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Biochemical and molecular variability of *Fusarium fujikuroi* isolates and their differential interactions with rice genotypes during infection

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Abstract

Bakanae of rice caused by *Fusarium fujikuroi* has become an important disease in major rice production regions worldwide. Twenty-nine isolates of *F. fujikuroi* were differentiated based on produced pigments, gibberellic acid (GA_3), molecular characteristics and correlated with bakanae disease symptoms of rice seedling. All isolates of *F. fujikuroi* produced pigments except six isolates. Also, all isolates differed in the production of gibberellic acid. The isolate FI 12 was the highest produced for GA3 and disease severity index. Molecular analysis showed that nine ISJ primers produced a total of 809 amplified bands. Current results indicated that the *Fusarium* isolates were clustered largely based on location and/or severity of isolates and level of GA3 production. The molecular results obtained proved the existence of high levels of genetic variation and high-resolution power of ISJ marker in detecting *Fusarium* molecular diversity. The differential behavior of bakanae pathogen in resistant and susceptible rice genotypes during infection was studied. Under greenhouse condition, Giza 177 was highly susceptible to rice genotypes while Giza 179 and Giza 178 were moderately resistant. Biochemical changes such as antioxidant enzymes and hydrogen peroxide (H_2O_2) was observed in rice seedling after 7, 15 and 21 days from inoculation. Enzymes activities were increased in Giza 179 and Giza 178 genotypes, but in H_2O_2 content was decreasing. Under field conditions, all genotypes showed increased chlorophyll content, number of tillers/hills and means of panicle length/10 healthy plants compared with infected plants. Giza 179 showed the highest grain yield.

Keywords: Bakanae, Rice, *Fusarium fujikuroi*, Gibberellic acid, cultivars and hybrid

INTRODUCTION

The causal pathogen of rice bakanae disease was identified as *F. moniliforme* Sheldon (**Nirenberg, 1976**) and *F. fujikuroi* as newly re-identified by Nirenberg (**Nirenberg, 1976**), the anamorph of the pathogen *Gibberella fujikuroi* Sawada. Bakanae disease is considered one of the most fungal diseases in India like a blast, brown spot, sheath blight and sheath rot of rice (**Sharma and Thind, 2007**). In recent years in India, bakanae was emerged as a serious threat to rice cultivation, especially on basmati rice and caused severe yield loss of more than 95% (**Gupta *et al.*, 2015**). In Egypt, *Fusarium fujikuroi* caused severe bakanae characteristic

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symptoms, such as diverse morphological changes including abnormal stem elongation, development of adventitious roots and chlorotic leaves due to excessive gibberellin production particularly on the fields of highly susceptible cultivars Sakha 101 and Giza 177 and only isolates of *F. fujikuroi* were able to cause bakanae infection (**Abeer and Gabr**, 2015). This bakanae pathogen can produce gibberellic acid (GA) and fusaric acid (FA). GA may isolate from elongation-promoting diterpenoids from the fungus *F. fujikuroi*. Both GA_3 and FA of all *Fusarium* spp. have an important role in producing elongation and stunting symptoms, respectively, and promoting pathogenicity. GAs production is mainly described so far in *F. fujikuroi* causal organisms of bakanae that produce the highest levels of gibberellic acid (GA3) (**Malonek, *et al*., 2005**). Because the causal pathogens had high genetic variation it is difficult to develop resistanc in bakanae rice varieties (**Serafica and Cruz, 2009**). The development of molecular marker technologies provides a precise, accurate and effective procedure for the assessment of genetic diversity. An interesting marker type is Intron Splicing Junction (ISJ) DNA markers. These markers are found in plants as semi-specific, based on sequences and crucial for post-transcription DNA processing (**Weining and Langridge, 1991**). On the exon-intron boundary, ISJ primers are partly complementary to the sequences. The amplified bands were scored either present or absent and treated as single dominant markers. In cereal species, ISJ markers were used to assess genetic variation (**Weining and Henry, 1995**). Both intra- and interspecific were used by semi-specific intron-exon splice junction markers (**Weining and Langridge, 1991).** The fungus *Uncinula necator* used ISJ markers to investigate genetic diversity **(Stummer, *et al.*, 2000**). To the best of our knowledge, no reports so far on using ISJ markers to assess the genetic diversity of *Fusarium* spp. Fungicides were an important way to control the disease, but they had a hazard effects on health and environment, especially microorganisms. There is another way to control this disease by using resistant varieties. But in Pakistan and India, the major varieties are susceptible to bakanae disease, so this approach is also not useful. Some candidate QTLs (qBK1, qB1 and qB2) were successfully identified which can be useful in resistance breeding programs (**Ji, *et al*., 2017**); and provided some horizontal resistance may be more practical than vertical resistance ***(*Naeem, *et al*., 2016)***.

This study aimed to determine the biochemical and pathogenic variability among the isolates based on the production of GA, pigments and molecular genetic diversity assess as well as to ascertain the correlation with bakanae symptoms in pathogenicity test. Also, evaluation of rice genotypes for bakanae disease and assessment of disease yield losses.

Materials and Methods

Laboratory, greenhouse and field studies

Experiments were conducted at the Rice Pathology Department, a laboratory and greenhouse at the Rice Research & Training Center (RRTC), while field experiments were performed at Sakha Agricultural Research Station, Egypt.

Collection of diseased samples

Infected rice samples with characteristic bakanae symptoms were collected from different cultivars and rice governorates in Egypt during 2015 and 2016 growing seasons described in Table (1).

Table 1: Sources of different *F. fujikuroi* isolates during two seasons

Isolate no.	Governorate	District	Rice genotypes	Year
FI1	Kafr El-Sheikh	Sakha	Sakha 101	2015
FI 2	Kafr El-Sheikh	Desouq	Giza 177	2015
FI 3	Kafr El-Sheikh	Sakha	Giza 178	2015
FI 4	Kafr El-Sheikh	Foaa	Sakha 104	2016
FI 5	Kafr El-Sheikh	Kafr El-Sheikh	Giza 177	2016
FI 6	Kafr El-Sheikh	Desouq	Sakha 101	2016
FI 7	Dakahlia	Met sweed	Giza 178	2015
FI 8	Dakahlia	Dekerns	Giza 178	2015
FI 9	Dakahlia	Talkha	Giza 178	2015
FI 10	Dakahlia	Dekerns	Sakha 101	2015
FI 11	Dakahlia	Mansoura	Sakha 104	2015
FI 12	Dakahlia	Mansoura	Sakha 101	2016
FI 13	Dakahlia	Met sweed	Sakha 101	2016
FI 14	Dakahlia	Met sweed	Sakha 104	2016
FI 15	Dakahlia	Sharbeen	Giza 178	2016
FI 16	Gharbia	Gemmiza	Giza 178	2015
FI 17	Gharbia	Basuooen	Sakha 101	2015
FI 18	Gharbia	Gemmiza	Sakha 101	2016
FI 19	Gharbia	Elmahla	Sakha 101	2016
FI 20	Beheira	Itai-El-Barood	Sakha 101	2015
FI 21	Beheira	Itai-El-Barood	Giza 177	2015
FI 22	Beheira	Mahmoudia	Giza 178	2016
FI 23	Beheira	Itai-El-Barood	Sakha 104	2016
FI 24	Damietta	Kafr Saad	Giza 177	2015
FI 25	Damietta	Kafr Saad	Giza 178	2015
FI 26	Damietta	Kafr Saad	Hybrid 1	2016
FI 27	Damietta	Kafr Saad	Giza 177	2016
FI 28	Damietta	Zarka	Sakha 101	2016
FI 29	Damietta	Zarka	Giza 177	2016

Isolation, purification, identification and pathogen culture preparation

Bakanae rice fungus was isolated from infected plants during 2015 and 2016 seasons from different rice growing governorates. The purification was carried out based on hyphal tip techniques according to **Hansen (1926)** and the pathogen (*F. fujikuroi)* was identified according to the morphological characteristics and microscopic examination at plant pathology laboratory at RRTC using the key of imperfect fungi **Summerell *et al*. (2003).** The pathogen was cultured in the PDA medium at 26±2^{o}C until mycelium covered the whole plate surface. Plates were exposed to continuous fluorescent light for two days to enhance sporulation. Inoculum of spore suspension was prepared by adding 10 ml sterilized water to each dish. Mycelia mats were gently scraped by spatula and filtered through cheesecloth. The spore suspension was adjusted to (4 x10^{5}/ml).

