Genetics & Biodiversity Journal

Journal homepage: https://journals.univ tlemcen.dz/GABJ/index.php/GABJ

ISSN: 2588-185X

OPEN ACCESS

Original Research Paper

Identification and pathogenicity of *Fusarium spp*. isolated from root and crown of rice in Golestan province

ZINATI FAKHRABAD F*[1], SALARI M[1], TALIYE F[1], PANJEKEH N[2], GAOUAR YADI M.[3] and SABBAGH S K[4].

[1.] *Department of Plant Protection, Faculty of Agriculture, University of Zabol, Iran.*
[2]. *Department of Plant Production, Gonbad Kavoos University.*
[3] *Department of Biology, Ecology and pathology of ecosystems laboratory. Tlemcen University, Algeria.*
[4]. *Department of Biology, Campus of Sciences. Yazd University, Iran.*

Corresponding author: Zinati fakhrabad, Department of Plant Protection, Faculty of Agriculture, University of Zabol, Iran. Email: zinati.fateme@gmail.com
Article history: Received: April 25th 2023, revised: October 31st 2024 Accepted: March 2nd 2024.

Abstract

In order to identify *Fusarium spp*. associated with root and crown's rot of rice, several fields from different regions of Golestan province were sampled during growing season (2015-2018). especially, samples with yellowing and abnormal elongation, abnormal growth, decay root and foot. For isolation of fungi, discolored segments (root and crown) were surface disinfected with 0.5% NaOCl. Then cultured on Potato Dextrose Agar with pH. For exact observation some characteristics of species were used from Carnation Leaf Agar medium. Among the 30 *Fusarium* isolates, 6 species were identified as: *Fusarium moniliforme*, F. fujikouri, F.verticiloides* *, F. *proliferatum*, F. concolor*, F.sacchari.* on the basis of their morphological characteristics. Literature data showed that the Bakanae disease of rice all over the world is caused by *F. moniliform* and probably some other *Fusarium* species from section Liseola or allied. To investigate the possibility of using a part of bases TEF gene for differentiation of *Fusarium* species, some parts were amplified using two primers ef1 and ef2. The equivalent size was amplified of 700 bp However, from pathogenicity tests that have been carried out by using variety Khazar of rice it was evident that *F.moniliform and F.proliferatum* was highly virulent and the only species involved in causing Bakanae disease.
Keyword: Bakanae disease, *Fusarium spp*. morphology identification

Introduction

The *Fusrium* genus is hyphomycetes soil born fungi which includes many species that has high economic importance in plant diseases and has a wide range of host plants, such as tomatoes, potatoes, crops, clove and grasses such as wheat, barley, oats, maize and sugarcane many of the infected vegetables and other plants (Nelson et al., 1983, Burgess et al., 1994). Species of this genus have a wide distribution in all regions of the world and many of its species such as chlamydospores in soil texture or passive and active mycelium in host debris and organic material present (Burgess et al., 1994, Summerell et al., 2003). *Fusarium* species pathogenic on plants and products, in addition they are the main cause of pollution mycotoxicoses in humans and other animals (Monds et al., 2005, Nelson et al., 1993). Some *Fusarium* species such as *Fusarium verticillioides* (= *F. moniliforme*) to cause seedling blight, root and stalk rot of corn and corn products decreased on average by 8-4% percent on year

Golestan province has the largest production area (13000 ha in 2015) of rice in IRAN. Several fungal diseases affect this crop; among these, *Fusarium* species are the agents of many different plant and seed diseases, such as bakanae. The most evident symptom of this disease is yellowing and abnormal elongation of infected seedlings, the result of gibberellic acid production; this led to the Japanese name bakanae, meaning 'foolish seedling'. In older plants, the roots, crowns, stems, leaf sheaths and panicles can be infected. The disease is seedborne and primarily seed transmitted (Desjardins et al., 1997). Although bakanae disease was first described over 100 years ago in Japan, it is still not clear which *Fusarium* species are associated with the different symptoms. Early work in Japan identified the pathogen as *Fusarium*

