our knowledge, previous

*Corresponding Author
Nik Mohd Izham Mohamed Nor, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia.
E-mail: nikizham @ usm.my

to our investigations, there are no reports on GA_3 and FA production by *Fusarium* species obtained from bakanae-diseased rice plants in Bangladesh. In fact, bakanae disease has already been reported as an emerging threat to rice production in Bangladesh [16].

The present study, therefore, was conducted to explore 18 *Fusarium* strains associated with bakanae disease in Bangladesh for GA_3 and FA production *in vitro*, to detect and quantify GA_3 and FA produced by the tested strains of *Fusarium* species, and to confirm the role of GA_3 and FA in bakanae disease development.

2. MATERIALS AND METHODS

2.1. Collection and Identification of *Fusarium* Strains from Bakanae Samples

Diseased rice plants with typical bakanae symptoms such as elongation, lanky, yellowish leaves, stunted and wilted plants were taken from various rice-growing locations in Bangladesh during 2019–2022. *Fusarium* strains were isolated from bakanae infected rice plants. The isolated strains were identified though morphological and molecular identification methods described by Husna *et al.* [17].

2.2. Virulence Assay for *Fusarium* Strains

In total, 121 *Fusarium* strains belonging to five species were assayed for the evaluation of their pathogenic behavior. Among them, 18 highly virulent *Fusarium* strains were selected for further analysis through virulence assay in the plant house method described by Husna *et al.* [17].

2.3. Production of GA3 and FA

2.3.1. Fusarium strains

Based on virulence assay, 18 *Fusarium* strains were identified as highly virulent and found to be associated with bakanae disease of rice. These strains were used in this study for analysis of GA_3 and FA production (Table 1).

2.3.2. Medium preparation

In this study, Czapek-Dox medium was used to stimulate GA_3 and FA production by *Fusarium* strains [18]. For 1 L Czapek-Dox medium preparation, the composition was as follows: $NaNO_3$: 2.0 g, Sucrose: 30.0 g, K_2HPO_4: 1.0 g, $FeSO_4$ $7H_2O$: 0.01 g, KCl: 0.5 g, $MgSO_4$ $7H_2O$: 0.5 g and distilled water :1 l.

2.3.3. Inoculum preparation

The *Fusarium* strains were cultured on PDA plates for 7 days at 25ºC ± 1°C with a 12:12 hours light:dark cycle. After that, the plates were immersed in 5 ml sterile distill water and spread with a glass rod (hockey stick shape). The conidial suspensions were pooled. The concentration was measured with a hemocytometer and adjusted to 1×10^5 conidia/ml.

2.3.4. Inoculation

A 1 ml suspension of conidia from each strain was added into 100 ml of sterilized Czapek-Dox media. For GA_3 production, strains were grown for 5 days in Czapek-Dox medium and shaken at 150 rpm, 30°C in a 12:12 hour light:dark cycle. Besides, for FA production, strains were allowed to grow for 10 days in Czapek-Dox medium and shaken at 180 rpm, 30°C in a 12:12 hour light: dark cycle by using a shaker [19]. The Czapek-Dox media containing 1 ml of sterile distilled water was used as a control.

2.3.5. Extraction, detection and quantification of GA3

2.3.5.1. Chemicals and reagents for GA_3

GA_3 standard (Sigma Chemical Co., USA), methanol, acetone, phosphoric acid, potassium hydroxide, ethyl acetate, hydrochloric acid (purity> 37%), and ultra-pure water (deionized) were purchased. All these chemicals were HPLC grade.

2.3.5.2. Extraction of GA_3

The GA_3 was extracted from *Fusarium* strains according to the method described by Husna *et al.* [20]. In brief, the fungal mycelial mat was discarded from Czapek-Dox medium and the filtrate pH was corrected to 2.5 with 1N HCL. Then, the filtrate was extracted with equal volumes of ethyl acetate using a separatory funnel. After rotary evaporation (50°C and 10 rpm) of ethyl acetate, the suspended residue was dissolved in 5 ml of acetone and kept at 4°C for ultra-performance liquid chromatography (UPLC) analysis.

2.3.5.3. Preparation of GA_3 standard solution

GA_3 in powder form was used as a standard for making stock solutions. Standard stock solution (1,000 µg/ml) was prepared in 5 ml methanol and kept at 8°C for further use. Five working standard solutions; 5, 50, 100, 250, and 500 µg/g were prepared in methanol and filtered by 0.2 µm polytetrafluorethylene filter. Finally, the filtrates were preserved at 4°C before use in UPLC.

2.3.5.4. UPLC analysis for GA_3

In UPLC analysis, quantification of GA_3 was performed according to the method described by Husna *et al.* [20]. In brief, a C18 column (reversed-phase) was used for chromatographic separations. Methanol

Table 1. Collection, isolation and identification *Fusarium* strains.