Quantitative analysis of gibberellic acid production

The isolates of bakanae pathogen were grown in sterilized Czapek-Dox medium (100 ml for 12 days at 26 ± 2°C) in three replicates. Individual isolates were placed on each flask with a 5 mm mycelial disc. Mycelial mat was separated after 12 days using Whatman filter paper no.1 and the filtrate was adjusted to pH 2.5 using 1 N HCl (**Muddapur *et al.*, 2015**). The filtrate with an equal volume of ethyl acetate was extracted. By spectrophotometric method, quantitative estimation of GA_3 was done (**Zainudin *et al.*, 2008b**). In a test tube 25 ml of filtrate, 2 ml of zinc acetate after 2 min 2 ml potassium ferrocyanide was added and centrifuged at 1000 rpm for 15 min. 30% of HCl was added for Equal volumes of supernatant and incubated at 2^0C for 75 min. 5% of HCl was treated for the blank sample and the absorbance of the test sample with bakanae pathogen and blank was measured at 254 nm in a spectrophotometer (Milton Roy, Spectronic, 1201 Digital). In the extract, the amount of GA present was calculated from the standard curve. The standered curve was prepared using a graded concentration of GA (1 mg in 10 ml- stock solution) and showed as µg/ml of the media.

Detection of molecular variability of *F. fujikuroi* isolates

Mycelia of all studied isolates of *F. fujikuroi* were inoculated into PD liquid medium and kept on an incubator-shaker at 150 rpm and 26 ± 2°C. The mycelia were collected by filtration, dried well and ground to a fine powder in the presence of liquid nitrogen. Fungal DNA was isolated, according **to Murray and Thompson (1980**), suspended in T.E. buffer and stored at -20°C untill use. The isolated DNA was quantified using electrophoresis on 0.8 % agarose gel. After several periods of dilution, the DNA concentration was adjusted to 30 ng / µl. The reaction was performed in 10 µl volume consisting of 30 ng of genomic DNA, 1 µM of each primer, and 5 µl of 2X *GoTaq* Green Master Mix (Promega, USA.). PCR amplification of ISJ primers was performed as follows: initial denaturation at 94°C for 5 min. followed by 45 cycles at 94°C for 1 min., 50°C for 1 min., and 72°C for 2 min., with a final extension step at 72°C for 7 min. PCR products were resolved using 1.5 Agarose gel electrophoresis stained with Ethidium Bromide and visualized on Biometra Gel Documentation system. Using the Jaccard similarity coefficient, data generated from the ISJ were analyzed (**Jaccard, 1908**). The similarity coefficients were used to construct a dendrogram using the Unweighted Pair Group Method with Arithmetic Average (UPGMA) employing NTSYS Pc2.11 program (**Ralf, 2005**). The polymorphic information content (PIC) for each primer was calculated to estimate its allelic variation as follows: PIC = 1- $\sum_{j=1}^{n} Pij^2$, where Pij is the frequency of the i^{th} allele for marker j with the summation extending over n alleles, calculated for each ISJ marker (**Anderson et al., 1993**).

Greenhouse experiment

Pathogenicity test

All the twenty-nine isolates were growing in PDA medium and incubated in an incubated at 26 ± 2°C for one week for inoculation. The harvest of *F. fujikuroi* conidia was suspended in sterile distilled water. Sakha 101 as highly susceptible rice cultivar to bakanae was evaluated

under artificial inoculation to assess the pathogenicity of all bakanae isolates. Seeds were sterilized by sodium hypochlorite solution 2% for 5 min and then washed with sterile water twice, then, was soaked in spore suspension (4 x 10^5 spores/ml) for 48 h. For check seeds were soaked in sterilized water. Control and inoculated seeds were sown in sterilized soils (autoclaved 2 times for 24 h intermittently at 121^{o}C, 15 psi,) in plastic pots (15x15 cm). One hundred seeds of each replicate were planted in each pot and arranged in a randomized complete design (RCD) with four replicates under greenhouse conditions. Every two weeks seedlings were fertilized with urea 46.5% N (3 g/ pot) and irrigated daily with tap water. The disease scale of seedlings was observed and scored from 0 to 4 (Table 2) according to **Zainudin *et al.* (2008a)** and the morphological disease symptoms were continuously observed. The germination percentage was recorded after 7 days of sowing and disease severity index (DSI) of rice seedling was calculated after inoculation within 40 days for each isolate following **Ooi (2002)**.

Evaluation of rice genotypes resistance to bakanae infection under greenhouse

Fourteen rice genotypes namely, Giza 177, Giza 178, Giza 179, Giza 181, Egyptian yasmine, Sakha 101, Sakha102, Sakha 103, Sakha 104, Sakha 105, Sakha 106, Sakha 107, Sakha 108 and hybrid 1 were inoculated with the most aggressive isolate of bakanae causal fungus (FI 12). Seeds of each genotype were sterilized by sodium hypochlorite solution 2% for 5 min and then washed with sterile water three times and soaked in spore suspension (4 x 10^5 spores/ml) for 48 h. To check genotypes, seeds were soaked in sterilized water for 48h. Inoculated and healthy seeds were sown in sterilized soil in plastic pots (15x15 cm). One hundred seeds were planted in each pot with four replicates and arranged in a randomized complete design (RCD) under greenhouse. The pots were fertilized two times with urea 46.5% N (3 g/ pot). The germination percent was recorded after 7 days of sowing and disease severity index (DSI) of rice seedlings was calculated after inoculation within 40 days for each cultivar following **Ooi (2002).**

Biochemical studies

Enzyme activities: Rice samples of each genotype were collected 7, 15 and 21 days after artificial inoculation with a spore suspension of *F. fujikuroi* (4 x 10^5 conidia/ml), then quickly frozen in liquid nitrogen and stored at -20°C as powder samples. Powder samples (0.5 g) for each genotype were homogenized in 3 ml of 0.1 M sodium phosphate buffer (pH 6.8). The homogenate was centrifuged at 10,000 rpm for 15 min and at 5°C. Supernatant served as an enzyme source for POX and PPO.

POX enzyme activity was determined according to the methods described by **Srivastava (1987)**. Peroxidase activity was expressed as changes in absorbance (mg/ min). The absorbance was measured at 425 nm and recorded at 0, 1, 2, 3, 4- and 5-min. intervals using a spectrophotometer (Milton Roy, Spectronic, 1201 Digital). Three replicates were maintained for each genotype.

PPO activity: The enzyme was determined according to the method adopted by **Matta and Dimond (1963)**. Polyphenoloxidase activity was expressed as changes in the absorbance and was measured at 495 nm, and recorded at 0, 1, 2, 3, 4- and 5-min intervals using a spectrophotometer (Milton Roy, Spectronic, 1201 Digital).

H_2O_2 content: Hydrogen peroxide content in the inoculated and healthy rice seedlings was determined according to **Velikova *et al.* (2000)**. In an ice bath, rice samples (0.5g) were homogenized and separated with 5 ml of 0.1% (w/v) trichloroacetic acid. The homogenate sample was centrifuged at 10,000 rpm for 15 min, and 0.5 ml of the supernatant was added to 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1 M potassium iodide. The absorbance of the supernatant was measured at 390 nm.

Field condition

Evaluation of certain rice genotypes resistance to bakanae infection under field conditions

The experiment was carried out under field conditions during 2017 and 2018 seasons under artificial inoculation to evaluate bakanae resistance of fourteen rice genotypes. The seeds of each genotype were soaked in spore suspension at ($4x10^5$ spore/ml) of the most aggressive isolate (FI 12) for 48 h and incubated at 30±2°C for 48 h then, sown in sterilized soil for 30 days as an infected seedling in pots (60 x 30 x 15 x cm). The healthy seeds were soaked in sterilized water for 48 h and incubated at 30±2°C for 48 h then sown in sterilized soil for 30 days as healthy seedlings in the same size pots in a greenhouse. After 30 days of sowing, all inoculated and healthy genotype seedlings were transplanted into field plots (3.5 x 3.5 m/ plot) as single plants. A completely randomized block design with four replicates was used. All other agronomic practices were followed as recommended during the growing seasons. Disease incidence (%) and DSI, chlorophyll content, number of tillers/hill, and stem length were assessed and recorded after maximum tillers. Meanwhile, the panicle length is recorded at the heading stage and grain yield at the maturing stage for each genotype.

Chlorophyll content: Total chlorophyll content of leaves was determined in mg using a chlorophyll meter (SPAD-502) **(Kalboush, 2007**).

Disease assessment: - Disease incidence was assessed according to **Teng and James (2001)** with slight modifications:

$$\text{Disease incidence \%} = \frac{\text{total number of infected plants}}{\text{total number of tested plants}} \text{ X } 100$$

DSI was calculated for each genotype following **Ooi (2002)**. DSI was calculated using the following formula:

$$\text{DSI} = \frac{\sum (\text{number of plants in the specific scale} \times \text{disease scale})}{\text{Total number of tested plants}}$$

Table 2. Symptoms of rice plants were scored based on disease scale 0–4

Disease scale	Disease symptoms
0	healthy and uninfected plants (no external symptoms)
1	normal growth but leaves are beginning to show yellowish–green
2	abnormal growth, elongated, thin and yellowish-green leaves; seedlings also shorter or taller than normal
3	abnormal growth, elongated; chlorotic, thin and brownish leaves; seedlings also shorter or taller than normal
4	seedlings with a fungal mass on the surface of infected plants or died

Data Analysis: Data were statistically analyzed using standard statistical analysis with MSTATC. in the table of the main treatments, **Duncan′s T. (1955)** was used to compare the significantly different averages.