moniliforme in a broad sense (Ou, 1985); however, this taxon comprises a number of distinct species, now collectively termed the *Gibberella fujikuroi* species complex. The presence of the Gibberella sexual stage can distinguish mating populations, or biological species, within this group (Leslie, 1995). Three mating populations of the *G. fujikuroi* complex have been associated with bakanae-diseased rice. Mating population C (MP-C) (anamorph *Fusarium fujikuroi*) was first identified in 1977 among isolates from Taiwan rice. MP-A (anamorph *Fusarium verticillioides*) and MP-D (anamorph *Fusarium proliferatum*) were isolated from rice in Asia, and MP-D was also isolated from rice from Africa, Australia and the USA (Voigt et al., 1995; Desjardins et al., 1997). The sexual stages of these pathogens are named *G. fujikuroi*, *G. intermedia* and *G. moniliforme*, respectively. *Fusarium* species within this complex are also able to produce mycotoxins that can affect human and animal health (Leslie et al., 1992). In particular *F. fujikuroi* can produce moniliformin, beauvericin and fumonisins, *F. verticillioides* can produce fumonisins, and *F. proliferatum* is able to synthesize fumonisins, beauvericin and fusaproliferin. Recent work has revealed great diversity among *Fusarium* species, underestimated by earlier morphological criteria**.**). In these circumstances, it is better to identify regions with high phylogenetic utility. The translation elongation factor 1-a gene (EF-1a gene), which encodes an essential of the protein translation machinery, is a good single-locus identification tool in *Fusarium* because it shows high sequence polymorphism among closely related species, even compared to the intron-rich portions of protein-coding genes such as calmodulin, betatubulin and histone H3, and non-orthologous copies of the gene have not been detected in the genus.

Materials and methods

In order to identify *Fusarium spp*. associated with root and crown rot of rice, several fields in different regions of Golestan province were sampled during growing season (2015-2018). Samples with yellowing and abnormal elongation, abnormal growth, decay root and foot, death plant were collected. For isolation of fungi, discolored segments (root and crown) were surface disinfected with 0.5% NaOCl. Then cultured on Potato Dextrose Agar with pH. For exact observation some characteristics of species were used from Carnation Leaf Agar medium.

Culture media

A total of five selective and non-selective type of cultural media viz. Potato Dextrose Agar (PDA), Czapek Dox Agar (CDA) modified and Carnation Leaf Agar were used.

In this method, three layers of white blotter discs of 10 cm diameter were first jointly soaked in distilled water and sterilized at 15 p.s.i. for 20 minutes and kept in plastic petri-plates of 10 cm diameter. Seeds suspected to be infected were surface sterilized by dipping in 1% sodium hypochlorite solution or 0.1 % mercuric chloride solution for 30 seconds and subsequently rinsing three times in sterilized distilled water. Four hundred seeds from each sample were placed on three layers of moist blotters in 16 plastic Petri plates (25 seeds in each plate, 1 in the center, 9 in the inner circle, and 15 in the outer circle). In case of exotic samples, seed size was dependent on the quantity of seed in each sample. These plates were then incubated for 7 days at 20 ± 1°C under alternating cycles of 12-h light (Philips TL 40-W, florescent day light tubes hanging 20 cm apart from each other and at a distance of 41 cm from the incubation tables) and 12-h darkness. The incubated seeds were examined on the 8th day of incubation under stereo-binocular microscope at 6 to 50x magnification for seed borne fungi. A compound microscope was also used for confirmation of the identification.

