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Growth and biochemical variability among *Fusarium fujikuroi* isolates causing bakanae disease in aromatic rice

Vikram Singh[1*], Ashwani Kumar[2], Promil Kapoor[1], Mahaveer Singh Bochalya[3] & Neha Rohila[4]

[1]Department of Plant Pathology, Chaudhary Charan Singh Haryana Agricultural University, Hisar 125 004, Haryana, India
[2]Chaudhary Charan Singh Haryana Agricultural University, College of Agriculture, Kaul 136 021, Kaithal, Haryana, India
[3]Chaudhary Charan Singh Haryana Agricultural University, Cotton Research Station, Sirsa 125 055, Haryana, India
[4]Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar 125 004, Haryana, India

***Correspondence:** vikramrohilla56@gmail.com

Abstract

During the kharif seasons of 2020 and 2021, a study was conducted at the Department of Plant Pathology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, to investigate bakanae disease of aromatic rice, which is a major constraint on rice production in Haryana and neighbouring states, particularly in early-transplanted crops. During the kharif seasons of 2020 and 2021, 64 isolates were collected from major aromatic rice-growing areas in Haryana. Out of these, 26 isolates were selected to analyse their growth rates and biochemical variability. The growth rate among isolates varied between 12.95–25 mm/48 hr on potato dextrose agar. Isolate FM 18 (25.00 mm/48hr) showed the fastest growth rate, whereas the slowest was observed in FM 31 (12.95 mm/48 h). Maximum colony diameter was shown by FM 18 (83.66 mm) and minimum by FM 31 (55.82 mm). Biochemical constituents (mg/g dry mycelium), including total sugars, total phenols, free amino acids and protein content, were estimated using standard methods. Among the biochemical constituents (mg/g of dry mycelium), total sugars, phenols, free amino acids and protein content varied between 3.77 (FM 53)–12.08 (FM 63), 3.18 (FM 60)–11.98 (FM 20), 26.31 (FM 25)–58.83 (FM 53) and 70 (FM 36)–112 (FM 40) respectively. The present investigation confirms significant biochemical and growth rate variations among the 26 isolates. This characterisation provides a deeper understanding of the pathogen's variation, serving as a vital basis for creating focused management plans and enhancing disease control techniques.

Keywords: bakanae; Haryana; rice; total sugars; variability

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Introduction

In the major rice-growing countries of Asia and Africa, *Fusarium fujikuroi* is the causal agent of bakanae disease, also known as rice seedling disease (1). Although the disease was first described in 1898, historical records indicate that it had been present in Japan since 1828 (2, 3). In India, the disease was first reported in 1931 (4). Crop production is seriously threatened by this disease, which is common in areas that produce rice and can result in yield losses of up to 75 % (5, 6). In the field, infected seeds serve as the primary inoculum. Additionally, macroconidia and thick-walled hyphae can persist in soil and plant detritus, causing infections during the next growing season (7, 8). Under various climatic conditions worldwide, it is known to cause a variety of symptoms, such as rotting of seedlings before emergence, infection of grains at ripening, seedling blight, crown rot, rotting of roots, stunting of plants, etiolation, excessive or abnormal elongation of plants (hypertrophy), sterility, grain discolouration and production of empty panicles (9–12).

Adventitious root development from the lower portion of the culm has been reported as one of the symptoms of bakanae disease in India (4). Every country that produces rice, including Turkey, Pakistan, Thailand, Japan, Europe, America, Africa, Philippines, Nepal, Bangladesh, Cameroon, Nigeria, Vietnam, Indonesia, Malaysia, Sri Lanka, Ivory Coast, Uganda, Brazil, Spain, China, Trinidad, Iran, Venezuela, Mexico, etc., has reported cases of the bakanae disease

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(12, 13). Under ideal circumstances, disease can cause yield losses of 20–50 % in Japan, 70–80 % in Australia, 3.7–14.7 % in Thailand, 5–23 % in Spain, 3.0–95.4 % in India, 40 % in Nepal, 6.7–58.0 % in Pakistan and up to 75.0 % in Iran (14). Bakanae disease yield losses vary significantly due to differences in regional climates, cultivar susceptibility and local agricultural practices. High temperature and humidity accelerate fungal growth, while highly aromatic varieties like basmati are especially prone to severe infection. Additionally, the implementation of preventative measures, such as fungicidal seed treatments and specific nursery management methods, keeps losses lower in certain countries. The successful development of new high-yielding cultivars requires an understanding of its naturally occurring populations. For the management of the pathogen, a precise understanding of its variability is essential.

The isolates were identified based on cultural characters, such as colony colour and morphological characteristics, such as macro- and microconidial size and shape, with the help of microscopy and pathogenicity tests were also verified on rice. Molecular confirmation of these was also done in our previous study by using translation elongation factor 1-alpha (TEF-1α) molecular marker (15). Variability based on morphological, genetic and cultural traits for distinguishing *F. fujikuroi* isolates has been described by a number of studies (16, 17). In the previous study we used the β-tubulin molecular marker to distinguish between these isolates. A positive result was observed for 20 isolates, whereas 6 isolates did not show amplification in the β-tubulin region (18).

The current investigation was conducted to distinguish *F. fujikuroi* isolates based on their biochemical diversity, as information regarding the metabolic profiling of this pathogen remains insufficient. The selection of total sugars, total phenols, free amino acids and proteins is directly based on their vital roles in fungal physiology and pathogenicity. Total sugars act as primary carbon energy reserves needed for spore germination and host tissue invasion, while total phenols function as defensive compounds that protect the fungal cell wall from host-induced oxidative stress. Regarding nitrogen metabolism, free amino acids form the mobile cytoplasmic pool required for rapid cellular regulation, whereas protein concentrations reflect the catalytic machinery and enzymes essential for plant colonisation. Profiling these foundational pools directly uncovers the distinct metabolic strategies driving pathotype fitness and field virulence.