Results and Discussion

Isolation and identification of the causal organism and Pathogenicity tests

The characteristics for 29 isolates obtained from different rice growing areas were identified as *F. fujikuroi* isolate (FI) and designated as FI1, FI 2-----to FI 29. These isolates were characterized based on produced GA_3, pigments and symptoms on rice seedling (Fig. 1). Pathogenicity test of twenty-nine bakanae isolates collected from different rice cultivars and locations were carried out using Sakha 101 cv as the most susceptible one. All isolates produce specific pigments except for six isolates (no. 3, 5, 7, 13, 18 and 23). Meanwhile all isolates produced GA3 and the isolates FI 12 recorded the highest production of GA_3 (28.6 µg/ml) followed by FI 22 (25.0 µg/ml). The tested isolates varied in their virulence. Isolate number FI 12 from Dakahlia governorate, proved to be the most aggressive isolate with the highest disease severity index (3.76). Also, there is no significant difference between (FI 6 and 22) in the disease severity index (3.48 and 3.36, respectively). The disease incidence % and seedling death % were given the same trend of the disease severity index. All the data are shown in Table (3). However, FI 18 isolated from Gharbia governorates as were the least virulent one which gave the least disease severity index and GA3 among all tested isolates as indicated in Table (3).

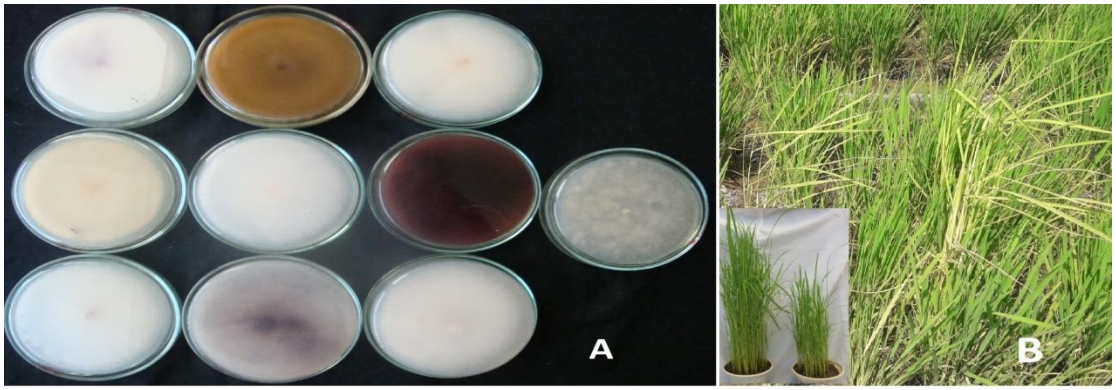

Fig. 1: Different isolates of F. *fujikuroi* with pigment colour (A) and bakanae disease symptoms in rice seedling and adult plants (B)

Different bakanae isolates were characterized as *F. fujikuroi* reproduced a wide variability in their appearance and different levels of virulence (**Venturini, *et al*., 2013**). All the isolates varied in their production of GA. Also, the observations that *Fusarium* spp. was caused by bakanae in rice could produce GA but not all the isolates had produced FA (**Kaur, *et al*., 2014**). ***Puyam et al***. (**2017**) reported that the pathogenicity test for isolates of *F. fujikuroi* correlated with variation in their production of GA and numbers of elongated rice plants. Our current results demonstrate the tremendous potential for high levels of gibberellic acid production, that highly associated with the highest percentage of infection and seedling death. These results are in harmony with **Wiemann, *et al*., 2013** when investigated GAs production is a key virulence factor in *F. fujikuroi*, host-cell colonization and invasive growth of rice root infection.

Table 3. Comparative production of gibberellic acid and pigment by *Fusarium fujikuroi* isolates and pathogenicity test on Sakha 101 rice seedlings

No.	Governorates	Pigments	GA_3 (µg/ml)	Germination %	Seedling death %	Disease incidence (%)	Disease Severity Index
FI1	Kafr El-Sheikh	+	15.0^{h}	68.00^{c-i}	10.34^{e-i}	8.15^{hi}	0.900^{hi}
FI 2	Kafr El-Sheikh	+	13.8^{hi}	82.66^{abc}	14.00^{b-f}	13.34^{hi}	0.800^{hi}
FI 3	Kafr El-Sheikh	-	14.8^{h}	71.00^{b-i}	13.00^{b-g}	20.00^{gh}	1.200^{gh}
FI 4	Kafr El-Sheikh	+	10.5^{j}	80.00^{a-e}	5.00^{ij}	5.34^{ij}	0.320^{ij}
FI 5	Kafr El-Sheikh	-	17.6^{fg}	81.67^{a-d}	0.00^{j}	13.00^{hi}	0.780^{hi}
FI 6	Kafr El-Sheikh	+	23.0^{c}	77.00^{b-f}	20.34^{ab}	58.00^{ab}	3.480^{ab}
FI 7	Dakahlia	-	3.24^{l}	85.34^{ab}	0.00^{j}	13.34^{hi}	0.800^{hi}
FI 8	Dakahlia	+	20.2^{de}	73.67^{b-h}	12.00^{c-h}	53.34^{abc}	3.20^{abc}
FI 9	Dakahlia	+	18.5^{fg}	61.34^{f-j}	6.00^{hij}	40.34^{c-f}	2.420^{c-f}
FI 10	Dakahlia	+	14.6^{h}	65.4^{d-j}	18.0^{bcd}	46.67^{bcd}	2.80^{bcd}
FI 11	Dakahlia	+	21.2^{d}	59.4^{g-j}	15.34^{b-e}	27.67^{fg}	1.660^{fg}
FI 12	Dakahlia	+	28.6^{a}	49.34^{j}	26.00^{a}	62.67^{a}	3.760^{a}
FI 13	Dakahlia	-	23.0^{c}	87.34^{ab}	0.00^{j}	14.00^{hi}	0.840^{hi}
FI 14	Dakahlia	+	9.36^{j}	55.67^{ij}	16.0^{b-e}	38.34^{def}	2.300^{def}
FI 15	Dakahlia	+	18.6^{efg}	65.00^{e-j}	18.34^{bc}	45.67^{bcd}	2.740^{bcd}
FI 16	Gharbia	+	6.34^{k}	55.4^{ij}	19.67^{b}	35.67^{def}	2.140^{def}
FI 17	Gharbia	+	14.4^{h}	73.67^{b-h}	19.0^{bc}	30.34^{ef}	1.820^{efg}
FI 18	Gharbia	-	1.70^{m}	86.34^{ab}	0.00^{j}	0.00^{j}	0.00^{j}
FI 19	Gharbia	+	19.2^{ef}	61.34^{f-j}	19.0^{bc}	55.67^{ab}	3.340^{ab}
FI 20	Beheira	+	17.5^{g}	71.00^{b-i}	12.00^{c-h}	41.67^{cde}	2.50^{cde}
FI 21	Beheira	+	12.6^{i}	82.34^{abc}	8.00^{f-i}	34.00^{def}	2.04^{def}
FI 22	Beheira	+	25.0b	51.00j	16.0b-e	56.00ab	3.360ab
FI 23	Beheira	-	19.2^{ef}	61.67^{f-j}	15.0^{b-f}	40.34^{c-f}	2.420^{c-f}
FI 24	Damietta	+	17.5^{g}	64.00^{e-j}	12.67^{b-h}	46.34^{bcd}	2.76^{bcd}
FI 25	Damietta	+	4.00^{l}	75.67^{b-g}	14.34^{b-f}	36.67^{def}	2.20^{def}
FI 26	Damietta	+	14.7^{h}	62.34^{f-j}	14.67^{b-f}	44.34^{bcd}	2.66^{bcd}
FI 27	Damietta	+	12.67^{i}	58.00^{hij}	11.00^{d-i}	47.00^{bcd}	2.82^{bcd}
FI 28	Damietta	+	10.8^{j}	55.34^{ij}	5.00^{ij}	46.00^{bcd}	2.76^{bcd}
FI 29	Damietta	+	10.0^{j}	51.34^{j}	6.67^{g-j}	14.00^{hi}	0.88^{hi}
Healthy seedling (Control)		-	0.0^{n}	94.67^{a}	0.00^{j}	0.00^{j}	0.00^{j}

In column means followed by the same letters are not statistically different at ($p \leq 0.05$) level according to Duncan's multiple range test.

