

SPORE GERMINATION OF BLIGHT PATHOGEN¹

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ABSTRACT - Influence of physical factors and substrates on conidial germination of pearl millet (*Pennisetum americanum* (L.) Leeke) blight pathogen (*Curvularia penniseti* (Mitra) Boedijn) was investigated. Though conidial germination was noticed in wide ranges of temperature, pH and relative humidity, maximum germination occurred at 30°C, pH 6.5 and 100 per cent R.H. Washing of conidia upto six cycles (centrifugation at 3000 rpm for 15 minutes at each cycle) did not cause significant increase or decrease in their germination. Of the two substrates tested, germination was always higher on glass slides than on pearl millet leaf segment.

Index terms: conidial germination, *Pennisetum americanum*, *curvularia penniseti*.

GERMINAÇÃO DE ESPOROS DO PATÓGENO DA FERRUGEM

RESUMO - Estudou-se a influência dos fatores físicos e de substratos sobre a germinação de esporos (=conídios) do patógeno da ferrugem (*Curvularia penniseti* (Mitra) Boedijn) em painço-pérola (*Pennisetum americanum* (L.) Leeke). Embora se tenha observado germinação de conídios em diversos graus de temperatura, pH e umidade relativa, a maior quantidade de germinações ocorreu a 30°C, pH 6,5 e 100% de umidade relativa. Até seis ciclos (centrifugação a 3.000 rpm por 15 minutos a cada ciclo), a lavagem dos conídios não causou significativo aumento nem diminuição em sua germinação. Dos substratos testados, foi mais abundante a germinação em lâminas de vidro do que no segmento da folha do painço-pérola.

Termos para indexação: germinação de conídios, *Pennisetum americanum*, *Curvularia penniseti*.

INTRODUCTION

Blight of pearl millet (*Pennisetum americanum* (L.) Leeke) incited by *Curvularia penniseti* (Mitra) Boedijn causes severe damage to the millet particularly when the crop is reaching maturity. A poor understanding of biology of the pathogen has hampered development of effective strategies for disease control. Since spore germination is the first and major event in the pre-penetration phase of host-pathogen interaction and no information is available on this aspect of the pathogen, the present investigation was undertaken to study spore germination under the impact of various factors.

MATERIALS AND METHODS

Effect of temperature, pH, relative humidity and spore washings on germination of conidia was determined on glass slides as well as on leaf segments. The latter were prepared from middle leaves of 2-month-old plants of pearl millet hybrid BJ 104. Leaf segments collected late in the day were cut to 3 cm length and were placed on glass slides. The glass slides supporting leaf segments and glass slides alone were incubated in the same manner.

In all the experiments except the one on effect of relative humidity, mature conidia collected by taping 15-day-old monoconidial culture of *C. penniseti* were suspended in sterilized distilled water. The spore concentration was adjusted to 2.2×10^5 conidia/ml. A drop of spore suspension was placed on a glass slide as well as on a leaf segment and these were accommodated in a humid chamber.

Temperature: In order to study effect of temperature on spore germination, the humid chambers containing conidia on glass slides and on leaf segments were placed at 7, 15, 20, 25, 30, 35 and 40°C.

pH: Germination was assessed at different pH levels ranging from 4.0 to 9.0 with the interval of 0.5. The spore suspensions were prepared in solutions adjusted to different pH levels. The humid chambers having glass slides and leaf segments with spore suspensions were incubated at $30 \pm 1^\circ\text{C}$.

Relative humidity: Levels of RH ranging from 50 to 100 per cent were produced by sulphuric acid - distilled water mixtures (Buxton & Mellanby 1934). Ten ml of sulphuric acid solution of respective concentration were placed into a Petri dish (10 cm). Two glass rods were positioned on the bottom of each Petri dish to support glass slide. Glass slides/leaf segments containing dry conidia were placed in Petri dishes with sulphuric acid solutions of respective concentration. The Petri dishes were then sealed with an adhesive tape and incubated at $25 \pm 1^\circ\text{C}$.

Spore washing: The spores were suspended in sterilized distilled water, shaken gently for 15 minutes, centrifuged (at 3000 rpm for 15 minutes) and supernatant poured-off. This cycle was repeated for six times. Conidia

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after one to six cycles of washings were separately collected. The conidia were finally resuspended in sterilized distilled water and germination studies performed using glass slides and leaf segments by keeping them in humid chambers at $30 \pm 1^\circ\text{C}$.

In all the experiments, germination was assessed ten hours after incubation. The slides were taken out, a drop of lactophenol was added to the spore suspension and per cent germination was assessed under the microscope with a spore sample size of 200. Each treatment was replicated four times.

TABLE 1. Germination of conidia of *C. penniseti* at different temperatures after incubation of ten hours.

Temperature (°C)	Average per cent germination on two substrates		Mean
	Glass slide	Pearl millet leaf segment	
7	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
15	48.64 (44.21)	36.92 (37.40)	42.78 (40.80)
20	72.97 (58.68)	69.65 (56.56)	71.31 (57.62)
25	88.44 (77.04)	76.34 (60.90)	82.39 (68.97)
30	97.11 (80.41)	89.41 (70.99)	93.26 (75.70)
35	62.71 (52.35)	54.21 (47.41)	58.46 (49.88)
40	36.56 (37.18)	30.35 (33.40)	33.45 (35.29)
Mean	58.06 (48.98)	50.98 (43.80)	

L.S.D. at 5% for temperature 2.68
for substrate 1.43
for temperature x substrate 3.80.