Materials and Methods

The present study was carried out during the kharif seasons of 2020 and 2021 at Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana.

Survey and collection of diseased samples

During kharif 2020 and 2021, a total of 64 bakanae-infected plant samples from five commonly grown aromatic varieties PB 1121, PB 1509, PB 1401, PB 1718 and Basmati 521 were collected from major aromatic rice-growing districts in Haryana, Karnal (3), Kurukshetra (1), Kaithal (1), Jind (6), Fatehabad (11), Sirsa (14), Sonipat (3) Yamunanagar (2), Panipat (5), Hisar (10) and Bhiwani (8) (Table 1, Fig. 1).

Table 1. List of diseased samples collected from different districts of Haryana

Isolates Id. No.	Districts	Locations (Villages)	Varieties
FM 2 to FM 11	Hisar	Dhad, Kharak Punia, Gurana and Kheri Jalab	PB 1121
FM 12 to FM 17	Jind	Intal Khurd, Intal Kalan and Ikkas	PB 1121
FM 18 to FM 28	Fatehabad	Saniana and Pirthala	PB 1121 and PB 1401
FM 29 to FM 36	Bhiwani	Kungar, Durjanpur, Alakhpura and Bhiwani	PB 1121 and PB 1509
FM 37 to FM 50	Sirsa	Patli Dabar,Vaidwala, Mochiwala, Jodhkan, Bajekan, Fatehpuria, Rasulpur, Rania, Ootu, Ramsinghpuria and Naiwala	PB 1121, PB 1401 and PB 1718
FM 51 to FM 55	Panipat	Bapoli, Panipat, Samalkha and Chhichhrana	PB 1121, PB 1509 and PB 1718
FM 56 to FM 58	Sonipat	Sonipat, Kumaspur and Mukinpur	PB 1121
FM 59 to FM 61	Karnal	Taraori and Sikri	PB 1121 and PB 1718
FM 62 to FM 63	Yamunanagar	Kartarpur and Yamunanagar	PB 1121 and PB 1509
FM 64	Kaithal	Kaul	Basmati 521
FM 66	Kurukshetra	Babain	PB 1509

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Fig. 1. Field view of bakanae disease-infected plants from Haryana districts during survey.

Isolation, purification and maintenance

The infected samples were cut into pieces that were between 3 mm and 4 mm in size and $MgCl_2$ (0.1 %) was used to surface sterilise them. The excised fragments were placed uniformly on potato dextrose agar medium in Petri dishes. Plates were placed in a biochemical oxygen demand (BOD) incubator and kept at 25 ± 2 °C. The single spore culture method was used to create purified cultures and the related fungus was detected under a microscope and kept for additional research (Fig. 2).

Colony diameter was measured every 48 hr until the eighth day. This measurement was used to compute the growth rates of the isolates.



Fig. 2. Pure culture of the pathogen.

Biochemical constituents

To evaluate metabolic variability, the mycelial mat from each *F. fujikuroi* isolate cultivated in potato dextrose broth (PDB) was harvested 10 days post-inoculation. To ensure absolute baseline uniformity and prevent environmental variations from skewing metabolite accumulation, all 26 fungal isolates were grown under strictly identical culture conditions. Liquid cultivation was conducted using a standardised inoculum size consisting of two 5 mm mycelial plugs per flask taken from the margins of active 5-day-old agar cultures. The flasks were kept at a constant incubation temperature of 25 ± 2 °C under uniform static incubator conditions for a set culture duration of exactly 10 days under continuous dark conditions. The harvested mats were washed with distilled water, oven-dried at 60 °C for 72 hr and finely ground into a powder.

Replication design and statistical rigour

To determine the differences between these isolates, the different biochemical components such as total phenols, total sugars, protein and free amino acids were measured.

All quantitative metabolic evaluations were performed using three independent biological replicates per isolate. Each biological replicate represents an entirely separate broth culture run to ensure true biological variation was captured. Additionally, each biological sample was analysed in triplicate technical runs during the spectrophotometric assays to minimise pipetting and instrumental error, yielding a total of nine data measurements per isolate profile.

Biomass optimisation and standardisation

While the selected isolates exhibited significant differences in radial growth velocities on solid agar media, their biomass accumulation in liquid media was fully optimised. Cultures were grown in sufficient broth volumes for 10 days to yield abundant mats, ensuring that an identical, standardised mass of exactly 0.5 g of dried mycelium powder was consistently weighed out on a digital analytical balance for every single isolate. This precise physical standardisation guaranteed absolute extraction consistency across all isolates, independent of their baseline growth rates.

Extraction of biochemical constituents

The standardised dry mycelium (0.5 g) was extracted for 40 min at 70 °C in 80 % ethanol. The procedure was repeated 3 times and the mycelium was removed by filtering the supernatant first through muslin cloth and then through Whatman No. 1 filter paper. Distilled water was added to bring the final volume to 50 mL.

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Spectrophotometric calibration and quality controls

These alcoholic extracts were used for the estimation of the above-mentioned biochemicals as per the standard method described below. For every separate colourimetric measurement batch, negative control blanks (consisting of the respective reagents and buffers without any fungal extract) were systematically processed alongside the test samples to calibrate the spectrophotometer and subtract baseline absorbance values. Positive quantitative calibration was established using reference standard curves generated from pure chemical grades of bovine serum albumin (for proteins), leucine (for free amino acids), tannic acid (for total phenols) and glucose (for total sugars).

Estimation of proteins

Protein was extracted from the finely powdered material according to the protocol described by previous researchers (19).

