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Original Research Article

Physiological Dynamics of *Ustilaginoidea virens* Causing False Smut Disease of Rice

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A B S T R A C T

False smut, an emerging disease of rice caused by *Ustilaginoidea virens* (teleomorph: *Villosiclava virens*). To reveal the favorable physiological condition for growth of *U. virens*, this study was conducted. The best pH for the growth of fungus was pH 6 (68.82 mm) which was followed by pH 7 (62.42 mm) and minimum growth found at pH 8 (43.98 mm) after 21 days of incubation. Potato sucrose agar (67.36 mm) was best medium for the growth of fungus and followed by potato dextrose agar (63.58 mm), minimum growth were found in case of banana dextrose agar (34.50 mm) which was approximately half of the growth of potato sucrose agar (67.36 mm) after 21 days of incubation.

Keywords

Ustilaginoidea virens, false smut, media, pH, mycelial growth

Introduction

Ustilaginoidea virens is the causal organism of false smut of rice, belongs to the phylum Ascomycetes and its perfect stage is *Villosiclava virens* (Tanaka *et al.*, 2008) and it is an emerging disease of rice. In India false smut disease has been observed in severe form since 2001 in all major rice-growing states due to wide spread cultivation of hybrid rice and heavy application of nitrogenous fertilizer. In northern Indian states as a whole, disease incidence (percentage of false smut-infected tillers) varied from 2 to 75 per cent. Disease incidence of 10-20% and 5-85% respectively has been also reported from Punjab and Tamil Nadu on different rice cultivars (Ladhalakshmi *et al.*, 2012). In some rice growing districts of Bihar, 15-50 percent losses occurs due to false smut of rice when comes as medium to severe form (Laha *et al.*, 2013). The fungus overwinters in soil by means of sclerotia and chlamydospores. Sclerotia produce ascospores, which are primary source of infection to rice plants, whereas secondary infection may come from air-borne chlamydospores (Ashizawa *et al.*, 2010). Infection starts before fertilization, during flowering. Infection of the fungus transforms individual grains of the panicle into greenish spore balls (false smut balls or pseudomorphs) with a velvety appearance, the spore balls are almost smooth when young, and become warty and dark-green while the spore balls are mature. The ball surface is covered by powdery dark-green chlamydospores, conidia and mycelia. A sclerotium can sometime appear on the surface of smut balls (Atia, 2004). The effects of temperature and pH on the

mycelial growth and conidial germination of this fungus have been reported (Li *et al.*, 2008), whereas little research has been reported on the effects of nutrient and environmental factors on *U. virens*. So, an understanding of the environmental conditions required for mycelial growth is needed to identify the conditions required for spikelet infection and to develop appropriate control measures for the disease (Fu *et al.*, 2013).Therefore, the aims of this study were to determine the effect of the culture medium and pH on mycelial growth of *U. virens*.

Materials and Methods

The studies were carried out in the laboratory of Department of Plant Pathology, Bihar Agricultural University, Sabour, Bhagalpur during 2016-17 to find out the favorable culture media and pH for mass multiplication of *Ustilaginoidea virens*. False smut infected panicles were collected from Bihar Agricultural University farm, Sabour, Bhagalpur having Rajendra Mahsuri -1 variety of rice and used for isolation of the pathogen.

Isolation protocol developed by Baite and Sharma, 2015 were used. Hyphal tip method was used for sub culturing of the fungus in slants or Petri plates in order to get the pure culture of the fungus. The pathogen was identified by their cultural characteristics and by Indian type Culture Collection, IARI, New Delhi. Pathogenicity was proved by dipping of detached flowered panicles into spore suspension (5×10^5 spores/ml) of isolated culture.

Effect of pH (Hydrogen ion concentration)

Mycelial growth of *U. virens* was evaluated at five different pH levels 4.0, 5.0, 6.0, 7.0 and 8.0. Five replicates (n=5) of each pH levels were maintained. Mycelial growth (mm) of fungus was recorded at 7 days, 14 days and 21days after incubation.

Effect of different media

This experiment was conducted for finding suitable medium for growth of *U. virens*. Five different media *viz.*, potato dextrose agar (200g potato, 20g dextrose, 20g agar agar, 1000ml distilled water), potato sucrose agar (200g potato, 20g sucrose, 20g agar agar, 1000ml distilled water), radish root dextrose agar media (200g radish peeled, 20g dextrose, 20g agar agar, 1000ml distilled water), yeast extract agar media (10g yeast extract, 20g peptone**,** 15g dextrose**,** 15g agar agar**,** 1000ml distilled water**)**and banana dextrose agar media (200g banana peeled**,** 20g dextrose, 15g agar agar, 1000ml distilled water) were taken for evaluating the mycelial growth of the isolated fungus under laboratory condition.

Five replicates of each media were maintained and their mycelial growth (mm) was recorded at 7 days, 14 days and 21days after incubation.