Molecular Analysis

The molecular analysis using nine Intron Splice Junction (ISJ) primers showed the existence of a considerable amount of molecular diversity among the 29 tested *Fusarium* isolates (FI) and banding patterns of ISJ 5 shown in Fig. 2. The figure also shows high levels of detected polymorphism among *Fusarium* isolates. Summary of molecular results is shown in Table (4). The nine primers produced a total of 809 amplified bands with an average of 89.89 amplified bands/ primer. The highest amplified bands were recorded for ISJ 6 with 145 amplified bands across the tested *Fusarium* isolates, while the lowest number of the amplified product was obtained with ISJ 12 with only 20 amplified bands. A total of 82 genetic loci (alleles) were detected across fungus genome. The number of alleles (genetic loci ranged from 4 for ISJ 12 to 14 in ISJ 5, with an average of 9.11 alleles. The results also showed that all markers and detected alleles were polymorphic (Table 4). The polymorphism information content (PIC) had an average value of 0.83 for the tested markers. The PIC values ranged from 0.54 for the primer ISJ 12 to 0.91 for ISJ 5 and ISJ 7 (Table 4)

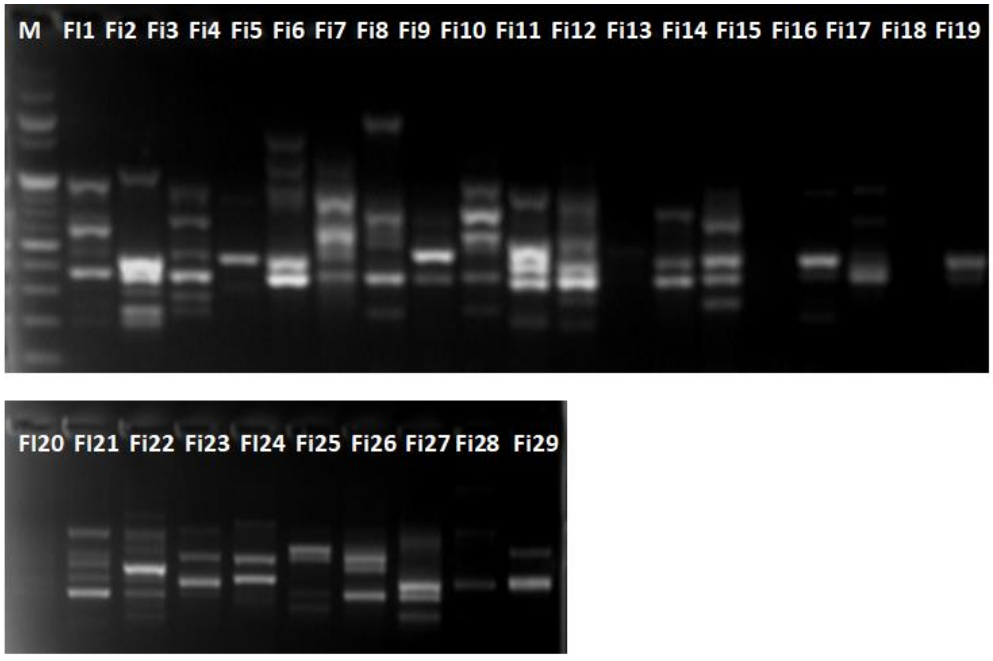

Fig. 2: The banding patterns of ISJ 5 among the 29 *Fusarium* isolates, M: 100 bp ladder

Table 4: Summary of molecular analysis results of ISJ markers among 29 *Fusarium* isolates

ISJ marker	# of amplified bands	# of amplified alleles	# of polymorphic alleles	Polymorphism %	PIC value
ISJ 1	60	7	7	100	0.83
ISJ 2	68	8	8	100	0.85
ISJ 5	115	14	14	100	0.91
ISJ 6	148	8	8	100	0.86
ISJ 7	124	12	12	100	0.91
ISJ 9	98	8	8	100	0.84
ISJ 10	89	13	13	100	0.89
ISJ 11	87	8	8	100	0.83
ISJ 12	20	4	4	100	0.535
Total	**809**	**82**	**82**		
Average	**89.89**	**9.11**	**9.11**	**100**	**0.83**

Table (5) represents the similarity matrix among the fungus isolate pairs according to **Jaccard similarity Coefficient (Jacard, 1908).** The isolate FI 9 and FI 11 were found to be the closest pair of isolates based on their similarity (0.59), while the pairs on the other hand; FI 20 with each of FI 1, FI 2 and FI 18 had the lowest similarity coefficient (0.01). The phylogenic tree was generated employing Jaccard similarity coefficient using anunweighted pair group method with an arithmetic average [UPGMA] and SAHN [sequential, agglomerative, hierarchical, and nested clustering] from the NTSYSpc (ver.2.10) program (**Rohlf, 2005).** The generated clustering of the isolates is presented in Fig (3). The isolate FI 20 was the most diverse isolate and formed a single cluster A at 0.06 similarity level and all other isolates were in a major cluster B. at 0.11 similarity level, the main cluster B was subdivided into two clusters B1 and B2. B1 only had FI 18 and B2 had the remaining 27 isolates. The isolates were largely clustered based on location, level of GA3 production and /or virulence. For instance, isolates collected from Damietta governorates were clustered together in a single cluster. Most isolates from different governorates tended to cluster nearby. However, the isolate FI 18 that was distinct had a zero-severity index and the lowest level of GA3 production. These results indicate that the clustering was largely based on location, level of GA3 production and/or severity of isolates. At 62% similarity level, ISJ markers were able to distinguish all isolates. The molecular results obtained here proved the existence of high levels of genetic variation. This explains the high level of the mutation rate of *Fusarium* fungus. This explains the ability of the fungus to produce new isolates/races and a wide range of hosts

(**Niehaus, *et al*., 2016**). The results also demonstrate the high-resolution power of ISJ primers in detecting molecular diversity. Also, the high PIC values confirmed this fact. Similar results on the high levels of diversity of *Fusarium* species were also reported by **Amatulli, *et al*. (2010)**.

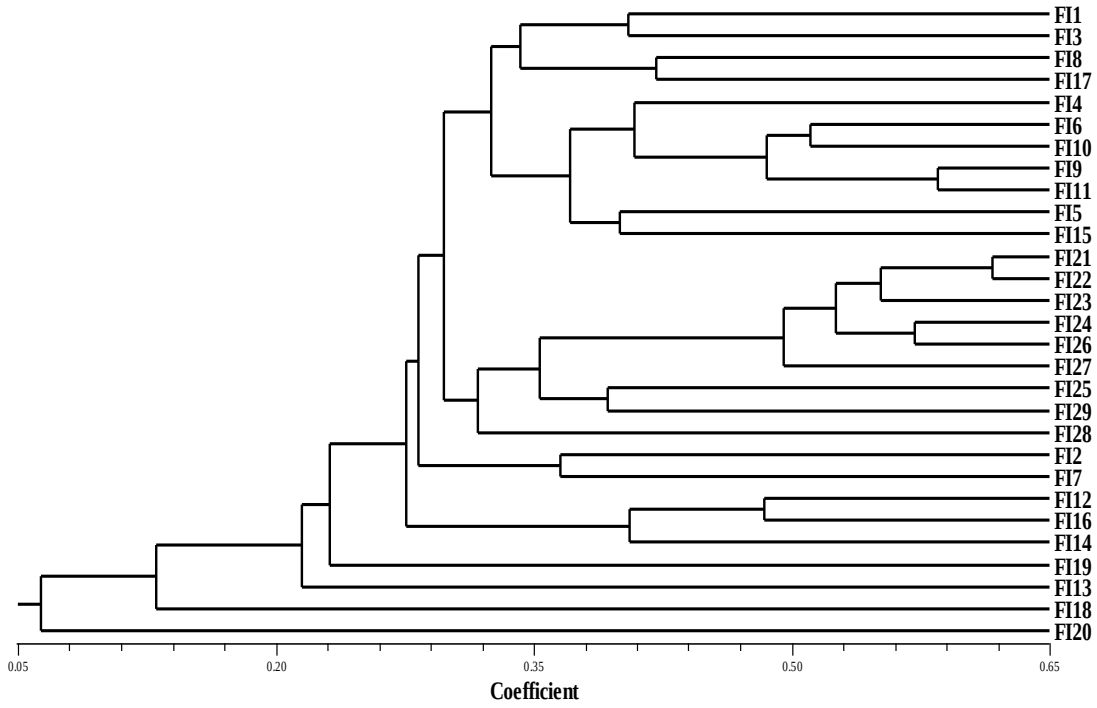

Fig. 3 : Dendrogram explaining the genetic relationships among the tested isolates using ISJ primers using UPGMA method

Table 5: Similarity coefficient among *Fusarium* isolate pairs according to Jaccard similarity coefficient