Extraction

Twenty-five milligrams of the oven-dried samples were completely macerated in a mortar and pestle in 0.1 molar (M) potassium phosphate buffer pH 7.3 to extract the protein. The homogenised sample was centrifuged for 10 min at 1500 revolutions per minute (rpm). The pellet was then rinsed with the same buffer and centrifuged again after the supernatant was decanted. The resulting supernatants were pooled and used for estimation of protein.

Reagents

Reagent A : Sodium carbonate (Na_2CO_3) in 0.1 N sodium hydroxide (NaOH).

Reagent B : 0.5 % copper sulphate in 1.0 % potassium sodium tartrate.

Reagent C : This was prepared fresh by mixing 50 mL of reagent A with 1 mL of reagent B

Procedure

To 1 mL aliquot of each extract 5 mL of Reagent C was added and the content mixed thoroughly. The absorbance was measured after 30 min at 660 nanometre (nm) using a spectrophotometer. The amount of protein was determined from a standard curve prepared using graded concentrations (0–200 µg) of bovine serum albumin, with final calculations expressed in mg/g dry mycelium.

Estimation of free amino acid content

The amino acid content was estimated using the method described by previous researchers (20). A 20 mg portion of the oven-dried fungal inoculum was thoroughly macerated and homogenised in 80 % ethanol to extract the free amino acids. For 10 min, the homogenate was centrifuged at 5000 rpm. The collected supernatants were combined and utilised to estimate the amount of free amino acids.

Reagents

Reagent A : 5.0 % weight per volume (w/v) solution of ninhydrin in acetone.

Reagent B : 5 mL of 0.01 M potassium cyanide (KCN) solution, prepared in 60 % ethanol, was diluted to 250 mL with acetone

Reagent C : Prepared by mixing Reagents A and B in the ratio of 1:5 just before use

Reagent D : 0.2 M citrate buffer (pH 5.0)

Procedure

A 0.5 mL portion of Reagent D and 1.0 mL of Reagent C were mixed with a 0.5 mL aliquot of the ethanolic extract from each isolate and heated in a water bath for 20 min. After cooling to room temperature, the final volume was made to 5 mL with distilled water. The absorbance at a wavelength of 570 nm was recorded. A reference curve was prepared with 0–10 micromoles of leucine and the free amino acids content of the samples was expressed in terms of leucine equivalents.

Estimation of total phenol

Total phenol content was estimated using the method described by previous researchers (21).

Procedure

Five millilitres of distilled water and half a millilitre of Folin-Dennis reagent were added to half a millilitre of alcoholic extract. After 3 min, 1 mL of saturated sodium carbonate solution was added, and the final volume was made up to 25 mL. The mixture was allowed to stand for 1 hr. At a wavelength of 740 nm, the absorbance was measured. Tannic acid was used to create a standard curve.

Estimation of total sugar

Total sugar content was estimated using the method described by previous researchers (22).

Procedure

One millilitre of 5 % phenol of concentrated sulphuric acid (H_2SO_4) was added to half a millilitre of alcoholic extract. After holding for 20 min, the extract was left to cool. A standard curve made using glucose was used to calculate the quantity of total sugar after the final volume was adjusted to 25 mL using distilled water and the absorbance was measured at 490 nm wavelength.

Biochemical variation among different isolates on the basis of total phenol, total sugar, total proteins and amino acids was estimated (mg/g of dry mycelium).

Statistical analysis

The experiments were laid out in a completely randomised design (CRD) with 3 replications and the data were analysed using Duncan's Multiple Range Test (DMRT) at a 5 % level of significance with 52 error degrees of freedom for solid media growth rates and 54 error degrees of freedom for colourimetric biochemical analyses. All experimental data were subjected to statistical analysis using OPSTAT software (23).

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Results

Growth rate and colony diameter

On potato dextrose agar (PDA), the 26 selected isolates (Table 2) showed differences in colony diameter and growth rate (Table 3). On PDA, the growth rate ranged from 12.95 to 25 mm/48 hr. Isolates ranged from slow growers to very fast growers based on growth rate. Isolates FM 18, FM 59 and FM 40 exhibited the fastest growth rates, measuring 25.00, 20.91 and 20.47 mm/48 hr respectively. Isolate FM 31 exhibited the slowest growth rate (12.95 mm/48 hr), followed by FM 44 (15.97 mm/48 hr) and FM 20 (15.96 mm/48 hr). Isolate FM 18 recorded the maximum colony diameter (83.66 mm), followed by FM 40 (83.41 mm), FM 59 (82.96 mm) and FM 31 (55.82 mm). Additionally, the isolates were grouped according to their growth rates (Table 4). Isolate FM 31 was classified as a medium grower (10.1–14.99 mm/48 hr). Fast growers (15.00–19.99 mm/48 hr) included FM 3, FM 7, FM 10, FM 12, FM 16, FM 20, FM 25, FM 28, FM 34, FM 36, FM 44, FM 50, FM 51, FM 52, FM 53, FM 56, FM 60, FM 62, FM 63, FM 64 and FM 66. Isolates FM 18, FM 40 and FM 59 were classified as very fast growers (> 20.00 mm/48 hr).

Duncan Multiple Range test indicated that most of the isolates were significantly different (p = 0.05).

Biochemical variations in *Fusarium fujikuroi* isolates

Quantitative analysis revealed significant diversity in the accumulation of biochemical constituents across the evaluated isolates (Table 5, Fig. 3). To establish metabolic patterns, the isolates were classified into high- and low-performing categories based on their biochemical thresholds (Table 6).

The following biochemical parameters were assessed from the dried mycelium of the pathogen.