Fusarium species	Strain	Location GIS coordinates
F. fujikuroi	BD023R	23°36′53″N, 91°6′25″E
	BD043R	23°13′3″N, 91°18′22″E
	BD047R	23°43′40″N, 90°28′23″E
	BD050R	23°13′3″N, 91°18′22″E
	BD056R	23⁰ 12′ 48″N, 91⁰ 26′ 52″
	BD058R	23⁰ 37′ 5″ N, 90⁰ 29′ 59″ E
	BD066R	24⁰ 20′ 44″ N, 91⁰ 25′ 9″ E
	BD080R	23⁰ 55′ 39″ N, 90⁰ 34′ 26″ E
	BD087R	24⁰ 53′ 5″N, 91⁰ 54′ 45″E
	BD094R	24⁰ 11′ 14″N, 91⁰ 10′ 41″E
	BD111R	25⁰ 26′ 12″N, 89⁰ 18′ 51″E
	BD113R	23⁰ 51′ 2″N, 89⁰ 1′ 57″E
	BD117R	23⁰ 16′ 8″N, 89⁰ 10′ 30″E
	BD118R	24⁰ 16′ 52″N, 91⁰ 5′ 27″E
F. proliferatum	BD006R	23°45′14″N, 88°57′07″E
F. verticillioides	BD013R	23°45′14″N, 88°57′07″E
F. commune	BD019R	23°44′54″N, 88°57′26″E
F. sulawesiense	BD026R	23°36′53″N, 91°6′25″E

(20%) with 10 mM H_3PO_4 and pH 2.3 KOH was used as the mobile phase [21]. Then, the mobile phase solution was filtrated. The samples were run at 0.1 ml/minute for 10 minutes. The injected rate was 10 µl/sample. GA_3 was identified by comparing its retention time and UV spectrum to the standard GA_3 sample. For GA_3 quantification, the retention durations and peak heights of the samples were compared to those of GA_3 standards using a calibration curve.

2.3.6. Extraction, detection, and quantification of FA

2.3.6.1. Chemicals and reagents for FA

FA standard (ACROS ORGANICS, 99%), methanol, ethanol, phosphoric acid, ethyl acetate, hydrochloric acid (purity> 37%), and ultra-pure water (deionized) were purchased. All these chemicals were HPLC grade.

2.3.6.2. Extraction of FA

The FA was extracted from *Fusarium* strains according to the method described by Husna *et al.* [20]. In brief, the fungal mycelial mat was discarded from Czapek-Dox medium and the filtrate pH was corrected to 3.5–4.0 with 2N HCL. Then, the filtrate was extracted with equal volumes of ethyl acetate and shaken well in a separatory funnel. The separatory funnel was kept undisturbed for 30 minutes to divide into two layers. The upper layer containing ethyl acetate was taken in a conical flask. After rotary evaporation (50°C and 10 rpm) of ethyl acetate, the suspended residue was dissolved in 5 ml of ethanol and kept at 4°C for UPLC analysis.

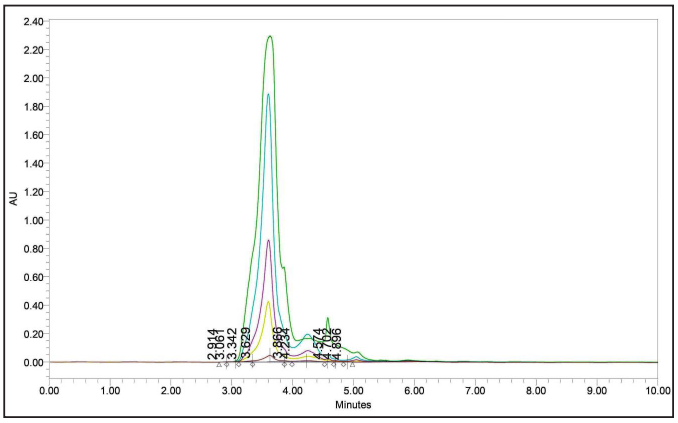

Figure 1. Overlay of GA_3 standard peaks for different concentrations (5, 50, 100, 250, 500 µg/g).

2.3.6.3. Preparation of FA standard

FA in powder form was used as a standard for making stock solutions. Standard stock solution (1,000 µg/ml) was prepared in 5 ml methanol and kept at 8°C for further use. Five working standard solutions; 5, 10, 20, 50, and 100 µg/g were prepared in methanol and filtered by 0.2 µm polytetrafluorethylene filter. Finally, the filtrates were preserved at 4°C before use in UPLC.

2.3.6.4. UPLC analysis for FA

In UPLC analysis, quantification of FA was performed according to the method described by Husna *et al.* [20]. In brief, a C18 column (reversed phase) was used for chromatographic separations. Methanol (20%) and 2% H_3PO_4 (> 85%) were utilized as mobile phases. An isocratic ratio of mobile phases Methanol: H_3PO_4 (70%:30%) was utilized [4]. Then, the mobile phase solution was filtrated. The samples were run at 0.1 ml/minute for 10 minutes. The injected rate was 10 µl/sample. FA was identified by comparing its retention time and UV spectrum to the standard FA sample. For FA quantification, the retention durations and peak heights of the samples were compared to those of FA standards using a calibration curve.