6	FI 1	FI 2	FI 3	FI 4	FI 5	FI 6	FI 7	FI 8	FI 9	FI	FI	FI	FI	FI	FI	FI	FI	FI	FI	FI	FI	FI	FI	FI	FI	FI	FI
FI 1	1.00																										
FI 2	0.31	1.00																									
FI 3	0.41	0.31	1.00																								
FI 4	0.23	0.27	0.29	1.00																							
FI 5	0.35	0.40	0.38	0.37	1.00																						
FI 6	0.31	0.39	0.34	0.36	0.40	1.00																					
FI 7	0.26	0.37	0.26	0.17	0.28	0.35	1.00																				
FI 8	0.31	0.31	0.38	0.23	0.36	0.35	0.26	1.00																			
FI 9	0.27	0.40	0.42	0.41	0.37	0.50	0.31	0.33	1.00																		
FI 10	0.33	0.33	0.28	0.43	0.37	0.51	0.29	0.26	0.50	1.00																	
FI 11	0.30	0.33	0.44	0.43	0.32	0.49	0.21	0.28	0.59	0.45	1.00																
FI 12	0.31	0.20	0.18	0.22	0.27	0.29	0.20	0.28	0.27	0.31	0.19	1.00															
FI 13	0.24	0.23	0.27	0.18	0.27	0.20	0.23	0.21	0.24	0.22	0.21	0.23	1.00														
FI 14	0.30	0.28	0.37	0.15	0.16	0.26	0.25	0.27	0.33	0.30	0.27	0.39	0.27	1.00													
FI 15	0.28	0.26	0.33	0.36	0.40	0.42	0.26	0.31	0.41	0.33	0.36	0.28	0.14	0.12	1.00												
FI 16	0.28	0.21	0.34	0.30	0.33	0.38	0.16	0.32	0.40	0.37	0.31	0.48	0.24	0.42	0.37	1.00											
FI 17	0.34	0.26	0.34	0.33	0.35	0.38	0.27	0.42	0.33	0.37	0.34	0.35	0.25	0.28	0.34	0.42	1.00										
FI 18	0.09	0.04	0.06	0.13	0.12	0.14	0.07	0.06	0.12	0.13	0.11	0.14	0.13	0.03	0.18	0.24	0.14	1.00									
FI 19	0.19	0.22	0.16	0.29	0.18	0.15	0.14	0.20	0.18	0.21	0.15	0.27	0.18	0.26	0.21	0.27	0.31	0.04	1.00								
FI 20	0.01	0.01	0.07	0.13	0.03	0.03	0.06	0.04	0.08	0.12	0.06	0.09	0.05	0.08	0.06	0.07	0.06	0.01	0.05	1.00							
FI 21	0.30	0.33	0.35	0.32	0.34	0.43	0.38	0.39	0.37	0.38	0.38	0.30	0.20	0.27	0.40	0.33	0.42	0.16	0.30	0.08	1.00						
FI 22	0.34	0.29	0.31	0.33	0.40	0.37	0.25	0.22	0.33	0.42	0.37	0.26	0.20	0.23	0.34	0.32	0.40	0.20	0.32	0.08	0.62	1.00					
FI 23	0.25	0.31	0.33	0.30	0.37	0.32	0.34	0.26	0.35	0.31	0.26	0.20	0.20	0.18	0.38	0.24	0.29	0.14	0.34	0.08	0.60	0.50	1.00				
FI 24	0.36	0.24	0.40	0.39	0.40	0.34	0.26	0.26	0.30	0.36	0.31	0.20	0.25	0.22	0.36	0.37	0.37	0.22	0.28	0.06	0.49	0.58	0.47	1.00			
FI 25	0.24	0.20	0.31	0.16	0.24	0.26	0.29	0.24	0.27	0.22	0.21	0.24	0.23	0.34	0.20	0.35	0.22	0.10	0.19	0.09	0.30	0.38	0.31	0.41	1.00		
FI 26	0.35	0.31	0.35	0.25	0.32	0.36	0.31	0.24	0.39	0.38	0.28	0.33	0.18	0.35	0.33	0.42	0.33	0.20	0.28	0.07	0.52	0.60	0.50	0.57	0.49	1.00	
FI 27	0.32	0.30	0.41	0.28	0.29	0.38	0.30	0.24	0.29	0.37	0.35	0.24	0.16	0.32	0.32	0.33	0.35	0.18	0.23	0.08	0.47	0.52	0.48	0.46	0.36	0.55	1.00
FI 28	0.15	0.16	0.21	0.26	0.21	0.18	0.17	0.15	0.19	0.15	0.16	0.17	0.12	0.10	0.22	0.28	0.22	0.19	0.15	0.09	0.30	0.29	0.34	0.34	0.31	0.28	0.33
FI 29	0.22	0.14	0.29	0.24	0.23	0.20	0.24	0.23	0.23	0.21	0.17	0.18	0.26	0.29	0.18	0.30	0.27	0.16	0.30	0.10	0.30	0.34	0.27	0.33	0.39	0.33	0.42

Evaluation of rice genotypes toward infection with *F. fujikuroi* under greenhouse

A highly virulent isolate of *F. fujikuroi* (FI 12, isolated from sakha101 cv. in Dakhlia Gov.) was used for evaluating susceptibility and resistance of fourteen rice genotypes under artificial inoculation. Data in Table (6) indicated that susceptibility varied in toward inoculation with the selected isolate. However, Giza 177 showed the highest disease incidence and disease severity index (60.67% and1.28, respectively) followed by E. yasmine as a disease incidence and disease severity index (38.0% and 1.206, respectively). On the other hand, Giza 179 and Giza 178 were the least disease incidence (7.67, 11.34%, respectively) and disease severity index (0.37, 0.38, respectively). The germination rate decreased in inoculated genotypes compared with healthy genotypes. Bakanae is a seed-borne as well as soil-borne disease. When seeds of rice plants are infected by the fungus, the most characteristic symptoms of the disease is the appearance of abnormally elongated taller and thin plants, markedly over-growing than their uninfected neighbours. The active metabolic product of the pathogen is gibberellins, which were isolated and proved to play an important role in the pathogenicity of this organism by many workers **(Wiemann *et al*., 2013 and Elshafey *et al*., 2018).**

Table 6. Evaluation of rice genotypes to bakanae disease infection under artificial inoculation of *Fusarium fujikuroi* under greenhouse during 2017 season

No.	Genotypes	Disease incidence (%)	Disease Severity Index	Germination %	
				Healthy	inoculated
1	Giza 177	60.67^{a}	1.28^{a}	79.30 $^{a-e}$	40.67 ^{i}
2	Giza 178	11.34^{d}	0.38^{g}	92.00^{ab}	71.3^{b-f}
3	Giza 179	7.67^{d}	0.37^{g}	94.60^{a}	79.34^{a-e}
4	Giza 181	36.34^{b}	1.086^{bc}	86.00^{a-d}	48.00^{ghi}
5	E.Yasmine	38.00^{b}	1.206^{ab}	87.34^{abc}	46.00^{hi}
6	Sakha 101	34.34^{bc}	0.99^{cd}	87.3^{abc}	54.67^{f-i}
7	Sakha 102	32.67^{bc}	0.976^{cd}	88.0^{abc}	57.34^{f-i}
8	Sakha 103	21.00^{cd}	0.946^{cd}	88.67^{abc}	58.00^{f-i}
9	Sakha 104	30.00^{bc}	0.95^{cd}	88.67^{abc}	61.34^{e-i}
10	Sakha 105	16.34^{d}	0.79^{de}	90.0^{ab}	68.67^{c-g}
11	Sakha 106	15.00^{d}	0.72^{ef}	91.34^{ab}	66.0^{d-h}
12	Sakha 107	14.00^{d}	0.72^{ef}	92.6^{ab}	60.0^{e-i}
13	Sakha 108	32.34^{bc}	0.97^{cd}	86.67^{abc}	48.67^{ghi}
14	Hybrid 1	13.34^{d}	0.593^{f}	91.3^{ab}	68.00^{c-g}

In column means followed by the same letters are not statistically different at ($p \leq 0.05$) level according to Duncan's multiple range test.

Hammoud and Gabr (2014) evaluated twenty entries against bakanae pathogen under greenhouse and field conditions. Sakha 101 as a susceptible cultivar gave the highest percentage and severity of infection followed by GZ 7769-2-1-1-2 and Sakha 104. On the other hand, all entries gave the lowest infection of bakanae rice disease under natural infection.

Biochemical studies

The activity of anti-oxidative enzymes

Peroxidase activity (POX) ranged from 0.704 to 3.30 mg/ min in the inoculated seedling (Table. 7). While, in healthy seedling, the POX activity ranged from 0.327 to 3.14 mg/ min.

The maximum increase in POX activity was recorded 15 days after inoculation and then decreased. The highest activity for POX was significantly induced in bakanae resistant genotypes in Giza 179, Giza178 (3.30 and 3.26 mg/min, respectively) after 15 days of inoculation. The lowst level of induction was recorded in Giza 177 and E. yasmine (1.408 and 1.702 mg/min, respectively) as susceptible rice genotypes after 15 days of inoculation. The activity of POX enzymes in healthy plants may be due to their presence in healthy plant tissues as constitutive enzymes. The second possible resistance mechanisms may be reactive oxygen species (ROS), and this resistance leads to activate of defence-related genes. These mechanisms also induced important signalling molecules (**Chen, *et al.*, 2014**). During plant-pathogen interactions, ROS can produce. Enzymatic antioxidants such as SOD and POX are considered important enzymes in scavenging various types of ROS **(Barna, *et al.*, 2012).** Elimination of ROS is mainly achieved by antioxidant compounds such as PPO and POX. Under stress conditions, a constitutively high antioxidant capacity can prevent damage due to ROS formation (**Harinasut, *et al.*, 2003**).