Isolation of the pathogen

Diseased rice ears were collected from different paddy fields in Golestan provinces in the North of Iran in summers of 2015- 2017. Five units of rice of each sample were surface sterilized with a 1% sodium hypochlorite solution for 1 min, rinsed twice in sterile distilled water and dried on sterile Whatman paper in a laminar flow cabinet. All sterilized samples were placed onto the selective medium peptone pentachloro nitrobenzene agar (PPA) (Nash & Synder 1962) and incubated at 25°C for 7 days. All *Fusarium* isolates were subcultured on Potato Dextrose Agar (PDA) (Merck, Darmstadt, Germany) and Carnation-leaf Agar (CLA) (Fisher et al. 1982), using a single spore technique (Leslie &Summerell 2006). PDA cultures were incubated at 25°C, and CLA and SNA cultures were incubated at 25°C with on/off black light (20W-220V)

cycles of 12 hours each for 2-4 weeks. Cultural characteristics were assessed visually and microscopically. Colony morphology (color, texture, sporodochia and growth rate) was recorded from cultures grown on PDA. The morphology of macroconidia, microconidia, conidio-genous cells and the chlamydospores was assessed from cultures grown on SNA and CLA. Morphological identification of isolates was made according to Nelson et al. (1983) and Leslie and Summerell (2006).

The roots of infected plant (showing elongation symptoms) were cut in small pieces and surface sterilized with 0.2 percent sodium hypochlorite solution for 1 min, rinsed twice with distilled water, dried and plated on potato dextrose agar medium. The plates were incubated at 28ºC for one week. The isolated target fungus was identified according to key provided by Booth (1971) and Nelson *et al.* (1993).

Fungal culture and DNA preparation and PCR

Liquid culture was initiated by adding 5 mm mycelial discs from the growing edge of single conidial colonies grown on PDA to 250 mm Erlenmeyer flasks, containing 50 ml of PDB (Potato Dextrose Broth) medium, and then were incubated at 28°C on an orbital shaker for two weeks. Mycelia from cultures were collected by vacuum filtration, washed by sterile distilled water, lyophilized, and finally ground in liquid nitrogen. Total genomic DNA was extracted from the powdered sample of mycelium, by using a modified Cetyl Trimethyl Ammonium Bromide (CTAB) procedure by Von Korf et al (2004). The DNA was resuspended in 50 µl of TE buffer and stored at -20°C. Species-specific PCR amplifications were carried out using primers EF1 (5'-ATGGGTAAGGAR(C/G)GACAAGAC-3') and EF2 (5'-GGARGTACCAGTS(T/A)ATCATG-3') and EF22 (5'-AGGAACCCTTACCGAGCTC-3') for detection of *Fusarium spp.* PCR reactions for both primer pairs performed in a final volume of 20 µl consisting of 1 PCR buffer, 0.5 µM primer, 0.2 mM of each deoxynucleotide triphosphate (dNTPs), 2.5 mM magnesium chloride (MgCl2), 0.125 U Go*Taq* DNA Polymerase, nuclease free water and 20 ng DNA template. Tubes in the PCR (Master Cycler Eppendorf Germany) was used and the reaction program consisted of 4 min at 94 ° C, 35 cycles of 35 seconds at 94 ° C, 55 seconds at 52 ° C, 2 min at 72 ° C and final 10 min at 72 ° C was performed. Gel electrophoresis was performed by using 1.5% agarose gel and immersed in 1X Tris Borate-acid EDTA (TBE) buffer amended with FloroSafe DNA stain according to manufacturer's instructions (1st BASE, Asia). Approximately 5 µL for each DNA ladder 100 bp (Thermo Scientific) and PCR products were loaded and electrophoresed for 35 min at 90 V. The gel was viewed and analysed using Syngene software by a gel documentation system under UV light (Syngene, Germany).