Results and Discussion

Effect of different pH on mycelial growth of *U. virens*

Some of the fungi are very specific to pH of the medium and some other can tolerate wide range of pH (acidic or alkaline). It was clearly observed from Table 1 that maximum growth of fungus achieved at pH 6 after 7 (13.98 mm), 14 (34.76 mm) and 21 (68.82 mm) days of incubation which was followed by pH 7 and minimum growth was observed at pH 8 after 7, 14 and 21 days of incubation. Beside this, pH 4 and 5 had also minimum mycelial growth than pH 6 and 7.

Table.1 Effect of different pH on mycelial growth (mm) of *U. virens*

pH	Colony diameter (mm)*		
	7 DAI**	14 DAI	21 DAI
4	10.66	22.86	49.98
5	10.98	25.38	52.90
6	13.98	34.76	68.82
7	11.88	28.54	62.42
8	8.22	19.9	43.98
CD (P=0.01)	0.63	0.79	1.03
CV (%)	3.19	1.67	1.03

*Mean of five replications, **DAI- Days after incubation

Table.2 Effect of different media on mycelial growth (mm) of *U. virens*

Media	Colony diameter (mm)*		
	7 DAI**	14 DAI	21 DAI
Potato dextrose agar	11.94	26.92	63.58
Potato sucrose agar	14.48	32.64	67.36
Radish root dextrose agar	10.68	23.84	61.90
Yeast extract agar	9.82	22.90	51.96
Banana dextrose agar	7.58	18.86	34.50
Mean	10.90	25.03	55.86
CD (P=0.01)	0.80	1.05	0.81
CV (%)	4.11	2.34	1.10

*Mean of five replications, **DAI- Days after incubation

These results shows that fungus grows best at slightly acidic to neutral pH and its growth reduced at highly acidic or alkaline pH. This result was in close conformity with the observations of Hong ping (2001) who also observed that pH 6.0 was best for faster growth of *U. virens*.

Ming-xia *et al.,* (2009) also revealed that the mycelia of *U. virens* can grow at pH value from 3 to 10 with optimum being pH 6.0. Jecmen (2014) also found that the optimum levels of pH for growth of all the isolates were ranged between 5.5- 6.5. Reduction in growth was observed for all isolates at pH 8.0. Baite and Sharma (2015) also found that optimum pH for growth of false smut fungus occurred at 6.0. But, Fu *et al.,* (2013) opposed the finding that active mycelial growth was observed at pH 4.5 to 11 and optimal growth was observed at pH 7-8.

Effect of different media on mycelial growth of *U. virens*

In order to select the best medium suitable for the growth of the pathogen five media were tested. Out of five different media selected potato sucrose agar had highest mycelial growth at each 7 (14.48 mm), 14 (32.64 mm) and 21 (67.36 mm) days after incubation and followed by potato dextrose agar. Lowest mycelial growth was found in banana dextrose agar after each 7, 14 and 21 days of incubation. These results were revealed from the depicted Table 2 and Fig 2.Different media used for observing growth of fungus revealed that potato sucrose agar

was best media for the growth of fungus which was followed by potato dextrose agar, radish root dextrose agar, yeast extract agar and banana dextrose agar. This result was also supported by Li *et al.,* (2008) also reported that among the solid culture media tested, PSA supported the fastest mycelial growth. However, solid media with czapek, rice stalk extract and Wakimoto Toceshi were not suitable for mycelial growth.

Fu *et al.,* (2013) also found that potato sucrose agar was the best medium for fast mycelial growth and wakimoto toceshi media and potato dextrose agar also favoured mycelial growth, whereas czapek's dox agar media was not suitable. Baite and Sharma (2015) also found that the fastest growth rate was achieved with potato sucrose agar medium at 2.54 mm/day.

Rani *et al.,* (2015) also found that maximum colony diameter (68.74 mm) and sporulation (6.86 × 105 spores/ml) of *U. virens* was produced on potato sucrose agar followed by potato dextrose agar. Khedkar *et al.,* (2017) also found that *U. virens* having highest growth on PDA while its excellent sporulation occurs in oat meal agar media. Rui-ling *et al.,* (2009) found out that potato dextrose broth and the rice extract medium in the solid media are best suitable for the growth of this fungus suitable for the growth of the fungus.

Kumar (2012) found out that among the different media tested, oat meal agar showed maximum growth of mycelium (42.30 mm) with growth rate of 0.90 mm/day, followed by Rice yeast dextrose agar (37.00 mm) and Yeast protease peptone dextrose agar (32.80 mm). EL-Shafey (2013) found that the growth behavior of false smut fungus were varied on the tested media as it was grown abundantly on rice bran medium followed by rice flour agar medium and oatmeal.

False smut caused by *U. virens* was a slow growing fungus and its maximum mycelia growth occurred at pH 6.0 in potato sucrose agar medium.

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