2.4. Rice Seedling Test for Shoot Length Assessment

After sterilization, rice seeds (BRRI 29 variety) were soaked for 24 hours in spore suspension (1×10^6 conidia/ml). Twenty-five seeds inoculated with *Fusarium* strains were placed on filter paper (3 layers, sterile, water-moisture) in Petri dishes, and then the Petri dishes were kept for incubation at 25ºC–26ºC under a 12 hours light and 12 hours dark, cycle [22]. The seeds treated with sterile distilled water served as control and the test was independently replicated thrice. The

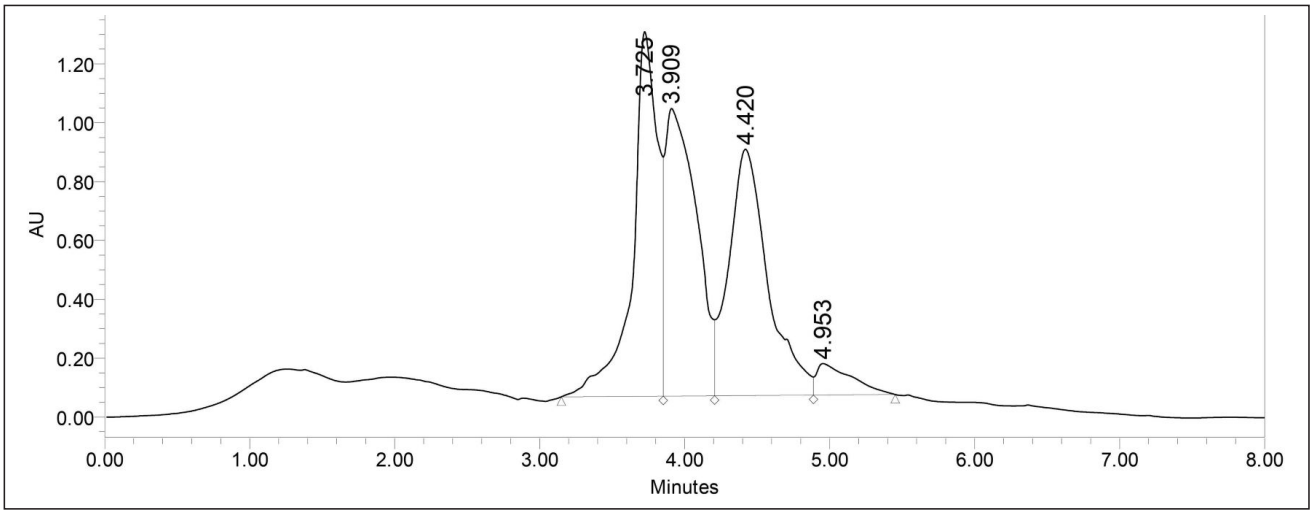

Figure 2. UPLC-photodiode array (PDA) chromatogram of GA_3 produced by *F. fujikuroi* (BD047R) detected at 3.7 minutes.

elongation and stunting symptoms were compared to the seedlings in control and assessed by measuring the seedling heights at 15 days after incubation.

2.5. Statistical Analysis

The GA_3 and FA concentrations were compared among the tested isolates. The standard curve was employed to calculate the concentration of each strain GA_3 and FA using Microsoft Excel 10. The correlation was worked out among GA_3, FA, and shoot length.

3. RESULTS

3.1. Isolation of *Fusarium* Strains

From the collected bakanae diseased rice plants, in total 121 strains of *Fusarium* were successfully isolated and identified through morphological and molecular methods described by Husna *et al.* [17]. Among them, 18 strains were selected based on virulence assay belonging to 5 *Fusarium* species (Table 1). These 18 *Fusarium* strains (14 strains from *F fujikuroi*, 1 strain from *F. proliferatum*, 1 strain from *F. verticillioides*, 1 strain from *Fusarium commune,* and 1 strain from *F. sulawesiense*) were further analyzed to produce GA_3 and FA.

3.2. GA3 Production

The synthesis of GA_3 by 18 *Fusarium* strains was assessed by comparing their retention time with GA_3 standard. A calibration curve using a GA_3 standard was created in order to accurately measure the concentration of GA_3 in different strains. The retention time of GA_3 ranged from 3.6 to 3.9 minutes for both standards and samples (Figs. 1 and 2).

In UPLC analysis, out of 18 strains, 15 strains of *Fusarium* species were capable of producing GA_3 at different concentrations in Czapek-Dox media. In contrast, GA_3 was not synthesized in the Czapek-Dox media inoculated with sterile distilled water that served as a control.

The production of GA_3 levels ranges from 14.43 to 327.87 µg/g. The highest concentration (327.87 µg/g) of GA_3 was produced by the strain BD066R of *F. fujikuroi*, while the lowest concentration (14.43 µg/g) was produced by the strain BD006R of *F. proliferatum*. The *Fusarium* strains of *F. fujikuroi* species produced higher concentrations of GA_3 compared to other species. All the strains of *F. fujikuroi* produced GA_3 except BD117R. Other than *F. fujikuroi* species, *F. proliferatum* and *F. verticillioides* produced GA_3 in low concentration while *F. commune* and *F. sulawesiense* could not produce GA_3 (Table 2).

3.3. FA Production

The synthesis of FA by 18 *Fusarium* strains was assessed by comparing their retention time with FA standard (Table 2). A calibration curve using an FA standard was created in order to accurately measure the concentration of FA in different strains. The retention time of FA ranged from 3.6 to 3.9 minutes for both standards and samples (Figs. 3 and 4).