Table 7. Peroxidase activity in the seedling of genotypes in healthy or inoculated by *F. fujikuroi*

NO.	Genotypes	POX (mg/ min)					
		Inoculated seedling			Healthy seedling		
		Time after inoculation in days			Time of measurement in days		
		7	15	21	7	15	21
1	Giza 177	0.704^{i}	1.408^{h}	0.896^{g}	0.327^{g}	0.65^{g}	0.50^{g}
2	Giza 178	1.650^{a}	3.260^{a}	1.98^{ab}	1.516^{a}	3.12^{a}	1.53^{a}
3	Giza 179	1.673^{a}	3.300^{a}	2.14^{a}	1.570^{a}	3.14^{a}	1.54^{a}
4	Giza 181	0.871^{ghi}	1.742^{fgh}	1.30^{ef}	0.69^{ef}	1.278^{f}	1.08^{e}
5	E.Yasmine	0.851^{hi}	1.702^{gh}	1.28^{f}	0.64^{f}	1.38^{ef}	0.80^{f}
6	Sakha 101	1.05^{efg}	1.948^{efg}	1.47^{def}	0.77^{def}	1.43^{ef}	1.21^{d}
7	Sakha 102	1.098^{ef}	2.100^{def}	1.51^{def}	0.71^{ef}	1.55^{def}	1.25^{cd}
8	Sakha 103	1.15^{def}	2.30^{cde}	1.58^{cde}	0.87^{cde}	1.73^{cde}	1.28^{cd}
9	Sakha 104	1.07^{ef}	2.196^{de}	1.58^{cde}	0.82^{de}	1.64^{c-f}	1.25^{cd}
10	Sakha 105	1.21^{de}	2.420^{cd}	1.60^{cd}	0.92^{cd}	1.84^{bcd}	1.3^{cd}
11	Sakha 106	1.31^{cd}	2.620^{bc}	1.61^{cd}	0.99^{bc}	1.996^{bc}	1.38^{c}
12	Sakha 107	1.46^{bc}	2.920^{ab}	1.62^{cd}	1.00^{bc}	2.00^{bc}	1.39^{bc}
13	Sakha 108	0.974^{fgh}	2.140^{de}	1.56^{cde}	0.85^{cde}	1.69^{cde}	1.27^{cd}
14	Hybrid 1	1.62^{ab}	3.240^{a}	1.80^{bc}	1.09^{b}	2.18^{b}	1.52^{ab}

In column means followed by the same letters are not statistically different at ($p \leq 0.05$) level according to Duncan's multiple range test.

For PPO activity profiles ranged from 0.039 to 0.932 Units/mg/min in healthy seedlings of 14 rice genotypes. The inoculated seedling, it ranged from 0.057 to 1.133 Unit/mg/min. Data

presented in Table (8) shown a gradual increase in PPO activity in healthy and inoculated seedlings with the pathogen spores (4 x 10^5 spores/ml).

However, the increment in the activity of PPO was higher in inoculated plants than in healthy ones. Also, a maximum increase in PPO activity was recorded 15 days after inoculation with *F. fujikuroi* and then decreased. The obtained data show that levels of PPO in Giza 178 and Giza 179 genotypes as resistant were higher than in Giza 177 and E. yasmine the highly susceptible ones. The results were supported by **Mohammadi and Kazemi (2002)** they found that quinones (antifungal compounds) and ligninproduced as results from the oxidation of PPO in plant cells during pathogen penetration so it enhances means of plant defense and resistance. Under stress, PPO activity increases and indicates the ability to oxidize and degrade the toxic substances such as phenolic compounds, which are generally accumulated in plants (**Weisany *et al.*, 2012). Castañera *et al*. (1996)** recorded that PPO plays a role in defense responses to biotic stresses by reducing the toxicity of quinones, bioavailability and alkylation of cellular proteins to the pathogen, cross-linking quinones with protein or other phenolic compounds and forming physical barriers**.**

Table 8. Polyphenoloxidase activity in rice genotypes in healthy or inoculated with *F. fujikuroi*.

No.	Genotypes	Polyphenoloxidase (Unit/mg/min)					
		Inoculated seedling			Healthy seedling		
		Time after inoculation in days			Time of measurement in days		
		7	15	21	7	15	21
1	Giza 177	0.057^{d}	0.058^{d}	0.097e	0.039^{c}	0.044^{d}	0.082^{f}
2	Giza 178	0.827^{a}	1.133^{a}	1.102^{a}	0.690^{a}	0.86^{a}	0.932^{a}
3	Giza 179	0.648^{ab}	0.965^{ab}	0.86^{b}	0.548^{ab}	0.627^{b}	0.744^{b}
4	Giza 181	0.132^{cd}	0.192^{d}	0.309^{cd}	0.049^{c}	0.080^{d}	0.086^{f}
5	E.Yasmine	0.079^{d}	0.168^{d}	0.224^{de}	0.048^{c}	0.053^{d}	0.083^{f}
6	Sakha 101	0.182^{cd}	0.220^{d}	0.336^{cd}	0.051^{c}	0.086^{d}	0.156^{ef}
7	Sakha 102	0.203^{cd}	0.425^{cd}	0.345^{cd}	0.092^{bc}	0.09^{d}	0.186^{def}
8	Sakha 103	0.218^{cd}	0.55^{bcd}	0.371^{cd}	0.109^{bc}	0.325^{c}	0.225^{def}
9	Sakha 104	0.206^{cd}	0.465^{bcd}	0.358^{cd}	0.099^{bc}	0.128^{d}	0.236^{def}
10	Sakha 105	0.221^{cd}	0.552^{bcd}	0.417^{cd}	0.111^{bc}	0.325^{c}	0.250^{def}
11	Sakha 106	0.245^{cd}	0.758abc	0.47^{c}	0.139^{bc}	0.382^{c}	0.299^{de}
12	Sakha 107	0.433^{bc}	0.820^{abc}	0.986^{ab}	0.147^{bc}	0.421^{c}	0.357^{d}
13	Sakha 108	0.21^{cd}	0.55^{bcd}	0.367^{cd}	0.094^{bc}	0.101^{d}	0.189^{def}
14	Hybrid 1	0.58^{ab}	0.82^{abc}	0.84^{b}	0.176^{bc}	0.786^{ab}	0.525^{c}

In column means followed by the same letters are not statistically different at ($p \leq 0.05$) level according to Duncan's multiple range test.

H_2O_2 content: The H_2O_2 content of inoculated rice genotypes ranged from 0.146 to 1.015 reduced mg/ min/ protein. While H_2O_2 content ranged from 0.102 to 0.649 reduced mg/ min/ protein in healthy rice genotypes. H_2O_2 content was higher in inoculated seedlings compared with healthy (Table, 9). Giza 179 (resistant genotype) proved to have the lowest content of H_2O_2 in inoculated and in healthy plants. There was no significant difference between Giza 178 and Hybrid1 (R) in H_2O_2 content. The highest H_2O_2 content was recorded in inoculated and in healthy Giza 177 and E. yasmine as bakanae susceptible to rice genotypes. Under salinity condition, concentration of H_2O_2 in leaf tissue were significantly increased with high salinity (**Weisany *et al.*, 2012**). Abiotic and biotic stress generally induces damage to plant tissues as a result of excessive ROS such as H_2O_2 produced at a high rate owing to ion imbalance and hyperosmotic stresses. ROS accumulation leads to lipid oxidation and harms cellular metabolism and physiology, thus detrimentally affecting the membrane integrity (**Munns *et al*., 2006**).

Table 9. H_2O_2 content in seedling of and rice genotypes in healthy or inoculated with *F. fujikuroi*.