Pathogenicity test

Pathogenicity tests were conducted in seed inoculation assays. The susceptible rice cultivar Khazar was used to assess the pathogenicity. Seeds were surface disinfected by immersion in 70 percent ethanol for 1 min, transferred to 1 percent sodium hypochlorite for 3 min and rinsed three times consecutively in sterile distilled water. Seeds were then left to dry in the flow cabinet in Petri dishes containing sterile filter paper. Fungal inoculum suspension was prepared from 15 days old plates of *F. fujikuroi*, flooded with sterile water and scraped with a sterile spatula. The resulting suspensions were filtered through two layers of sterile cotton lint and brought to a final concentration of 10^6 spores mL-1 in sterile distilled water. Thirty rice seeds were soaked in 10 ml of inoculum suspension for 18 hrs at room temperature. Control seeds were soaked in sterile water. Inoculated and control seeds were sown in pots (three pots per isolate/ten seeds per pot) containing autoclaved mixture of soil and sand in the ratio of 3:1. Fifteen days after inoculation, the number of germinated seeds was assessed.

Methodology

Samples with yellowing and abnormal elongation, abnormal growth, decay root and foot were collected (Figure1).

Present studies were undertaken to investigate the variability in *Fusarium spp.* isolated from the germplasm. Isolates from the Iranian Type Culture Collectiom were also included for comparison.

In order to characterize the 30 isolates of Bakanae disease, various morphological and cultural studies were conducted and results are presented in Table 2. The morphological characteristic of all the isolates like colony colour, colony texture, pigmentation, zonation, saltation and sporulation were recorded after 7 days of incubation at 20 ± 1°C on PDA (Figure 2). Colony characteristics on media have always been used as a method to differentiate the isolates/races of the fungal pathogen. Initially the colonies of all the isolates of Bakanae disease appeared bright white in colour but subsequently changes took place.

Figure 1: Bakanae symptoms disease in fileds Golestan province.

Figure2. Morphological identification characteristics of *Fusarium* spp. a) chlamidospor b) phiyahid c) continuum conidia

All test isolates were categorized into six major species on the basis of morphological characteristics (Figure 3).

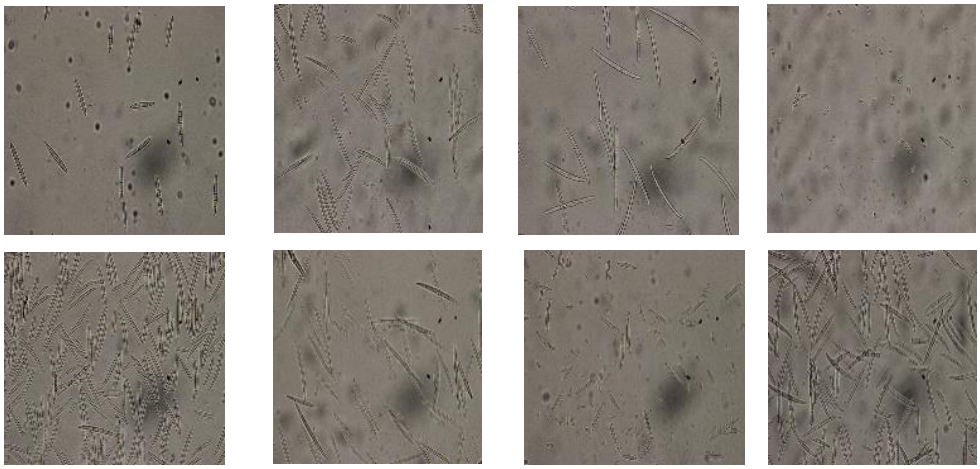

Figure 3. Produced macrocinidia and microconidia on Carnation-leaf Agar medium

Results

After a week of inoculation color of colony was pinkish for 2 isolates (namely S1 and S26) and violet for 8 isolates (i.e. S3, S5, S6, S15, S21, S25, S29) and white 18 isolates (i.e. S2, S4, S7, S8, S9, S13, S14, S16, S18, S19, S20, S22, S23, S24, S27, S28 and S30) while yellowish white in case of one isolates S11.

Detailed morphology texture of the isolates on PDA revealed that in isolates, the compact net mycelium was well developed. In all isolates showed compact net mycelium, condensedraised, floccose or undulated colony texture on PDA. Detailed pigmentation of the isolates revealed that out of 30 isolates showed three different pigmentations viz. purple, yellow and orange, respectively.

Table 1. symptoms and location adding bakanae isolates.