In UPLC analysis, out of 18 strains, 15 strains of *Fusarium* species were capable of producing FA at different concentrations in Czapek-Dox media. In contrast, FA was not synthesis in the Czapek-Dox media inoculated with sterile distilled water that was served as a control.

The production of FA levels ranged from 47.26 to 254.64 µg/g. The highest concentration (254.64 µg/g) of FA was produced by the strain BD019R of *F. commune* while the lowest concentration

Table 2. GA_3 and FA production by *Fusarium* strains.

Fusarium species	Strain	Concentration (µg/g)	
		GA_3[a]	FA
F. fujikuroi	BD023R	325.15	47.26
	BD043R	326.10	50.72
	BD047R	162.94	49.21
	BD050R	323.95	48.14
	BD056R	327.22	50.29
	BD058R	258.20	50.13
	BD066R	327.87	57.54
	BD080R	319.98	56.09
	BD087R	281.85	ND
	BD094R	80.86	ND
	BD111R	122.24	48.03
	BD113R	299.69	50.16
	BD117R	ND	253.34
	BD118R	322.86	50.51
F. proliferatum	BD006R	14.43	247.33
F. verticillioides	BD013R	27.63	223.5
F. commune	BD019R	ND	254.64
F. sulawesiense	BD026R	ND	ND
Control		ND	ND

[a]The sample of GA_3 were diluted with methanol into 50:50 ratio.

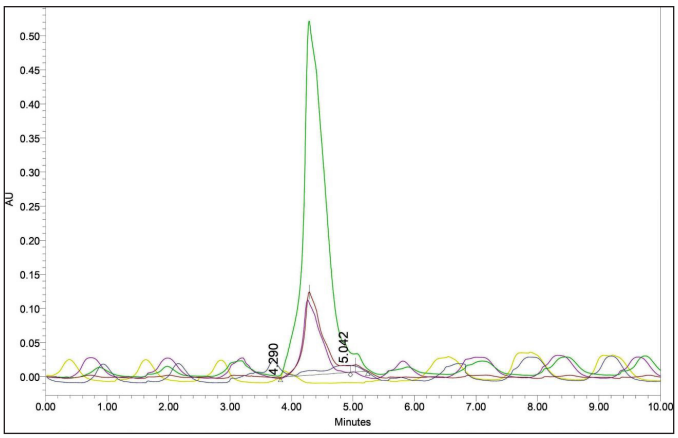

Figure 3. Overlay of FA standard peaks for different concentrations (10, 20 and 50 µg/g) with peaks of methanol and ethanol. Blue color peak indicates methanol and yellow color peak indicates ethanol.

(47.26 µg/g) of FA was produced by the strain BD023R of *F. fujikuroi*. The *Fusarium* strains in *F. fujikuroi* species produced lower concentrations of FA compared to other *Fusarium* species. All the strains of *F. fujikuroi* produced FA except BD087R and BD094R. Other than *F. fujikuroi* species, *F. proliferatum, F. verticillioides,* and *F. commune* produced FA while *F. sulawesiense* could not produce FA (Table 2).

Most of the tested *Fusarium* strains showed the capability to produce GA_3 and FA in different concentrations. The *Fusarium* strains of *F. fujikuroi*, in general, produced GA_3 at a high level while producing FA comparatively at a lower level. In contrast, *F. proliferatum* and *F. verticillioides* strains produced GA_3 at a low level but FA at a high

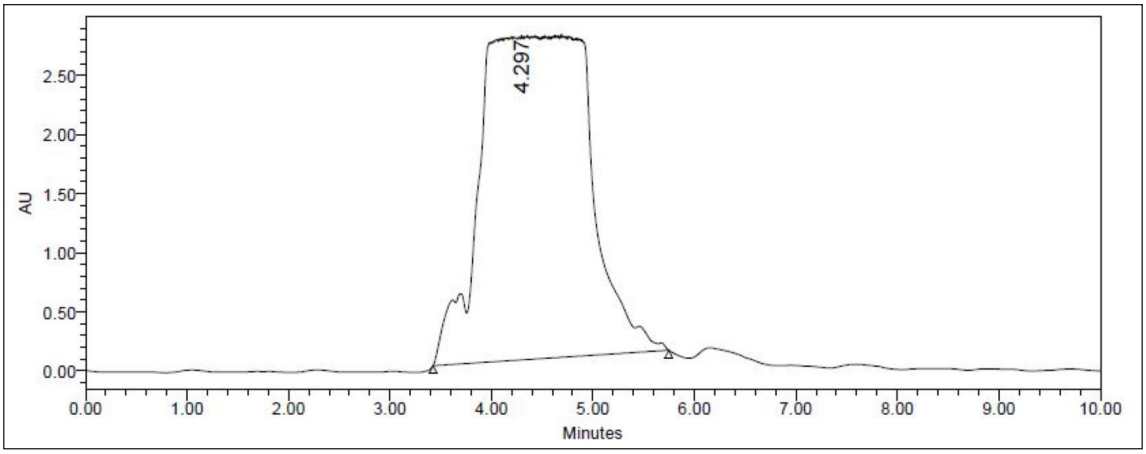

Figure 4. UPLC-photodiode array (PDA) chromatogram of FA produced by *F. fujikuroi* (BD043R) detected at 4.2 minutes.