Table 2. List of selected isolates for growth rate and biochemical variability

Sl. No.	Isolates Id. No.	Villages (Districts)	Varieties
1	FM 3	Dhad (Hisar)	PB 1121
2	FM 7	Gurana (Hisar)	PB 1121
3	FM 10	Kheri Jalab (Hisar)	PB 1121
4	FM 12	Intal Khurd (Jind)	PB 1121
5	FM 16	Ikkas (Jind)	PB 1121
6	FM 18	Saniana 1 (Fatehabad)	PB 1121
7	FM 20	Saniana 2 (Fatehabad)	PB 1401
8	FM 25	Pirthala 1 (Fatehabad)	PB 1121
9	FM 28	Pirthala 2 (Fatehabad)	PB 1121
10	FM 31	Kungar (Bhiwani)	PB 1509
11	FM 34	Alakhpura (Bhiwani)	PB 1121
12	FM 36	Barsi (Bhiwani)	PB 1121
13	FM 37	Patli dabar (Sirsa)	PB 1401
14	FM 40	Mochi wali (Sirsa)	PB 1121
15	FM 44	Bajekan (Sirsa)	PB 1121
16	FM 50	Naiwala (Sirsa)	PB 1718
17	FM 51	Bapoli (Panipat)	PB 1509
18	FM 52	Noltha (Panipat)	PB 1718
19	FM 53	Ahar (Panipat)	PB 1718
20	FM 56	Sonipat	PB 1121
21	FM 59	Tarawari (Karnal)	PB 1121
22	FM 60	Sikri (Karnal)	PB 1718
23	FM 62	Kartarpur (Yamunanagar)	PB 1509
24	FM 63	Sandhala (Yamunanagar)	PB 1121
25	FM 64	Rasina (Kaithal)	Basmati 521
26	FM 66	Babain (Kurukshetra)	PB 1509

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Table 3. Variability in colony diameter and growth rate of *Fusarium moniliforme* on potato dextrose agar (PDA)

Isolates Id. No.	Colony diameter				Growth rate (mm/48 hr)			
	2nd day	4th day	6th day	8th day	4–2 day	6–4 day	8–6 day	Average growth rate
FM 3	20.75	40.00	62.42	79.74	19.25	22.42	17.32	19.66^{abc}
FM 7	21.10	36.55	54.11	71.00	15.45	17.56	16.89	16.63^{bc}
FM 10	22.11	41.25	59.47	74.79	19.14	18.22	15.32	17.56^{bc}
FM 12	22.12	38.24	59.00	78.19	16.12	20.76	19.19	18.69^{abc}
FM 16	18.65	37.19	51.30	67.28	18.54	14.11	15.98	16.21^{bc}
FM 18	8.67	32.99	56.54	83.66	24.32	23.55	27.12	25.00^{a}
FM 20	15.43	31.87	48.98	63.30	16.44	17.11	14.32	15.96^{bc}
FM 25	16.67	29.90	52.45	67.21	13.23	22.55	14.76	16.85^{bc}
FM 28	18.93	34.80	53.23	71.44	15.87	18.43	18.21	17.50^{bc}
FM 31	16.98	35.20	43.70	55.82	18.22	8.50	12.12	12.95^{c}
FM 34	17.54	32.66	53.99	72.54	15.12	21.33	18.55	18.33^{bc}
FM 36	18.76	34.54	53.93	70.04	15.78	19.39	16.11	17.09^{bc}
FM 37	16.76	33.19	57.41	75.97	16.43	24.22	18.56	19.74^{ab}
FM 40	22.00	44.11	68.54	83.41	22.11	24.43	14.87	20.47^{ab}
FM 44	20.42	33.17	52.09	68.32	12.75	18.92	16.23	15.97^{bc}
FM 50	18.83	35.59	55.88	71.64	16.76	20.29	15.76	17.60^{bc}
FM 51	19.76	37.20	56.41	74.98	17.44	19.21	18.57	18.41^{bc}
FM 52	19.92	38.58	56.56	71.68	18.66	17.98	15.12	17.25^{bc}
FM 53	22.20	43.65	62.88	80.32	21.45	19.23	17.44	19.37^{abc}
FM 56	18.54	36.88	55.10	70.49	18.34	18.22	15.39	17.32^{bc}
FM 59	20.23	40.66	64.20	82.96	20.43	23.54	18.76	20.91^{ab}
FM 60	17.98	36.42	55.74	72.72	18.44	19.32	16.98	18.25^{bc}
FM 62	20.42	38.17	59.12	77.55	17.75	20.95	18.43	19.04^{abc}
FM 63	21.45	41.65	64.97	80.40	20.20	23.32	15.43	19.65^{abc}
FM 64	8.80	37.23	45.77	58.99	28.43	8.54	13.22	16.73^{bc}
FM 66	19.45	33.81	51.30	67.29	14.36	17.49	16.29	16.05^{bc}
C.D. (p = 0.05)	-	-	-	-	-	-	-	**5.548**
SE(m)	-	-	-	-	-	-	-	**1.95**

*Data followed by the same letter (s) are not significantly different (p = 0.05) according to Duncan's multiple range test (DMRT)

C.D. (p = 0.05): critical difference at the 5 % significance level; SE(m): standard error of the mean.

