

Studies on germination of teliospore of four smut fungi

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Regular survey during the last three years (1997-'99) a total of thirty-one smut fungi were collected and identified from various parts of North-East India of which four species viz., *Cintractia axicola*, *Farysia americana*, *Sporisorium sorghi* and *Ustilago cynodontis* were taken for germination studies. Studies showed that out of five different temperatures viz., 10°C, 15°C, 20°C, 25°C and 30°C, 25°C was most suitable for all the four species followed by 20°C except in case of *C. axicola* where the next best temperature was 30°C. It was also revealed that neutral pH at 25°C favoured highest germination percentage except in case of *C. axicola* where it was best in pH 6 at 30°C. Out of the four media tested to observe their growth it was found that Malt Agar supported best growth and sporulation followed by potato dextrose agar, water agar and potato sucrose agar.

Key words : Germination, *Cintractia*, *Farysia*, *Sporisorium*, *Ustilago*, temperature, pH, media

INTRODUCTION

Teliospores of different smut fungi usually take time for germination from a few hours to several days and even months. Few of the smut species required alternate freezing and thawing and others needed some time to break their dormancy. Some smut spores could not germinate readily after harvesting because of variable period of dormancy. Four smut species collected during the survey period (1997-99) from North-Eastern parts of India viz., *Cintractia axicola*, *Farysia americana*, *Sporisorium sorghi* and *Ustilago cynodontis* were taken up for their detailed germination studies under different temperatures, pH and combination of pH and temperature and media and temperature. Singh and Khare (1980) reported *U. cynodontis* to germinate readily within 30 hrs. in distilled water (DW) and on potato dextrose agar (PDA) while Thirumalachar and Pavgi (1950) suggested that *U. cynodontis* germinated very well at 25°C temperature. Freshly collected teliospores of *C. limitata* germinated readily at 30°C on potato sucrose agar (PSA) but not on simple DW or siril

extract and showed germination of 30% spores within 20 hrs. and maximum upto 50% after 48-60 hrs. (Wadhvani and Mehorotra, 1984). Shetty and Safeulla (1980) reported spores of *F. americana* to germinate between temperature range of 10-28°C but germtube could grow very rapidly only at and above 25°C. Best temperature, pH and combination of pH and temperature for germination of *C. axicola*, *F. americana*, *S. sorghi* and *U. cynodontis* are studied.

MATERIALS AND METHODS

Surface sterilization of teliospores

First teliospore dusts were filtered through cheese cloth to remove debris or host materials and then centrifuged at 1000 rpm for 2 min. to get them settled rapidly. Thereafter, the teliospores were placed in 0.25% aqueous solution sodium hypochlorite (NaOCI) for 45-60 sec. at room temperature and immediately after rinsed twice in sterile distilled water using minimal centrifugation to remove the NaOCI.

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Effect of temperature on germination

Sterilized teliospores suspension in distilled water was prepared through dilution method so as to get 8-10 spores per microscopic field. Single loop full of prepared spore suspension was poured in the cavity of sterilized groove slide. These slides were placed in petriplates and incubated at different temperatures viz., 10°C, 15°C, 20°C, 25°C and 30°C, ($\pm 1^\circ\text{C}$). For control spore suspension was not added in distilled water. At least 20 observations were taken for each temperature at regular interval and its average was taken as standard.

Effect of pH on germination

Sterile distilled water was adjusted to pH 4, 5, 6, 7 and 8 either by using 10% lactic acid or 10% NaOH buffer solution. The sterilized teliospores were then suspended in sterile distilled water with different pH. Suspension was adjusted in such a way so that it gave 8-10 spores per microscopic field per loop full. A single loop of prepared spore suspension was poured in the cavity of sterilized grooved-slide, placed in moisturized petriplates and incubated at 15°C, 20°C and 25°C ($\pm 1^\circ\text{C}$). For control spore suspension were not added. Germination percentages was then counted.

Effect of media on germination

Selected four media namely, malt extract agar (MA), potato carrot agar (PCA), potato dextrose agar (PDA) and water agar (WA) were taken for growth studies and radial measurements of the four smut fungi. To prevent bacterial infection antibiotic Ampicillin was mixed @ 10 mg per 200 ml media. Sterilized teliospores were suspended in sterile distilled water. An aliquot 150-250 μl spore suspension was uniformly spread on 9 cm petriplate containing 2 mm thick media. The petriplates were then kept at 20°C and 25°C ($\pm 1^\circ\text{C}$) temperature for incubation. Observation was taken after 4-5 days when sufficient growth was observed. Subculturing was done using cork borer. Uniform single culture disc along with the media were placed aseptically at the center of petriplate containing 2 mm thick media. These petriplates were incubated at 20°C and 25°C ($\pm 1^\circ\text{C}$) temperature for incubation. The

colony diameter and growth of the fungus was measured after 120 hrs for each genus. Three petriplates for each treatment were maintained. Average of the three measurements was taken.