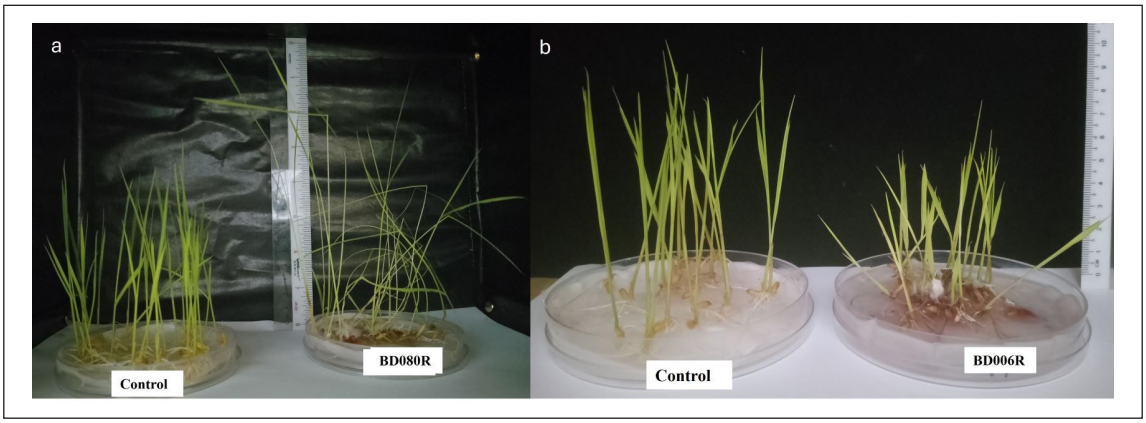

Figure 5. Rice seedling test for shoot length assessment; (a) The BD080R strain of *F. fujikuroi* inoculated seedlings showed elongation symptom; (b) The BD006R strain of *F. proliferatum* inoculated seedlings showed stunting symptom.

level. In *F. fujikuroi* species, the BD117R strain could not produce GA_3 while the BD087R and BD094R strains could not produce FA. The *F. commune* strain produced only FA, not GA_3 and *F. sulawesiense* could not produce GA_3 and FA.

3.4. Role of GA_3 and FA in Bakanae Symptoms Development

All 18 *Fusarium* strains were analyzed through rice seedling test by shoot length assessment to confirm the function of GA_3 and FA in bakanae symptoms development. The characteristic disease symptoms viz. elongation, stunted, rotted, wilted and the leaf tip turned yellow were observed in the seedlings inoculated with *Fusarium* strains. In the seedling test, the highest shoot length was recorded in the seedlings inoculated with *F. fujikuroi* BD080R strain which produced a higher amount GA_3 (319.98 µg/g) and a lower amount of FA (56.09 µg/g). In contrast, the lowest shoot length was found in the seedlings inoculated with *F. proliferatum* BD006R strain which produced a higher amount FA (247.33 µg/g) and a lower amount of GA_3 (14.43 µg/g). Thus, the produced elongation and stunting symptom were resembled with the typical bakanae symptom (Fig. 5). Again, most of the strains of *F. fujikuroi* species showed stem elongation symptoms while *F. proliferatum, F. verticillioides,* and *F. commune* strains showed stunting symptoms. Overall, the *F. fujikuroi* strains produced a high concentration of GA_3 but a relative low concentration of FA.

Table 3. Correlation co-efficient between GA_3 and FA produced by strains and shoot length of inoculated rice seedling by strains.

Correlation co-efficient	GA_3	FA	Shoot length
GA_3	1.000	−0.502	0.821
FA	−0.502	1.000	−0.635
Shoot length	0.821	−0.635	1.000

However, the *F. proliferatum* and *F. verticillioides* strains produced a low concentration of GA_3 but relatively high concentration of FA.

The strains BD023R, BD043R, BD047R, BD050R, BD056R, BD058R, BD066R, BD080R, BD087R, BD087R, BD094R, BD111R, BD113R, and BD118R (*F. fujikuroi*) inoculated rice seedlings showed a positive relationship between the shoot length and GA_3 production. The shoot length was high due to the effect of GA_3. The hypothesis that GA_3 contributes to elongated seedlings was validated by the positive correlation between GA_3 production and shoot length (0.821) (Table 3). The strain BD006R (*F. proliferatum*) and BD013R (*F. verticillioides*) inoculated rice seedlings produced reduced shoot length due to the effect of a low concentration of GA_3 but relative high concentration of FA. Again, the strain BD117R (*F. fujikuroi*) and BD19R (*F. commune*) could not produce GA_3 but produce only FA in high concentration, therefore these strains inoculated rice seedlings showed reduced shoot