Table 4. Grouping of the isolates on the basis of growth rate on potato dextrose agar (PDA)

Growth range	Groups	Isolates ID. No.
Less than 10 mm/48hr	Slow	**-----**
10.1–14.99 mm/48hr	Medium growers	FM 31
15–19.99 mm/48hr	Fast growers	FM 3, 7, 10, 12, 16, 20, 25, 28, 34, 36, 37, 44, 50 51, 52, 53, 56, 60, 62, 63, 64, 66
More than 20 mm/48hr	Very fast growers	FM 18, 40, 59

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Table 5. Variations in biochemical constituents of *Fusarium moniliforme* isolates

Biochemical constituents (mg/g of dry mycelium)				
Isolates	**Total sugar**	**Total phenol**	**Amino acids**	**Protein**
Blank	1.11^{l}	1.20^{n}	0^{i}	59.50^{j}
FM 3	11.11^{b}	7.79^{f}	53.40^{b}	85.75^{gh}
FM 7	11.09^{b}	7.80^{f}	53.43^{b}	84.67^{gh}
FM 10	9.17^{c}	7.51^{f}	32.46^{g}	85.75^{gh}
FM 12	6.89^{ef}	10.23^{c}	44.12^{de}	93.54^{f}
FM 16	5.89^{gh}	9.35^{d}	43.11^{e}	92.75^{f}
FM 18	7.83^{d}	5.96^{gh}	51.75^{b}	82.25^{h}
FM 20	11.49^{b}	11.98^{a}	30.87^{g}	106.75^{abcd}
FM 25	7.68^{d}	9.63^{cd}	26.31^{h}	110.25^{ab}
FM 28	6.78^{f}	8.65^{e}	25.67^{h}	109.54^{ab}
FM 31	4.54^{ij}	5.26^{hi}	26.75^{h}	104.78^{bcde}
FM 34	4.32^{ijk}	5.22^{hi}	26.60^{h}	103.25^{cde}
FM 36	6.41^{fg}	6.05^{g}	54.06^{b}	70.00^{i}
FM 37	7.57^{d}	4.21^{kl}	48.15^{c}	106.75^{abcd}
FM 40	5.74^{h}	4.12^{l}	51.45^{b}	112.00^{a}
FM 44	5.86^{gh}	4.25^{jkl}	52.45^{b}	111.23^{a}
FM 50	7.4^{de}	11.87^{ab}	46.35^{cd}	84.00^{gh}
FM 51	3.94^{jk}	5.02^{ij}	27.02^{h}	100.20^{e}
FM 52	3.93^{jk}	4.95^{ijk}	26.68^{h}	101.50^{de}
FM 53	3.77^{k}	4.86^{ijkl}	58.83^{a}	99.75^{e}
FM 56	3.80^{k}	11.92^{ab}	26.51^{h}	99.75^{e}
FM 59	4.81^{i}	3.21^{m}	26.72^{h}	99.75^{e}
FM 60	4.89^{i}	3.18^{m}	26.67^{h}	99.45^{e}
FM 62	5.96^{gh}	11.18^{b}	39.36^{f}	92.75^{f}
FM 63	12.08^{a}	4.38^{jkl}	48.36^{c}	89.25^{fg}
FM 64	11.34^{b}	11.21^{b}	28.05^{h}	105.00^{bcde}
FM 66	7.68^{d}	7.79^{f}	40.08^{f}	108.50^{abc}
C.D. (p = 0.05)	**0.060**	**0.058**	**0.363**	**0.753**
C.V. (%)	**0.53**	**0.49**	**0.57**	**0.47**
SE(m)	**0.0213**	**0.0205**	**0.1277**	**0.26**

*Data followed by the same letter (s) are not significantly different (p = 0.05) according to Duncan's multiple range test (DMRT)

C.D. (p = 0.05): critical difference at the 5 % significance level; SE(m): standard error of the mean; C.V. (%): Coefficient of variation (percentage).

Total Sugar

Total sugar content varied widely from 3.77 mg/g to 12.08 mg/g of dry mycelium. Isolate FM 63 synthesised the maximum total sugar level (12.08 mg/g), followed closely by FM 20 (11.49 mg/g) and FM 64 (11.34 mg/g). Conversely, the lowest sugar values were detected in isolates FM 53 (3.77 mg/g) and FM 56 (3.80 mg/g). The majority of the isolates (21 out of 26) accumulated low levels of sugar (< 10 mg/g). Isolates FM 3, FM 7, FM 20, FM 63 and FM 64 were classified as high-sugar accumulators, with sugar levels exceeding 10 mg/g (Table 6).

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Table 6. Grouping of different isolates based on biochemical constituent composition

Biochemical constituents mg/g dry mycelium							
Total sugars		Total phenols		Free amino acids		Protein content	
Low (< 10)	High (> 10)	Low (< 11.50)	High (> 11.50)	Low (< 50)	High (> 50)	Low (< 107)	High (> 107)
FM 10, 12, 16, 18, 25, 28, 31, 34, 36, 37, 40, 44, 50, 51, 52, 53, 56, 59, 60, 62, 66	FM 3, 7, 20, 63, 64	FM 3, 7,10, 12, 16, 18, 25, 28, 31, 34, 36, 37, 40, 44, 51, 52, 53, 59, 60, 62, 63, 64,66	FM 20, 50, 56	FM 10,12,16, 20, 25, 28, 31, 34, 37, 50, 51, 52, 56, 59, 60, 62, 63, 64, 66,	FM 3, 7, 18, 36, 40, 44, 53	FM 3, 7, 10, 12, 16, 18, 20 31, 34, 36, 37, 50, 51, 52, 53, 56, 59, 60, 62, 63, 64	FM 25, 28, 40, 44, 66