No.	Genotypes	H_2O_2 (mg/ min/ protein)					
		Inoculated seedling			Healthy seedling		
		Time after inoculation in days			Time of measurement in days		
		7	15	21	7	15	21
1	Giza 177	0.58^{a}	1.015^{a}	0.761^{a}	0.371^{a}	0.649^{a}	0.487^{a}
2	Giza 178	0.182^{de}	0.326^{de}	0.245^{de}	0.107^{e}	0.187^{e}	0.140^{e}
3	Giza 179	0.146^{e}	0.255^{e}	0.191^{e}	0.102^{e}	0.179^{e}	0.134^{e}
4	Giza 181	0.41^{bc}	0.718 ^{bc}	0.538^{bc}	0.258^{abc}	0.452^{abc}	0.338^{abc}
5	E.Yasmine	0.457^{ab}	0.80^{ab}	0.600^{ab}	0.320^{ab}	0.560^{ab}	0.420^{ab}
6	Sakha 101	0.356^{bcd}	0.623^{bcd}	0.429^{b-e}	0.240^{bcd}	0.420^{bcd}	0.315^{bcd}
7	Sakha 102	0.356^{bcd}	0.623^{bcd}	0.369^{b-e}	0.197^{cde}	0.377^{b-e}	0.283^{b-e}
8	Sakha 103	0.245^{cde}	0.429^{cde}	0.321^{cde}	0.149^{cde}	0.277^{cde}	0.207^{cde}
9	Sakha 104	0.282^{cde}	0.572^{b-e}	0.467^{bcd}	0.158^{cde}	0.301^{cde}	0.226^{cde}
10	Sakha 105	0.229^{de}	0.436^{cde}	0.327^{cde}	0.139^{cde}	0.261^{cde}	0.195^{cde}
11	Sakha 106	0.229^{de}	0.40^{cde}	0.300^{cde}	0.137^{cde}	0.243^{cde}	0.182^{cde}
12	Sakha 107	0.197^{de}	0.401^{cde}	0.300^{cde}	0.134^{cde}	0.235^{cde}	0.176^{cde}
13	Sakha 108	0.327^{bcd}	0.493^{b-e}	0.467^{bcd}	0.172^{cde}	0.345^{cde}	0.258^{cde}
14	Hybrid 1	0.187^{de}	0.344^{de}	0.258^{de}	0.122^{de}	0.214^{de}	0.16^{de}

In column means followed by the same letters are not statistically different at ($p \leq 0.05$) level according to Duncan's multiple range test.

Under field condition:

Evaluation of rice genotypes towards infection with bakanae disease and yield losses.

Fourteen rice genotypes were tested for rice bakanae disease under field condition for two seasons. Results from Table (10) indicated that Giza 177 as a susceptible genotype recorded the highest disease incidence percent and disease severity index for two seasons followed by E. yasmine. On the other hand, all genotypes gave the lowest DSI and disease incidence percent of bakanae rice disease under field condition. Concerning Giza 179 and 178 showed high resistance to bakanae disease. The effect of bakanae symptoms on growth and yield characteristics of tested rice genotypes was also investigated. Data summarized in Table (11) showed that between the 2017 and 2018 rice growing seasons, all rice genotypes showed an increasechlorophyll content, a number of tillers/hill and mean of panicle length/10 plants in healthy plants compared with infected plants. While all rice genotypes showed increased in a mean stem length/10 plants in infected plants compared with healthy plants. There are no significant differences between Giza 179 on growth and yield character in both healthy and infected plant followed by Giza178. Studies on varietal resistance screening revealed that basmati/scented germplasm and cultivars are more susceptible to bakanae disease compared to non-scented rice cultivars. The basmati rice cultivars affected by bakanae viz., Pusa Basmati 1121, Pusa Basmati 1176 and Pusa Basmati 1509 in Northern India, however, other basmati rice varieties *viz*., Pusa Basmati 1401, Pusa 2511, CSR 30, Dehradun basmati and Pakistani basmati were also found to be infected by the disease with 2.0-22.8% up to 40% incidence (**Gupta *et al*., 2015**). **Fiyaz *et al*. (2014)** screened 12 genotypes against bakanae pathogen. The genotypes (Athad apunnu, C101A51, Chandana, IR 58025B, Panchami, PAU 201, Pusa 1342, and Varun Dhan) were found to be highly resistant, whereas the genotypes (BPT 5204, Himju, Peeli Badam and Suphala) were resistant to bakanae disease.

Table 10. Evaluation of rice genotypes to infection with bakanae disease during 2017 and 2018 seasons.

NO.	Genotypes	Disease incidence (%)		Disease Severity Index	
		2017	2018	2017	2018
1	Giza 177	25.376^{a}	27.00^{a}	0.43^{a}	0. 61^{a}
2	Giza 178	4.390^{fg}	6.633^{fg}	0.058^{b}	0.163^{de}
3	Giza 179	1.000^{g}	3.100^{g}	0.042^{b}	0.150^{e}
4	Giza 181	18.780^{b}	19.00^{bc}	0.243^{ab}	0.531^{abc}
5	E.Yasmine	19.08^{b}	21.00^{ab}	0.27^{ab}	0.567^{ab}
6	Sakha 101	16.44^{bc}	18.34^{bc}	0.225^{ab}	0.501^{abc}
7	Sakha 102	14.133^{bcd}	17.34^{bcd}	0.189^{b}	0.467^{abc}
8	Sakha 103	11.167^{cde}	13.667^{cde}	0.168^{b}	0.370^{b-e}
9	Sakha 104	15.227^{bcd}	13.67^{cde}	0.171^{b}	0.401^{b-e}
10	Sakha 105	10.213^{c-f}	11.67^{def}	0.132^{b}	0.298^{b-e}
11	Sakha 106	9.197^{def}	8.00^{efg}	0.123^{b}	0.290^{b-e}
12	Sakha 107	4.490^{efg}	7.00^{fg}	0.086^{b}	0.283^{b-e}
13	Sakha 108	13.34^{bcd}	15.00^{bcd}	0.171^{b}	0.457^{a-d}
14	Hybrid1	4.747^{fg}	7.00^{fg}	0.068^{b}	0.233^{cde}

In column means followed by the same letters are not statistically different at ($p \leq 0.05$) level according to Duncan's multiple range test.

Table 11. Effect of infection with bakanae pathogen on agronomic characters for rice genotypes under field conditions

No.	Genotypes	Inoculated / Healthy	Chlorophyll content (SPAD value)		No. of tillers/ hill		Mean of stem length/10 plants		Mean of panicle length/10 plants	
			2017	2018	2017	2018	2017	2018	2017	2018
1	Giza 177	Inoculated	34.3^{b}	33.0^{c}	11.3^{l}	11.40^{l}	85.4^{efg}	87.67^{c-g}	15.717^{j}	15.6^{4i}
		Healthy	38.9^{ab}	38.6^{abc}	13.3^{ghi}	13.4^{ghi}	83.00^{fgh}	83.00^{e-l}	17.73^{g-j}	17.75^{ghi}
2	Giza 178	Inoculated	40.0^{ab}	39.0^{abc}	26.6^{bc}	25.7^{cd}	92.00^{b-e}	92.67^{bcd}	18.45^{e-h}	18.67^{e-h}
		Healthy	40.4^{ab}	39.6^{abc}	27.3^{bc}	27.0^{cd}	76.00^{hi}	78.34^{hij}	20.67^{b-e}	21.00^{a-f}
3	Giza 179	Inoculated	36.3^{ab}	36.0^{abc}	38.0^{a}	37.4^{a}	92.00^{b-g}	93.34^{bcd}	19.28^{e-h}	19.34^{d-g}
		Healthy	40.6^{ab}	40.0^{abc}	38.3^{a}	37.0^{a}	90.0^{b-g}	91.67^{b-e}	19.47^{d-h}	19.67^{c-g}
4	Giza 181	Inoculated	35.0^{c}	34.6^{bc}	24.3^{cde}	25.4^{cd}	95.67^{abc}	95.67^{abc}	20.03^{c-g}	19.83^{b-g}
		Healthy	36.5^{ab}	36.0^{abc}	29.6^{bc}	29.67^{cd}	88.00^{c-f}	88.00^{c-g}	23.00^{a}	23.00^{a}
5	E.Yasmine	Inoculated	23.2^{b}	22.6^{d}	13.7^{ghi}	13.67^{gkl}	93.4^{b-e}	93.34^{bcd}	22.18^{abc}	21.67^{a-d}
		Healthy	35.3^{ab}	34.0^{bc}	17.0^{ghi}	17.67^{e-j}	75.67^{hi}	77.34^{ij}	22.44^{ab}	22.00^{abc}
6	Sakha 101	Inoculated	41.2^{ab}	40.0^{abc}	12.7^{hi}	13.00^{kl}	79.4^{ghi}	79.67^{f-j}	20.11^{c-f}	20.34^{b-g}
		Healthy	41.3^{ab}	41.6^{abc}	15.4^{ghi}	15.34^{h-l}	74.00^{i}	74.00^{j}	20.63^{b-e}	20.00^{b-g}
7	Sakha 102	Inoculated	38.8^{ab}	38.3^{abc}	23.4^{c-f}	21.4^{fed}	92.67^{b-e}	99.34^{ab}	18.83^{e-h}	19.00^{d-g}
		Healthy	41.2^{ab}	41.0^{abc}	25.6^{bcd}	24.00^{de}	77.4^{ab}	92.67^{bcd}	19.62^{d-h}	19.50^{c-g}
8	Sakha 103	Inoculated	39.3^{ab}	38.3^{abc}	17.0^{ghi}	19.00^{fgh}	102.67^{a}	102.67^{a}	19.55^{d-h}	19.34^{d-g}
		Healthy	42.4^{ab}	41.6^{abc}	19.6^{d-g}	19.67^{fgh}	92.34^{b-e}	92.34^{bcd}	21.62^{e-d}	21.34^{a-e}
9	Sakha 104	Inoculated	40.7^{ab}	40.0^{abc}	16.6^{ghi}	17.00^{g-k}	92.34^{b-e}	91.67^{b-e}	18.00^{f-j}	18.34^{fgh}
		Healthy	41.9^{ab}	41.3^{abc}	18.7^{e-h}	18.67^{f-l}	87.00^{d-g}	87.00^{c-h}	18.89^{e-h}	19.00^{d-g}
10	Sakha 105	Inoculated	38.9^{ab}	38.3^{abc}	16.0^{ghi}	16.00^{g-k}	95.00^{a-d}	94.34^{abc}	18.86^{e-h}	19.00^{d-g}
		Healthy	40.2^{ab}	40.0^{abc}	18.0f^{gh}	19.67^{fgh}	85.00^{efg}	85.00^{d-l}	20.72^{b-e}	20.00^{b-g}
11	Sakha 106	Inoculated	37.2^{ab}	38.3^{abc}	14.4^{ghi}	14.67^{il}	92.00^{b-e}	89.00^{cde}	16.02^{ij}	16.34^{hi}
		Healthy	41.4^{ab}	40.0^{abc}	19.7^{d-g}	20.00^{fg}	87.67^{c-g}	85.00^{d-i}	17.88^{f-j}	18.00^{ghi}
12	Sakha 107	Inoculated	37.6^{ab}	37.6^{abc}	23.4^{c-f}	21.4e^{fcd}	93.34^{b-e}	93.34^{bcd}	17.50^{hij}	18.34^{fgh}
		Healthy	45.0^{a}	43.3^{a}	27.4^{bc}	26.00^{cd}	86.67^{d-g}	87.34^{c-h}	19.11^{e-h}	19.34^{d-g}
13	Sakha 108	Inoculated	41.0^{ab}	38.6^{abc}	23.4^{c-f}	26.4^{cd}	89.34^{c-f}	88.67^{c-f}	19.89^{c-h}	20.00^{b-g}
		Healthy	42.6^{ab}	41.6^{ab}	26.4^{bc}	26.4^{cd}	85.00^{efg}	83.00^{e-i}	20.58^{b-e}	20.34^{b-g}
14	Hybrid 1	Inoculated	37.0^{ab}	37.0^{abc}	29.7^{bc}	29.37^{cd}	89.67^{d-f}	87.34^{c-h}	22.13^{abc}	22.34^{ab}
		Healthy	38.9^{ab}	38.6^{abc}	31.4^{b}	31.34^{b}	79.4^{ghi}	79.00^{g-j}	22.44^{ab}	22.34^{ab}