Species	Cultivare	Sympotum	Growth Stage	Location	Name isolate	S.No
_	Domsiyah	Abnormal elongation	tillering	Galikesh	s1	1
_	Domsiyah	Abnormal elongation	tillering	Galikesh	s2	2
F. moniliforme	Domsiyah	Abnormal elongation	tillering	Yanghagh	s3	3
F.proliferatum	Domsiyah	Abnormal elongation	tillering	Yanghagh	s4	4
F. moniliforme	Domsiyah	Abnormal elongation	tillering	Esmailabad	s5	5
F. moniliforme	Domsiyah	Death plant	booting	Sarje	s6	6
F. moniliforme	Domsiyah	Death plant	booting	Sarje	s7	7
F.fujikuri	Domsiyah	Abnormal elongation	booting	Azadshahr	s8	8
F.fujikuri	Fajr	Abnormal elongation	tillering	Azadshahr	s9	9
F.fujikuri	Domsiyah	Death plant	tillering	Azadshahr	s10	10
F.fujikuri	Domsiyah	Death plant	tillering	Azadshahr	s11	11
F.fujikuri	Domsiyah	Abnormal elongation	emergance	Azadshahr	s12	12
F.proliferatum	Domsiyah	Abnormal elongation	tillering	Kalale	s13	13
F.proliferatum	Domsiyah	Death plant	tillering	Kalale	s14	14
F.moniliforme	Hashemi	Abnormal elongation	booting	Kordkoye	s15	15
Fusarium Sacchari	Domsiyah	Abnormal elongation	tillering	Aliabad	s16	16
F. moniliforme	Domsiyah	Abnormal elongation	tillering	Aliabad	s17	17
F. moniliforme	Domsiyah	Death plant	booting	Aliabad	s18	18
F. moniliforme	Fajr	Death plant	booting	Aliabad	s19	19
F.proliferatum	Domsiyah	Abnormal elongation	tillering	Ramiyan	s20	20
F.proliferatum	Domsiyah	Abnormal elongation	tillering	Ramiyan	s21	21
F.concolor	Domsiyah	Death plant	tillering	Ghareghach	s22	22
F. moniliforme	Domsiyah	Death plant	tillering	Ghareghach	s23	23
F.proliferatum	Domsiyah	Death plant	Grain filling	Gorgan	s24	24
F.concolor	Fajr	Death plant	Grain filling	Gorgan	s25	25
F.fujikuri	Fajr	Death plant	tillering	bandargaz	s26	26
F.verticiloides	Fajr	Abnormal elongation	booting	Bandargaz	s27	27
F. moniliforme	Fajr	Death plant	tillering	Agheghala	s28	28
_	Fajr	Death plant	tillering	Agheghala	s29	29
F. moniliforme	Domsiyah	Death plant	tillering	Agheghala	s30	30

In order to characterize the 30 isolates of Bakanae disease, various morphological and cultural studies were conducted and results are presented in Table 2. The morphological characteristic of all the isolates like colony colour, colony texture, pigmentation, and sporulation were recorded after 7 days of incubation at 20 ± 1°C on PDA. Colony characteristics on media have always been used as a method to differentiate the isolates/races of the fungal pathogen. Initially the colonies of all the isolates of *Fusarium spp* appeared bright white in colour but subsequently changes took place. All test isolates were categorized into six major species on the basis of colony characters (colony colour ,texture and…), however, summarized *Fusarium spp.* isolates grouping based on morphological characteristics.