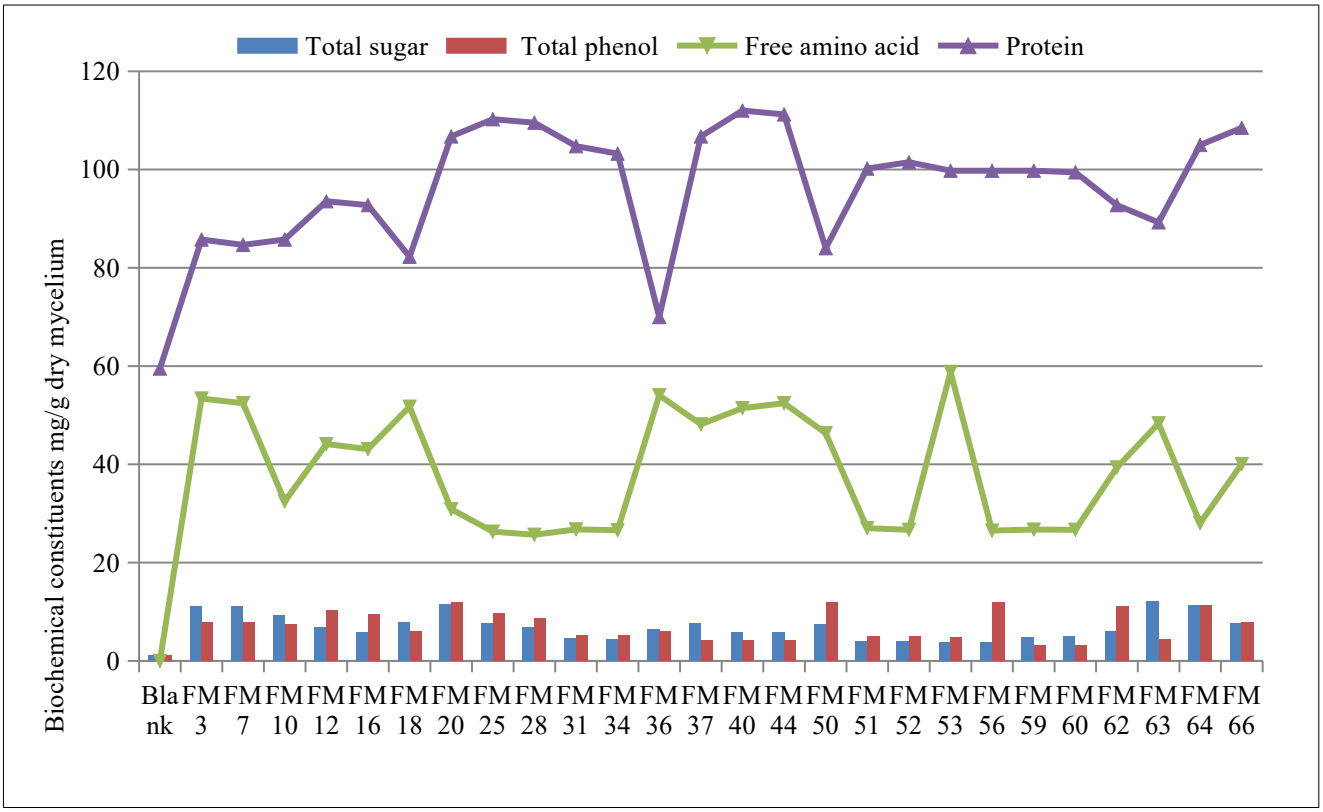


Fig. 3. Variability on the basis of biochemical constituents among different isolates of *Fusarium moniliforme*.

Total phenol

The accumulation of total phenolic compounds ranged between 3.18 mg/g and 11.98 mg/g of dry mycelium. The highest phenolic contents were tightly clustered in isolates FM 20 (11.98 mg/g), FM 56 (11.92 mg/g) and FM 50 (11.87 mg/g), placing them in the high-phenol group (> 11.50 mg/g) (Table 6). The baseline minimum was observed in isolate FM 60 (3.18 mg/g).

Free amino acids

Free amino acid profiles displayed a more than two-fold variation, spanning from 26.31 mg/g to 58.83 mg/g. Isolate FM 53 yielded the peak amino acid concentration (58.83 mg/g), followed by FM 36 (54.06 mg/g) and FM 7 (53.43 mg/g). The lowest concentrations were confined to a group led by FM 25 (26.31 mg/g), FM 56 (26.51 mg/g) and FM 34 (26.60 mg/g). Seven isolates reached the high amino acid classification (> 50mg/g) (Table 6).

Protein

Mycelial protein concentrations ranged from a minimum of 70.00 mg/g to a maximum of 112.00 mg/g. Peak protein accumulation was concentrated in the high-protein cluster (> 107mg/g), consisting of isolates FM 40 (112.00 mg/g), FM 44 (111.23 mg/g) and FM 25 (110.25 mg/g) (Table 6). Isolate FM 36 registered the lowest absolute protein value (70.00 mg/g).

Discussion

Several researchers have reported variability based on cultural and morphological characteristics for differentiating *Fusarium fujikuroi* isolates. However, information on biochemical variability of the pathogen is lacking, so the present study was conducted to differentiate the isolates on the basis of biochemical constituents and growth rate.

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At first, 64 bakanae-infected samples were gathered from various Haryana districts. Twenty-six isolates were chosen for evaluation of growth rate and biochemical variability. The current study found that 26 isolates from the state of Haryana's primary aromatic rice-growing regions varied significantly in terms of growth rate and biochemical components. On potato dextrose agar (PDA), the isolates' colony diameter and growth rate varied. On PDA, the growth rate fluctuated between 12.95 and 25 mm/48 hr. Isolates ranged from slow growers to very fast growers based on growth rate. FM 18, FM 59 and FM 40 all displayed very fast growth, measuring 25.00, 20.91 and 20.47 mm respectively. In contrast, FM 31 exhibited the slowest growth rate, at 12.95 mm/48 hr.

Isolate FM 18 recorded the maximum colony diameter (83.66 mm), whereas FM 31 recorded the minimum (55.82 mm). Different isolates exhibited distinct differences in the biochemical contents (mg/g of dry mycelium). The range of total sugars was 3.77 (FM 53) to 12.08 (FM 63), with FM 63 having the highest total sugar content (12.08 mg). The range of total phenols was 3.18 (FM 60) to 11.98 (FM 20). The greatest concentrations of total phenols were found in FM 20 and FM 56, at 11.98 and 11.92 mg respectively. In the present study**,** the free amino acid concentration ranged from 26.31 (FM 25) to 58.83 (FM 53), with isolate FM 53 having the greatest amino acid content (58.83 mg).