In column means followed by the same letters are not statistically different at ($p \leq 0.05$) level according to Duncan's multiple range test.

The effect of infection with bakanae on yield losses to different rice genotypes are indicated in Table (12). Giza 179 provided the highest grain yield and the lowest yield losses affected with bakanae compared with the other genotypes followed by Hybrid 1, Sakha 102 and106 decreasing the yield losses in 2017 season. While in 2018 season Sakha 106 gave the lowest yield losses after Giza 179. Sakha 101 showed the highest percent in yield losses during two seasons (13.88 and 11.76, respectively), in season 2018 Sakha 104 recorded the highest losses in grain yield (12.23%). In India, some states such as Uttar Pradesh, Assam, Andhra Pradesh, Tamilnadu, Haryana and Punjab recorded yield losses due to bakanae rice disease ranging between 15-25% (**Sunder *et al*., 2014**). The infected seed of different basmati rice cultivars by bakanae pathogen appear to be part of yield losses and correlated with the highest percentage (1-24%) and affected seeds quality (**Bashyal *et al*., 2014**). Also, in India, the incidence of bakanae disease ranged from 1.2-40%, especially in basmati rice cultivars during 2008-2014 in Northern states (**Gupta *et al*., 2015**).

Table 12. Effect of infection with bakanae pathogen on yield and yield losses of rice genotypes under field condition.

No.	genotypes	Yield (ton/fed)				Yield losses (%)	
		2017		2018		2017	2018
		Healthy	Inoculated	Healthy	Inoculated		
1	**Giza 177**	3.58d	3.28d	3.83ef	3.60bcd	8.38	6.00
2	**Giza 178**	4.37bcd	4.23abc	4.57a-d	4.44ab	3.20	2.84
3	**Giza 179**	**5.07a**	**5.00a**	**4.97ab**	**4.90a**	**1.38**	**1.41**
4	**Giza 181**	4.17bcd	3.84bcd	3.89ef	3.60bcd	7.91	7.45
5	**E.Yasmine**	3.96bcd	3.66bcd	3.90ef	3.80bcd	7.57	2.56
6	**Sakha 101**	4.32bcd	3.72bcd	4.42b-e	3.90bcd	13.88	11.76
7	**Sakha 102**	4.48bcd	4.37ab	4.34b-e	4.00bcd	2.45	7.83
8	**Sakha 103**	3.87cd	3.55bcd	4.00c-f	3.94bcd	8.27	2.50
9	**Sakha 104**	4.17bcd	3.91bcd	4.17c-f	3.66bcd	6.23	12.23
10	**Sakha 105**	3.93bcd	3.70bcd	3.93def	3.83bcd	5.61	2.54
11	**Sakha 106**	3.72d	3.62bcd	3.67f	3.60bcd	**2.75**	**1.90**
12	**Sakha 107**	4.91abc	4.64ab	4.40b-e	4.20abc	3.96	4.50
13	**Sakha 108**	4.63bcd	4.45ab	4.63abc	4.37ab	3.88	5.62
14	**Hybrid 1**	5.01ab	4.91a	5.13a	5.00a	**2.00**	**2.53**

In column means followed by the same letters are not statistically different at ($p \leq 0.05$) level according to Duncan's multiple range test.

Conclusion

From the obtained results of the present work, it could be concluded that bakanae caused by *Fusarium fujikuroi* (Nirenberg) is emerging as a serious disease of rice in Egypt. Different bakanae isolates were characterized as *Fusarium* by diversity, variability and appearance of different levels of virulence. The molecular results obtained here proved the existence of high levels of genetic variation. This explains the high level of the mutation rate of *Fusarium* fungus. High disease incidences (25.37- 27.0%) found in Giza 177. The yield losses were found in Sakha 101(13.88%) during the rice-growing seasons. The pathogen primarily survives in seed but is also known to survive in soil. So studies on genotypescreening revealed that aromatic rice such as Egyptian yasmine is more susceptible to bakanae disease compared to coarse grain unscented genotypes like Giza 179 and Giza 178. Also, these two cultivars can produce a high number of tillers/hill and grain yield so maybe this good growth and yield compensates the losses of the bakanae disease.

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الملخص العربي

التباين البيوكيميائي و الجزيئي لعزلات Fusarium fujikuroi وتفاعلاتها التفاضلية مع الطرز الوراثيه للأرز أثناء الإصابة

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يعتبر مرض البكانا في الارز المتسبب عنه (*Fusarium fujikuroi*)مرضًا مهمًا في مناطق إنتاج الأرز الرئيسية في جميع أنحاء العالم. تم تمييز تسعة وعشرين عزلة من *F. fujikuroi* على أساس الأصباغ المنتجة، وحمض الجبريليك (GA3)، والخصائص الجزيئية المرتبطة بأعراض مرض البكانا في بادرات الأرز. أنتجت جميع عزلات *F. fujikuroi* أصباغ ماعدا ستة عزلات. كما اختلفت جميع العزلات في إنتاج حمض الجبريليك. كانت العزله FI 12 هي ألاعلى في إنتاج حمض الجبريللين ومؤشر الشدة المرض. أظهر التحليل الجزيئي أن تسعة بادئات ISJ أنتجت ما مجموعه 809 حزم. تشير النتائج الحاليه إلى أن عزلات *Fusarium* تم تجميعها بشكل كبير بناءً على موقع و / أو شدة العزلات ومستوى إنتاج الجبريللين. أثبتت النتائج الجزيئية التي تم الحصول عليها وجود مستويات عالية من التباين الجيني وقوة الاستبانة العالية لبادئات ISJ في الكشف عن التنوع الجزيئي للفيوزاريوم. تمت دراسة السلوك التفاضلي لمرض البكانا في الطرز الوراثية المقاومة والحساسة للأرز أثناء الإصابة. تحت ظروف الصوبه الزجاجيه كانت الصنف جيزة 177 عالية الحساسية للاصابه بمرض البكانا والجيزة 179 والجيزة 178 ذات المقاومة المتوسطة. تم ملاحظة التغيرات الكيميائية الحيوية مثل إنزيمات مضادات الأكسدة وبيروكسيد الهيدروجين (H2O2) في بادرات الأرز بعد 7 و15 و21 يومًا من التلقيح. زادت أنشطة الإنزيمات في جيزة 179 و 178 جيزة، لكن محتوى H_2O_2 كان في تناقص. في ظل الظروف الحقلية، أظهرت جميع الطرز الوراثية زياده في محتوي الكلورفيل، الفروع /الجوره ومتوسط طول السنابل/ 10نباتات في كل الاصناف السليمه مقارنة بالنباتات المعديه. كان الصنف جيزة 179 أعلى في محصول حبوب.