Table 2. morphological and characterization Fusarium spp. Isolates

phiyalid	Continuum conidi	macroconidi	microconidi	clamidospore	Growth rate after 5 day	Back colony color	On colony color	Species	Isolate name	S.No
					1.5	pinkish	white	_	s1	1
					2.1	white	white	_	s2	2
		*	*		1.6	violet	violet	***F. moniliforme***	s3	3
			*		1.5	white	white	***F.proliferatum***	s4	4
		*	*		1.5	violet	violet	***F. moniliforme***	s5	5
			*		1.8	violet	violet	***F. moniliforme***	s6	6
			*		1.3	white	white	***F. moniliforme***	s7	7
			*	*	1.6	white	white	***F.fujikuri***	s8	8
		*	*	*	1.6	white	white	***F.fujikuri***	s9	9
				*				***F.fujikuri***	s10	10
*		*	*	*	2.7	yellow	white	***F.fujikuri***	s11	11
*			*	*	2			***F.fujikuri***	s12	12
			*	*	1.6	white	white	***F.proliferatum***	s13	13
			*	*	1.7	white	white	***F.proliferatum***	s14	14
		*	*		1.7	violet	violet	***F.moniliforme***	s15	15
		*	*	*	1.2	white	white	***Fusarium Sacchari***	s16	16
			*		1.8	white	white	***F. moniliforme***	s17	17
		*	*		1.7	white	white	***F. moniliforme***	s18	18
			*		1.6	white	white	***F. moniliforme***	s19	19
				*	1.6	white	white	***F.proliferatum***	s20	20
			*		1.5	violet	violet	***F.proliferatum***	s21	21
		*	*		1.6	white	white	***F.concolor***	s22	22
			*	*	1.5	white	white	***F. moniliforme***	s23	23
	*	*	*	*	1.9	white	white	***F.proliferatum***	s24	24
		*	*	*	1.7	violet	violet	***F.concolor***	s25	25
			*		2.2	pinkish	white	***F.fujikuri***	s26	26
*		*	*	*	2.8	white	white	***F.verticiloides***	s27	27
	*		*		2	white	white	***F. moniliforme***	s28	28
					1.9	violet	violet	_	s29	29
			*		1.8	white	white	***F. moniliforme***	s30	30

To investigate the possibility of using a part of bases TEF gene for differentiation of *Fusarium* species, some parts were amplified using two primers ef1 and ef2. The equivalent size was amplified of 700 bp (Figure 4).

The rice suffers from many seed-borne diseases, which cause reduction in germination at initial stage directing to poor crop stand and at adult stage resulting in both qualitative and quantitative loss in grain yield. *Fusarium spp.* is seed-borne and soil borne pathogen and can cause reduction in germination and attack mature plants and developing seeds. Pathogenicity tests were conducted in seed inoculation by symptoms no germination, damping off and abnormal elongation on different isolates (Figure 5). Symptoms showed S13 and S18 important in bakanae disease and can cause reduction in germination seeds (table 3).

Table 3. Effect on seed treatment

Seed test					Name isolate	S.No
Abnormal elongation	**Damping off**	**No germination**	**Colony number**	**Seed number**		
0	4	3	5	7	s1	1
0	6	1	7	7	s2	2
1	3	3	3	7	s3	3
0	4	3	4	7	s4	4
2	4	2	3	8	s5	5
6	2	0	0	8	s6	6
0	4	3	3	7	s7	7
2	2	3	3	8	s8	8
1	6	0	3	7	s9	9
0	8	0	6	8	s10	10
1	3	3	3	7	s11	11
2	1	3	2	7	s12	12
0	0	8	8	8	s13	13
1	1	5	4	7	s14	14
0	2	5	0	7	s15	15
1	4	3	2	8	s16	16
0	8	0	2	8	s17	17
0	1	7	5	8	s18	18
0	4	4	3	8	s19	19
1	5	2	1	8	s20	20
1	6	0	1	7	s21	21
2	0	5	5	7	s22	22
1	3	3	3	7	s23	23
7	0	0	0	7	s24	24
2	4	3	3	8	s25	25
3	0	3	3	7	s26	26
1	3	3	2	7	s27	27
0	8	3	8	8	s28	28
2	7	3	3	8	s29	29
2	3	2	2	7	s30	30