Statistical analysis

Replicated data obtained from above experiments were statistically analyzed to get the values of critical differences (CD) at 1% and 5% level of significance for interpreting the results.

RESULTS AND DISCUSSION

Effect of temperature

Germination of spores of *C. axicola* was highest (34.2%) at 25°C as compared to 23% at 30°C whereas zero at 10°C and 20°C (Table 1). Wadhvani and Mehrotra (1984) reported that freshly collected and 24 hrs chilled teliospores of *C. limitata* germinated readily at 30°C on PSA but not in simple distilled water, soil extract or seed exudes. Nene and Bhelwa (1976) reported that *C. axicola* could produce germtube within 6-7 hrs of soaking in distilled water but there was no detailed report regarding germination of *C. axicola* under various temperature, pH or media.

Table 1 : Effect of different temperatures on spore germination of four smut species (in per cent).

Temperature (°C)	<i>Cintractia axicola</i>	<i>Farysia americana</i>	<i>Sporisorium sorghi</i>	<i>Ustilago cynodontis</i>
10	0.0	0.0	13.4	10.7
15	0.0	0.0	36.6	13.1
20	3.5	24.0	66.9	79.9
25	34.2	39.3	69.9	86.2
30	23.0	7.0	48.4	64.7
Control	0.0	0.0	0.0	0.0
CD at 1%	1.24	2.25	1.76	1.73
CD at 5%	0.87	1.77	1.23	1.22

In case of *F. americana* slightly higher germination (39.3%) was observed at 25°C and very low (7%) at 30°C. But, it germinated well (24%) at 20°C as compared to *C. axicola* (3.5%) (Table 1). Shetty and Safeeulla (1980) reported germination of spores of *F. americana* between 10-28°C but germtube could grow very rapidly only at and above 25°C. They also reported fresh spores to possess high germination (75%), retention of viability upto 45

days and there after germination percentage declined. Similarly viability was lost after 60 days but was retained upto 150 days if kept in polythene bag at 5°C. The present studies, however, did not prove it as the specimens were kept in brown paper packet at room temperature and taken for studies 90 days after collection.

Both *S. sorghi* and *U. cynodontis* showed much higher percentages of germination at 25°C being 69.9% and 86.2% respectively. Germination of *S. sorghi* and *U. cynodontis* was also high at 20°C being 66.9% and 79.9% respectively. It was also high at 30°C as against 10°C and 15°C (Table 1). Present study gets supported from the earlier work of Thirumalachar and Pavgi (1950) where it was suggested that *U. cynodontis* germinates very well at 25°C in persence of leaf and stem extracts of *Tradescantia* sp. in the media. Singh and Khare (1980) reported that *U. cynodontis* germinated readily within 30 hrs in distilled water. Chauhan and Pawar (1979) reported 100% germination of *Sphacelotheca sorghi* at 22°C and 24°C but no germination at 5°C after 48 hrs whereas low above 30°C. It was clear from the persent study that all the four smut fungi viz., *C. axicola*, *F. americana*, *S. sorghi* and *U. cynodontis* showed highest germination percentage at 25°C followed by 20°C except in case of *C. axicola* where it was recorded at 30°C.

Effect of pH

Effect of different pH (4-8) was studied at 15°C, 20°C and 25°C on *C. axicola* (Table 2), *F. americana* (Table 3), *S. sorghi* (Table 4) and *U. cynodontis* (Table 5). It was observed that germination of spores of *C. axicola* at 25°C were 34.0% and 30.9% at pH 6 and 7 respectively whereas at pH 4 and 8 spores failed to germinate both at 15°C and 20°C. Similar pattern of germination was observed in *F. americana* with a slightly less germination per cent (16.4% and 19.1%) at 20°C both at pH 6 and 7. Lowest per cent of germination was observed at pH 7 in *S. sorghi* and *U. cynodontis* at 15°C whereas no germination was observed at pH 4, 5, and 8 at 15°C in *C. axicola* and *F. americana*. It was further observed that pH 7 at 25°C favoured maximum germination of spores of all the four last species except *C.*

axicola where it was at pH 6. Chauhan and Pawar (1979) reported that neutral pH was found to be best for germination 100% of *Sphacelotheca sorghi* whereas it decreased with increasing as well as decreasing pH. Overall results obtained showed that pH 6 and 7 both at 20°C and 25°C favoured germination of spores of three test species and *C. axicola* where only at 25°C.