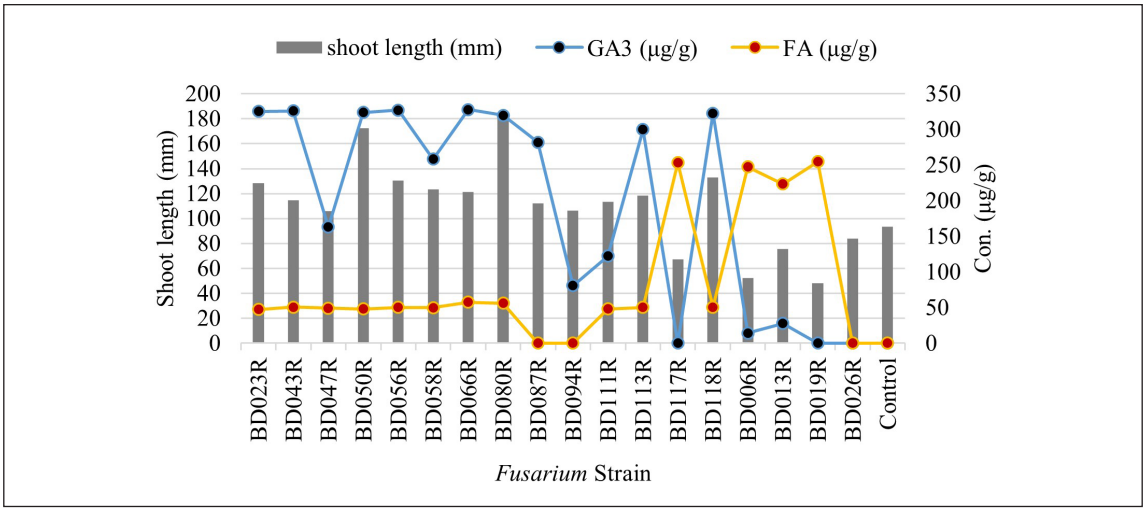

Figure 6. Effect of GA_3 and FA production on shoot length (mm) of rice seedling inoculated with *Fusarium* strains.

length i.e. stunting symptoms. The strain BD026R (*F. sulawesiense*) inoculated rice seedlings showed about normal shoot growth without the production of any GA_3 and FA (Fig. 6).

4. DISCUSSION

Fusarium species could produce variable amounts of GA_3 and FA in bakanae diseased rice plants [23]. In this study, 18 *Fusarium* strains belonging to 5 *Fusarium* species isolated from Bangladesh were examined to produce GA_3 and FA *in vitro* conditions. The GA_3 and FA produced by the *Fusarium* strains were detected and quantified as these were associated directly with bakanae symptom development.

In this study, the UPLC analysis was performed to detect and quantify GA_3 and FA. The obtained results from UPLC analysis depicted that all the strains of *F. fujikuroi*, *F. proliferatum,* and *F. verticillioides* were able to produce GA_3 in high concentration. Interestingly, the strains of *F. commune* and *F. sulawesiense* could not produce GA_3. Likewise in an earlier report, *Fusarium* species including *F. fujikuroi*, *F. proliferatum,* and *F. verticillioides* were found to produce GA_3 in bakanae diseased rice plants [3,4]. Thus, GA_3 can be used as a physiological marker to distinguish one *Fusarium* species from others.

In contrast, in this study, the *Fusarium* species like *F. proliferatum* and *F. verticillioides* produced FA in high concentration. In earlier, similar findings were observed by several authors [2,3, 24]. Interestingly, *F. commune* could not produce GA_3 but produced FA in high concentration. *F. commune* was reported to be associated with bakanae disease but there was no report earlier on this pathogen for GA_3 and FA production. In fact, GA_3 and FA production are governed by the expression of gene clusters responsible for producing GA_3 and FA [25]. The variable GA_3 and FA profiles were observed in *Fusarium* species due to the large number of genetic differences between and within the species [26]. In addition, intraspecific variability among the *Fusarium* strains effect on the variability in the production of GA_3 and FA [27]. According to Bhalla *et al.* [28], the GA_3 production by the different strains of *Fusarium* was varied due to different metabolic pathways of gibberellin production. Besides, the physiological variability of the *Fusarium* strains was linked with the pathogenic variability. We can get information about the potentiality of a strain to become pathogenic in disease development by understanding the pathogenic and physiological variability of that strain.

In the rice seedling test, we found that the shoots were elongated when inoculated with *F. fujikuroi* strains that produced GA_3 in higher concentrations. In contrast, the stunted shoots were found when inoculated with *F. proliferatum, F. verticillioides,* and *F. commune* that produced FA in higher concentration.

The GA_3 and FA are responsible for the pathogenicity and the generation of pathogenic diversity in the pathogen. The disease symptoms index mostly relies on the specific type and concentration of GA_3 and FA produced, as well as the interactions between the host and pathogen [29,30]. The synthesis of GA_3 and FA varies across the strains and is directly linked to their potential to induce bakanae disease [13,23,28]. The symptoms of bakanae disease are related to an imbalance of the phytohormone GA_3 [31]. According to Shakeel *et al.* [9] *F. fujikuroi* was well recognized for producing GA_3. Thus, previous several studies established that the role of GA_3 causing elongation symptoms and FA causing stunted seedlings inoculated by *Fusarium* strains isolated from bakanae disease [3,32]. Thus, our investigation confirmed earlier findings that GA_3 and FA are involved in bakanae disease symptom development.