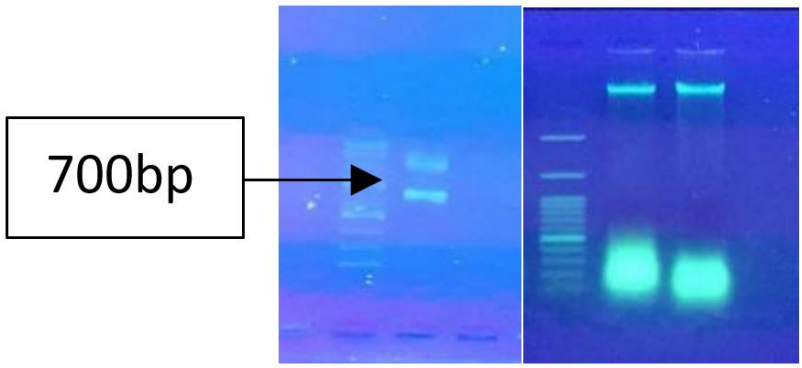

Figure 4. product DNA extraction and Size of sections amplified by primers ef1, ef2 and ef22

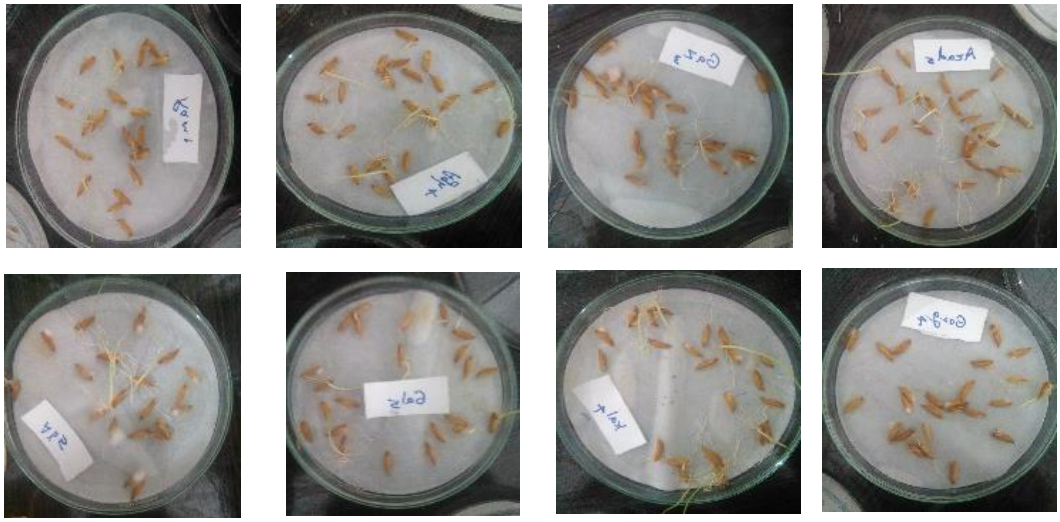

Figure 5. Pathogenicity tests were conducted in seed inoculation by symptoms no germination, damping off and abnormal elongation on different isolates.

Conclusion

Other studies have been performed by Bashyal et al, 2014, indeed, three *Fusarium* spp., viz. *F. verticillioides, F. fujikuroi* and *F. proliferatum* were found associated with bakanae disease of rice in India. Maximum numbers of slender and chlorotic leaves were produced by *F. fujikuroi* (90 %), whereas crown rot and stem rot was produced by *F. verticillioides* (50 %). *F. proliferatum* produced both elongation and rotting symptoms. Information on the bakanae disease, its distribution, characterization and identification could be helpful for the development of management strategies.

Some isolates were also tested under glasshouse by Kirti Rawat et al., (2022) for disease inhibition studies. *F. equiseti*, *Fusarium sp*. and *Trichoderma sp.* gave a disease inhibition of, 87%, 66% and 94%, respectively. *Tf2* and *Tf1* isolate dominantly inhibited the disease with 95% whereas *Tf3* also inhibited successfully with 70%. Through the results of our study, we can deduce that the *T. flavus* (*Tf1, Tf2, Tf3*) isolates and the endophytes *F. equiseti*, *Fusarium sp*. and *Trichoderma sp*. may represent an important biocontrol agent to control the bakanae disease of rice and also implicated that could further be befitting to capitalize them for field evaluations.