Protein content ranged from 70 mg (FM 36) to 112 mg (FM 40), with isolate FM 40 having the greatest protein level (112 mg). In general, isolates were classified into two groups (low and high) based on their biochemical components. Additionally, very little is known about the metabolic characteristics of this disease.

The significant variations in biochemical constituents among the 26 *F. fujikuroi* (2) isolates reveal distinct metabolic survival strategies within the local population. For instance, elevated sugar reserves in isolates like FM 63 and FM 20 can serve as primary metabolic sinks that accelerate spore germination and fuel rapid biomass accumulation. Conversely, the high accumulation of total phenols in FM 20 and FM 56 likely provides critical protection against host-induced oxidative stress. Interestingly, a physiological trade-off exists as the fastest-growing isolates (FM 18 and FM 40) maintain low internal sugar and phenolic reserves, indicating a clear metabolic preference for rapid resource turnover and immediate vegetative expansion over secondary storage.

Nitrogen utilisation profiles reinforce these unique physiological adaptations. The hyper-accumulation of free amino acids in FM 53 provides an immediate reservoir of mobile nitrogen blocks to quickly drive protein synthesis and enzymatic pathogenetic pathways during host tissue invasion. On the other hand, peak mycelial protein concentrations in fast-growing isolates like FM 40 point to a structural strategy where nitrogen resources are immediately optimised into functional cell machinery and pathogenetic proteins to maximise tissue colonisation.

These variations have vital practical implications for managing bakanae disease in Haryana. Because of this high metabolic heterogeneity, resistance breeding programs cannot rely on screening germplasm against a single standard strain; varieties must be tested against multiple biochemical groups (e.g., high-protein vs. fast-growing strains) to ensure broad, stable resistance. Furthermore, agricultural interventions can be tailored to disrupt these metabolic balances during early nursery growth phases using targeted nutrients or systemic resistance elicitors.

Similarly, many researchers have studied biochemical variability; for example, variations in the biochemical components of various *Neovossia indica* isolates were reported (24). Biochemical heterogeneity between various *Sclerotium oryzae* isolates was also documented (25). Biochemical diversity in the *Ascochyta rabiei* pathogen was also evaluated (26). In a previous study, wild *Agaricus* spp. demonstrated high chemical composition. The *Agaricus qilianensis* BR47 showed the highest protein content among the studied strains (29.52 %). *Agaricus bispours* BR9 was marked by a high phenol content (1.93 $mg.g^{-1}$ of dry weight) (27). In a previous study, it was found that *Fusarium oxysporum* and *F. moniliforme* contained approximately 30 % more protein and total amino acids than *Aspergillus niger* (28). The production of various biochemical compounds such as proteins, glucans and glucanases, from the mycelium of 4 strains of Basidiomycetes species, *Agaricus bisporus*, *Agaricus subrufescens*, *Pleurotus eryngii* and *Pleurotus ostreatus*, during batch culture in shaking flasks, was studied. All strains presented a similar pattern of total protein production, with *A. bisporus* exhibiting the highest percentage of total protein (29).

Conclusion

This study successfully demonstrates significant vegetative growth rates and biochemical heterogeneity among 26 *Fusarium fujikuroi* isolates collected from major aromatic rice-growing tracts of Haryana. The primary findings reveal that radial growth velocities vary widely from 12.95 to 25.00 mm/48 hr on potato dextrose agar, establishing a distinct physiological gradient from medium to very fast growers, led by the hyper-extended isolate FM 18. Furthermore, extensive metabolic diversity was quantified across all key fungal constituents, with total sugars spanning 3.77 to 12.08 mg/g, total phenols ranging from 3.18 to 11.98 mg/g, free amino acids varying between 26.31 and 58.83 mg/g and soluble proteins distributed across 70.00 to 112.00 mg/g of dry mycelium. An apparent physiological trade-off was observed among the top performers, where the fastest-growing pathotypes (FM 18 and FM 40) maintain low internal sugar reserves and reduced phenolic baselines, signalling rapid metabolic turnover and a preference for directing cellular assets toward immediate hyphal expansion rather than

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secondary storage or pigmentation pathways. Ultimately, mapping these diverse physiological and metabolic profiles provides an essential baseline for understanding the pathogen's natural variations, serving as a critical reference for future epidemiological profiling and targeted localised disease management programs.

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Authors' contributions

VS and AK conceptualised the study. VS, AK and PK prepared the original draft and performed formal analysis. VS handled data curation. VS, AK, PK, MSB and NR developed the methodology and contributed to writing, review and editing the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest

Authors do not have any conflict of interests to declare.