In our study, GA_3 contributes to elongate symptom was validated by the positive correlation between GA_3 production and shoot length whereas FA production contributes to producing reduced shoot length. According to Puyam *et al.* [3] the production of GA_3 and FA were positively correlated with elongation and stunting type of symptoms, respectively. In addition, Quazi *et al.* [4] found a correlation between the quantity of FA and the development of bakanae symptoms Wu *et al.* [33] also found that FA concentration reduced plant height and root length. Additionally, it was reported that bakanae susceptible plants have a negative correlation between plant height and FA concentration [34]. *Fusarium* strains produced high quantities of FA that prevented GA_3 transportation from early leaves to internodes of bakanae-infected plants, and thus, plants became stunted [4].

5. CONCLUSION

The production of GA_3 and FA by *Fusarium* strains causing bakanae disease of rice in Bangladesh was reported in this study as the first attempt. The production of GA_3 and FA varied among the 18 *Fusarium* strains. This study also exposed the co-occurrence of GA_3 and FA among the tested strains of *F. fujikuroi, F. proliferatum,* and *F. verticillioides*. The development of bakanae disease symptoms

was significantly influenced by the concentration levels of GA3 and FA. Thus, the information on the GA_3 and FA-producing *Fusarium* strains for bakanae disease development will contribute in effective management practices for higher rice yields.

6. ACKNOWLEDGMENTS

This research work has been funded by USM Research Grant: 1001.PBIOLOGI.8011097. The Org. for Women in Science for the Developing World (OWSD) also funded.

7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

11. PUBLISHER'S NOTE

All claims expressed in this article are solely those of the authors and do not necessarily represent those of the publisher, the editors and the reviewers. This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

REFERENCES

1. Wiemann P, Sieber CM, von Bargen KW, Studt L, Niehaus EM, Espino JJ, *et al.* Deciphering the cryptic genome: genome-wide analyses of the rice pathogen *Fusarium fujikuroi* reveal complex regulation of secondary metabolism and novel metabolites. PLoS Pathog 2013;9(6):e1003475.
2. Zainudin NAIM, Razak AA, Baharuddin S. Secondary metabolite profiles and mating populations of *Fusarium* species in section Liseola associated with bakanae disease of rice. Malays J Microbiol 2008;4(1):6–13; http://doi.org/10.21161/mjm.01708
3. Puyam A, Pannu PPS, Kaur J, Sethi S. Variability in production of gibberellic acid and fusaric acid by *Fusarium moniliforme* and their relationship. J Plant Pathol 2017;99(1):103–8; http://doi.org/10.4454/jpp.v99i1.3811
4. Quazi SAJ, Sariah M, Ahmad ZABM, Hawa J. Detection of fungal metabolites from Bakanae diseased plants and their relationship with Bakanae disease symptoms expression. Am J Biosci Bioeng 2016;4(6):77–89; http://doi.org/10.11648/j.bio.20160406.14
5. Volante A, Tondelli A, Aragona M, Valente MT, Biselli C, Desiderio F, *et al.* Identification of bakanae disease resistance loci in japonica rice though genome wide association study. Rice 2017;10(29):1–16; http://doi.org/10.1186/s12284-017-0168-z
6. Bacon CW, Porter JK, Norred WP, Leslie JF. Production of fusaric acid by *Fusarium* species. Appl Environ Microbiol 1996;62(11):4039–43; http://doi.org/10.1128/aem.62.11.4039-4043.1996
7. Zainudin NAIM, Razak AA, Salleh B. Bakanae disease of rice in Malaysia and Indonesia: etiology of the causal agent based on morphological, physiological and pathogenicity characteristics. J Plant Prot Res 2008;48(4):475–85.
8. Machado CMM, Oishi BO, Pandey A, Soccol CR. Kinetics of *Gibberella fujikuroi* growth and gibberellic acid production by solid-state fermentation in a packed-bed column. Biotechnol Prog 2004;20:1449–53.
9. Shakeel Q, Mubeen M, Sohail MA, Ali S, Iftikhar Y, Bajwa RT, *et al.* An explanation of the mystifying bakanae disease narrative for tomorrow's rice. Front Microbiol 2023;14:1153437.
10. Nelson PE, Desjardins AE, Plattner RD. Fumonisins, mycotoxins produced by *Fusarium* species: biology, chemistry, and significance. Annu Rev Phytopathol 1993;31:233–52.
11. Singh VK, Singh HB, Upadhyay RS. Role of fusaric acid in the development of 'Fusarium wilt' symptoms in tomato: physiological, biochemical and proteomic perspectives. Plant Physiol Biochem 2017;118:320–32.
12. Amatulli MT, Spadaro D, Gullino ML, Garibaldi A. Molecular identification of *Fusarium* spp. associated with bakanae disease of rice in Italy and assessment of their pathogenicity. Plant Pathol 2010;59(5):839–44; http://doi.org/10.1111/j.1365-3059.2010.02319.x
13. Kaur J, Pannu PPS, Sucheta S. Morphological, biochemical and molecular characterization of *Gibberella fujikuroi* isolates causing bakanae disease of basmati rice. J Mycol Plant Pathol 2014;44(1):78–82.
14. Rojas C, Hedden P, Gaskin P, Tudzynski B. The P450–1 gene of *Gibberella fujikuroi* encodes a multifunctional enzyme in gibberellin biosynthesis. Proc Nat Acad Sci 2001;98(10):5838–44; http://doi.org/10.1073/pnas.091096298
15. Mohd Hawa M, Nor NMIM, Azuddin NF, Latiffah Z. Mycotoxin production by *Fusarium proliferatum* and *Fusarium fujikuroi* causing stem rot of *Hylocereus polyrhizus* in Malaysia. Malays Appl Biol 2023;52(3):13–22; http://doi.org/10.55230/mabjournal.v52i3.2644
16. Husna A, Miah MA, Nor NMIM. Rice bakanae disease: an emerging threat to rice production in Bangladesh. Asian J Med Biol Res 2020;6(4):608–10.
17. Husna A, Zakaria L, Mohamed Nor NM. *Fusarium commune* associated with wilt and root rot disease in rice. Plant Pathol 2021;70(1):123–32.
18. Ooi KHH. Pencirian dan pengawalan kimia *Fusarium oxysporum*, penyebab penyakit layu vascular pada rosel. Ph.D. thesis, Universiti Sains Malaysia, Malaysia, 2002.
19. Burmeister H, Grove MD, Peterson RE, Weisleder D, Plattner RD. Isolation and characterization of two new fusaric acid analogs from *Fusarium moniliforme* NRRL 13,163. Appl Environ Microbiol 1985;50(2):311–4; http://doi.org/10.1128/aem.50.2.311-314.1985
20. Husna A, Miah MA, Zakaria L, Nor NMIM. *Fusarium andiyazi*, a pathogenic species associated with rice Bakanae disease in Malaysia. Curr Microbiol 2024;81(10):308; http://doi.org/10.1007/s00284-024-03823-5
21. Barendse GWM, Van de Werken PH, Takahashi N. High-performance liquid chromatography of gibberellins. J Chromatogr A 1980;198(4):449–55.
22. Qiu J, Lu Y, He D, Lee YW, Ji F, Xu J, *et al. Fusarium fujikuroi* species complex associated with rice, maize, and soybean from