References

Bashyal, B., Aggarwal, R., Banerjee, S., Gupta, S., Sharma, S. 2014. Pathogenicity, Ecology and Genetic Diversity of the *Fusarium* spp. Associated with an Emerging Bakanae Disease of Rice (*Oryza sativa* L.) in India. In: Kharwar, R., Upadhyay, R., Dubey, N., Raghuwanshi, R. (eds) Microbial

Diversity and Biotechnology in Food Security. Springer, New Delhi. https://doi.org/10.1007/978-81-322-1801-2_27

Booth, C. 1971. The Genus Fusarium. Commonwealth Mycological Inst., Kew Surrey, England. 1-237 pp.

Burgess LW, Summerell BA, Bullock S, Gott KP and Bakhous D. 1994. Laboratory Manual for Fusarium Research. Fusarium Research Laboratory. Department of Crop Science, University of Sydney and Royal Botanic Gardens 133 pp.

Desjardins AE, Plattner RD, Nelson P, 1997. Production of fumonisin B1 and moniliformin by Gibberella fujikuroi from rice from various geographic areas. Applied and Environmental Microbiology 63, 1838–42.

Leslie JF, 1995. Gibberella fujikuroi: available populations and variable traits. Canadian Journal of Botany 73, S282–91.

Leslie JF, Plattner RD, Desjardins AE, Klittich CJR, 1992. Fumonisin B1 production by strains from different mating populations of Gibberella fujikuroi (Fusarium moniliforme). Phytopathology 82, 341–5.

Mondes RD, Cromey MG, Lauren DR, Menna MD and Marshall J. 2005. Fusarium graminearun, F. cortaderiae and F. pseudograminearum in New Zealand. Molecular phylogenetic analysis, mycotoxin chemotypes and co-existence of species. Mycological Research 109: 410/420.

Nelson PE, Toussoun TA and Marasas WFO. 1983. Fusarium species, An Illustrated Mannual for Identification. Pennsylvania State University Press, University Park.193 pp.

Nelson, P.E., Desjardins, A.E. and Plattner, R.D. 1993. Fumonisins, mycotoxins produced by *Fusarium* species: biology, chemistry and significance. *Ann. Rev. Phytopathol.* **31**: 233-252.

Ou SH, 1985. Rice Diseases. Kew, UK: Commonwealth Mycological Institute.

Pasco C, Jouan B, Andrivon D, 2005. Resistance of potato genotypes to common and netted scab-causing species of Streptomyces. Plant Pathology 54, 383–92.

Rawat, K., Tripathi, S.B., Kaushik, N. et al., **2022**.Management of bakanae disease of rice using biocontrol agents and insights into their biocontrol mechanisms. *Arch Microbiol* **204**, 401 https://doi.org/10.1007/s00203-022-02999-3

Summerell BA, Salleh B and Leslie JF. 2003. A utilitarian approach to Fusarium idendtification. Plant Disease. 87: 117- 128.

Voigt K, Schleier S, Bruckner B, 1995. Genetic variability in Gibberella fujikuroi and some related species of the genus Fusarium based on random amplification of polymorphic DNA (RAPD). Current Genetics 27, 528–35.

Von Korff M, Udupa SM, Yahyaoui A, Baum M. 2004. Genetic variation among *Rhynchosporium secalis* populations of West Asia and North Africa as revealed by RAPD and AFLP analysis. J Phytopathol 152: 106-113.

Wulff, E.D., Sorensen, J.L., Lubeck, M., Nlelson, K.F., Thrane, U. and Torp, J.2010. *Fusarium* spp. associated with rice bakanae: ecology, genetic diversity, pathogenicity abd toxigenicity. *Environ. Microbiol.* **12**: 649-657.