Table 2 : Effect of different pH and temperature on germination of *Cintractia axicola* (in per cent).

Temp. (°C)	pH				
	4	5	6	7	8
15	0.0	0.0	0.0	0.0	0.0
20	0.0	0.0	4.0	4.7	0.0
25	0.0	0.0	34.0	30.9	0.0
Control	0.0	0.0	0.0	0.0	0.0
CD at 1%			0.825		
CD at 5%			0.613		

Table 3 : Effect of different pH and temperature on germination of *Farysia americana* (in per cent).

Temp. (°C)	pH				
	4	5	6	7	8
15	0.0	0.0	3.2	0.0	0.0
20	0.0	0.0	16.4	19.1	0.0
25	0.0	0.0	36.3	40.0	0.0
Control	0.0	0.0	0.0	0.0	0.0
CD at 1%			1.21		
CD at 5%			0.90		

Table 4 : Effect of different pH and temperature on germination of *Sporisorium sorghi* (in per cent).

Temp. (°C)	pH				
	4	5	6	7	8
15	0.0	0.0	6.1	19.4	0.0
20	0.0	4.7	26.3	49.3	19.1
25	12.3	32.2	56.4	72.6	43.2
Control	0.0	0.0	0.0	0.0	0.0
CD at 1%			1.03		
CD at 5%			0.77		

Table 5 : Effect of different pH and temperature on germination of *Ustilago cynodontis* (in per cent).

Temp. (°C)	pH				
	4	5	6	7	8
15	0.0	3.1	8.1	12.8	0.0
20	0.0	16.8	73.8	76.8	0.0
25	0.0	18.8	77.3	81.1	2.4
Control	0.0	0.0	0.0	0.0	0.0
CD at 1%			2.72		
CD at 5%			2.02		

Table 6 : Effect of different media and temperature on growth (in mm) of four smut species (after 120 hrs).

Media	Temp. (°C)	<i>Cintractia axicola</i>	<i>Farysia americana</i>	<i>Sporisorium sorghi</i>	<i>Ustilago cynodontis</i>
PDA	20	8.5 × 6.0	14.5 × 13.5	24.5 × 23.0	37.5 × 35.5
	25	34.0 × 32.5	17.5 × 15.0	26.0 × 26.5	39.5 × 38.0
PCA	20	4.0 × 3.0	11.5 × 9.0	12.0 × 10.0	21.5 × 21.0
	25	13.5 × 10.0	12.0 × 11.0	13.5 × 11.5	23.5 × 21.0
MA	20	12.5 × 10.5	21.0 × 19.0	32.0 × 31.5	38.0 × 36.5
	25	38.5 × 33.0	24.0 × 23.5	35.0 × 34.0	42.0 × 41.0
WA	20	7.5 × 6.0	11.0 × 10.5	17.5 × 16.5	26.0 × 24.5
	25	27.5 × 23.5	13.5 × 12.0	19.0 × 17.5	28.5 × 27.0

Effect of media

Four different media viz., malt extract agar (MA), potato carrot agar (PCA), potato dextrose agar (PDA) and water agar (WA) were tested for their growth (in mm) in petriplates at two cardinal temperatures, 20°C and 25°C ($\pm 1^\circ\text{C}$) against four different genera of smuts namely *C. axicola*, *F. americana*, *S. sorghi* and *U. cynodontis*. Results revealed from Table 6 that all the four genera supported best linear growth at 25°C in MA followed by PDA, WA and PCA respectively. Similar results were also obtained for all the four fungi tested at 20°C in the descending order as observed. Rao and Thakur (1983) reported colony size 11.5 × 18.6 mm and 12.6 × 9.4 mm on potato agar (PA) medium at 30°C and 35°C respectively and maximum 17.0 × 13.4 mm on PA at 35°C after 5 days whereas it was 13.8 × 11.8 mm and 8.9 × 8.2 mm in carrot agar (CA) and PDA respectively after 5 days. From the results (Table 6) it was

concluded that MA supported best growth for the four different smut fungi tested followed by PDA, WA and PCA. It was also concluded that there was relatively very less difference in the size of colony growth when temperature were maintained at 20°C and 25°C in respective media except in case of *C. axicola* where 25°C temperature favoured best linear growth of colony.

ACKNOWLEDGEMENTS

Authors are grateful to the Head, Division of Plant Pathology, IARI, New Delhi for providing necessary facilities to carry out the work.

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(Accepted for publication November 04 2002)