Jiangsu Province, China: phylogenetic, pathogenic, and toxigenic analysis. Plant Dis 2020;104:2193–201; http://doi.org/10.1094/PDIS-09-19-1909-RE

23. Lale G, Jogdand V, Gadre RV. Morphological mutants of *Gibberella fujikuroi* for enhanced production of gibberellic acid. J Appl Microbiol 2006;100(1):65–72.
24. Leslie JF, Plattner RD, Desjardins AE, Klittich CJ. Fumonisin B1 production by strains from different mating populations of *Gibberella fujikuroi* (*Fusarium* section Liseola). Phytopathology 1992;82(3):341–5.
25. Niehaus EM, Von Bargen KW, Espino JJ, Pfannmüller A, Humpf HU, Tudzynski B. Characterization of the fusaric acid gene cluster in *Fusarium fujikuroi*. Appl Microbiol Biotechnol 2014;98(4):1749–62; http://doi.org/10.1007/s00253-013-5453-1
26. Rabaaoui A, Asta CD, Righetti L, Susca A, Logrieco AF, Namsi A, *et al.* Phylogeny and mycotoxin profile of pathogenic *Fusarium* species isolated from sudden decline syndrome and leaf wilt symptoms on date palms (*Phoenix dactylifera*) in Tunisia. Toxins 2021;13(463):1–19.
27. Gálvez L, Urbaniak M, Waśkiewicz A, Stępień Ł, Palmero D. *Fusarium proliferatum*—causal agent of garlic bulb rot in Spain: genetic variability and mycotoxin production. Food Microbiol 2017;67:41–8; http://doi.org/10.1016/j.fm.2017.05.006
28. Bhalla K, Singh SB, Agarwal R. Quantitative determination of gibberellins by high performance liquid chomatography from various gibberellins producing *Fusarium* strains. Environ Monit Assess 2010;167(1–4):515–20; http://doi.org/10.1007/s10661-009-1068-5
29. Ou SH. Rice diseases. 2nd edition, Commonwealth Mycological Institute Kew, Surrey, UK, 1985.
30. Singh R, Sunder S. Foot rot and Bakanae of rice: an overview. Rev Plant Pathol 2012;5:565–604.
31. Quazi SAJ, Meon S, Jaafar H, Ahmad ZAB. The role of phytohormones in relation to bakanae disease development and symptoms expression. Physiol Mol Plant Pathol 2015;90:27–38.
32. Abo-Elnaga HIG, Ahmed NG. Pathogenicity, toxicity and gibberellic acid content of *Fusarium moniliforme* causing root rot and damping off of pepper. Plant Pathol 2007;6:318–23.
33. Wu H, Bao W, Liu D, Ling N, Ying R, Raza W, *et al.* Effect of fusaric acid on biomass and photosynthesis of watermelon seedlings leaves. Caryologia 2008;61(3):258–68; doi: http://doi.org/10.1080/00087114.2008.10589638
34. Lee YH, Crill JP, Lapis DB. Role of gibberellic acid and fusaric acid in rice plant inoculated with *Gibberella fujikuroi* (Sawada) Ito and Kimura. Plant Pathol J 1989;5(2):126–30.

How to cite this article:
Husna A, Miah MA, Zakaria L, Nor NMIM. *In vitro* production of gibberellic acid and fusaric acid by *Fusarium* spp. and their role in bakanae disease development. J Appl Biol Biotech. 2025; 13(1):235–242. DOI: 10.7324/JABB.2024.209502