Ethical issues

None

References

1. Sunani SK, Bashyal BM, Rawat K, Manjunatha C, Sharma S, Prakash G, et al. Development of PCR and loop mediated isothermal amplification assay for the detection of bakanae pathogen *Fusarium fujikuroi*. Eur J Plant Pathol. 2019;154:715–25. https://doi.org/10.1007/s10658-019-01694-2
2. Hori S. Some observations on bakanae disease of the rice plant. Mem Agric Res Sta (Tokyo). 1898;12:110–19.
3. Ito S, Kimura J. Studies on the bakanae disease of the rice plant. Rep Hokkaido Natl Agric Exp Stn. 1931;27:1–95.
4. Thomas KM. A new paddy disease in Madras. Madras Agric J. 1931;19:34–6.
5. Piombo E, Bosio P, Acquadro A, Abbruscato P, Spadaro D. Different phenotypes, similar genomes: three newly sequenced *Fusarium fujikuroi* strains induce different symptoms in rice depending on temperature. Phytopathology. 2020;110:656–65. https://doi.org/10.1094/PHYTO-09-19-0359-R
6. Peng Q, Waqas Younas M, Yang J, Zhu H, Miao J, Gu B, et al. Characterisation of prochloraz resistance in *Fusarium fujikuroi* from Heilongjiang Province in China. Plant Dis. 2022;106:418–24. https://doi.org/10.1094/PDIS-02-21-0372-RE
7. Chen CY, Chen SY, Liu CW, Wu DH, Kuo CC, Lin CC, et al. Invasion and colonisation pattern of *Fusarium fujikuroi* in rice. Phytopathology. 2020;110:1934–45. https://doi.org/10.1094/PHYTO-03-20-0068-R
8. Tadasanahaller PS, Bashyal BM, Yadav J, Krishnan Subbaiyan G, Ellur RK, Aggarwal R. Identification and characterisation of *Fusarium fujikuroi* pathotypes responsible for an emerging bakanae disease of rice in India. Plants. 2023;12:1303. https://doi.org/10.3390/plants12061303
9. Sun SK, Snyder WC. The bakanae disease of the rice plant. In: Nelson PE, Toussoun TA, Cook RJ, editors. Fusarium diseases, biology and taxonomy. University Park (PA): The Pennsylvania State University Press; 1981. p. 104–13.
10. Ou SH. Bakanae disease and foot rot. Rice diseases survey. Kew: Commonwealth Mycological Institute; 1985. p. 262–72.
11. Webster RK, Gunnell PS. Compendium of Rice Diseases. St. Paul (MN): APS Press; 1992. p. 62.
12. Desjardins AE, Manandhar HK, Plattner RD, Manandhar GG, Poling SM, Maragos CM. *Fusarium* species from Nepalese rice and production of mycotoxins and gibberellic acid by selected species. Appl Environ Microbiol. 2000;66(3):1020–25. https://doi.org/10.1128/AEM.66.3.1020-1025.2000
13. Cumagun CJR, Arcillas E, Gergon E. UP-PCR analysis of the seedborne pathogen *Fusarium fujikuroi* causing bakanae disease in rice. Int J Agric Biol. 2011;13(6):1029–32.
14. Singh R, Sunder S. Foot rot and bakanae of rice: an overview. Rev Plant Pathol. 2012;5:566–604.
15. Singh V, Kumar A, Kapoor P, Bochalya MS. Identification of *Fusarium* species associated with bakanae disease of Basmati rice by using molecular marker. Biol Forum. 2022;14(4):193–97. https://doi.org/10.56093/ijas.v95i6.137127
16. Singh V, Kumar A, Kapoor P, Bochalya MS, Kumar R. Cultural, morphological and molecular variability in *Fusarium moniliforme* isolates causing bakanae disease of aromatic rice in Haryana, India. Indian J Agric Sci. 2025;95(5):497–502. https://doi.org/10.56093/ijas.v95i6.137127
17. Puyam A, Pannu PPS, Kaur J, Sethi S. Cultural, morphological and molecular variability of *Fusarium moniliforme* Sheld. causing foot rot disease of basmati rice in Punjab. J Mycol Plant Pathol. 2017;47(4):369–81.
18. Singh V, Kumar A, Kapoor P, Bochalya MS. Comparative assessment of different isolates of *Fusarium moniliforme* Sheldon causing bakanae disease of aromatic rice using Beta-tubulin gene by PCR analysis. Environ Ecol. 2023;41(1B):513–18.
19. Marbach L, Mayer AM. Respiration and utilisation of storage materials in wild and cultivated pea seeds during germination. Plant Physiol. 1976;38:126–30. https://doi.org/10.1111/j.1399-3054.1976.tb04871.x
20. Yemm EW, Cocking EC. The determination of amino acids with ninhydrin. Anal Chem. 1955;80:203–13. https://doi.org/10.1039/an9558000209
21. Swain T, Hills EW. Phenolic constituents of *Prunus domestica* L. Quantitative analysis of phenolic constituents. J Sci Food Agric. 1959;10:63–8. https://doi.org/10.1002/jsfa.2740100110
22. Dubois M, Gills KA, Hamilton JK, Rabarg PA, Smith F. Colourimetric methods for determination of sugars and related substances. Anal Chem. 1958;28:350–56. https://doi.org/10.1021/ac60111a017
23. Sheoran OP. Statistical package for agricultural research workers. Hisar: CCS Haryana Agricultural University; 2004.

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https://doi.org/10.14719/pst.16216

24. Sharma SK, Singh DV, Aggarwal R, Srivastava KD. Pathogenic and biochemical variations in Neovossia indica. Indian Phytopathol. 2002;56:133–39.

25. Kumar A, Singh R, Jalali BL. Variability in growth rate and biochemical components of *Sclerotium oryzae* populations of rice and its relevance to virulence. J Mycol Plant Pathol. 2011;41(1):143–45.

26. Sangwan MS, Jalali BL, Nene YL. Biochemical variability in chickpea blight pathogen *Ascochyta rabiei* races. Indian Phytopathol. 1990;43:285.

27. Hola B, Murshed R, Jbour M. Chemical composition and antioxidant activity of some Syrian wild mushroom (*Agaricus* spp.) strains. Sci Rep. 2023;13(1):15896. https://doi.org/10.1038/s41598-023-43265-w

28. Christias C, Couvaraki C, Georgopoulos SG, Macris B, Vomvoyanni V. Protein content and amino acid composition of certain fungi evaluated for microbial protein production. Appl Microbiol. 1975;29(2):250–54. https://doi.org/10.1128/am.29.2.250-254.1975

29. Argyropoulos D, Psallida C, Sitareniou P, Flemetakis E, Diamantopoulou P. Biochemical evaluation of *Agaricus* and *Pleurotus* strains in batch cultures for production optimisation of valuable metabolites. Microorganisms. 2022;10(5):964. https://doi.org/10.3390/microorganisms10050964

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