Values in parentheses are angular transformations.

TABLE 2. Germination of conidia of *C. penniseti* at different pH levels after incubation of ten hours, at $30 \pm 1^\circ\text{C}$.

pH	Average per cent germination on two substrates		Mean
	Glass slide	Pearl millet leaf segment	
4.0	17.26 (24.53)	14.17 (22.06)	15.71 (23.29)
4.5	33.90 (35.59)	31.13 (33.89)	32.51 (34.74)
5.0	44.17 (41.64)	40.74 (39.64)	42.45 (40.04)
5.5	51.53 (45.84)	49.46 (44.65)	40.64 (45.25)
6.0	70.79 (57.26)	64.85 (53.61)	67.82 (55.43)
6.5	91.77 (73.35)	83.32 (65.91)	87.54 (69.63)
7.0	82.35 (65.14)	74.49 (59.64)	78.42 (62.39)
7.5	70.78 (57.26)	58.77 (50.03)	64.77 (53.64)
8.0	61.07 (51.37)	49.22 (44.52)	55.14 (47.94)
8.5	40.77 (39.66)	36.75 (37.30)	38.76 (38.48)
9.0	24.96 (29.45)	21.45 (27.53)	23.20 (28.49)
Mean	53.57 (47.41)	47.66 (43.52)	

L.S.D. at 5% for pH 1.18
for substrate 0.50
for pH x substrate 1.67.

Values in parentheses are angular transformations.

RESULTS AND DISCUSSION

Effect of temperature

Both on glass slides and leaf segments, germination was maximum at 30°C and was significantly different than at other temperatures (Table 1). There was no germination at 7°C . Germination at 25 and 35°C was significantly less than at 30°C . The fungus was able to display conidial germination even at 40°C . Similar observation has been recorded by Yang (1973) for *C. senegalensis*. Singh (1972) reported that *C. ovoidae*, *C. lunata* and *C. lunata* var. *aeria* showed maximum spore germination at 25°C . The temperature x substrate interactions were significant indicating that the germination at two kinds of substrates was significantly different. Germination at all the temperatures except at 20°C and 40°C was significantly higher on glass slide than on leaf segment.

Effect of pH

Conidial germination of the fungus was significantly less at pH 6.0 and 7.0 as compared to 6.5

(Table 2) which indicates sensitivity of the fungus to pH changes in respect of conidial germination. Least germination was recorded at pH 4.0. Singh (1972) recorded maximum spore germination in *C. ovoidae*, *C. lunata* and *C. lunata* var. *aeria* at pH 6.0. The pH x substrate interactions were significant. Germination at all pH levels except at pH 5.5 was significantly more on glass slide than on leaf segment.

Effect of RH

Conidia germinated at all the RH levels tested. Maximum germination was recorded at 100% RH (Table 3). These findings support views expressed by Choudhary (1937) who stated that spores needed high humidity for germination. Humidity x substrate interactions were significant.

TABLE 3. Germination of conidia of *C. penniseti* at different levels of relative humidity after incubation of ten hours $25 \pm 1^\circ\text{C}$.

Relative humidity	Average per cent germination on two substrates		Mean
	Glass slide	Pearl millet leaf segment	
50	4.61 (12.34)	1.66 (7.29)	3.13 (9.81)
60	18.27 (25.28)	10.06 (18.43)	14.16 (21.85)
70	32.08 (34.37)	27.03 (31.29)	29.55 (32.83)
80	44.99 (42.11)	38.58 (38.26)	41.78 (40.18)
90	70.43 (57.04)	62.79 (52.40)	66.61 (54.72)
100	93.38 (75.14)	81.08 (64.33)	87.23 (69.73)
Mean	43.96 (41.06)	36.36 (35.33)	

L.S.D. at 5% for humidity 1.75

for substrate 1.01

for humidity x substrate 2.48.

Values in the parentheses are angular transformations.

TABLE 4. Effect of spore washings on germination of conidia of *C. penniseti* after incubation of ten hours at $30 \pm 1^\circ\text{C}$.

No. of spore washing	Average per cent germination on two substrates		Mean
	Glass slide	Pearl millet leaf segment	
0	87.00 (69.51)	76.00 (60.74)	81.50 (65.12)
1	85.00 (69.01)	75.50 (60.36)	80.25 (64.68)
2	84.50 (66.81)	74.75 (59.90)	79.62 (63.35)
3	83.37 (65.94)	76.00 (60.70)	79.68 (63.32)
4	85.00 (67.25)	75.75 (60.51)	80.37 (63.88)
5	84.50 (66.82)	75.12 (60.09)	79.81 (63.45)
6	84.00 (66.42)	74.00 (59.34)	79.00 (62.88)
Mean	84.76 (67.39)	75.30 (60.23)	

L.S.D. at 5% for washing NS

for substrates 2.55

for washing x substrat 6.75

Values in the parentheses are angular transformations.

Effect of spore washing

There was no significant difference in germination of washed and unwashed conidia tested on glass slides or leaf segments (Table 4). This suggests that neither any germination inhibitor exists on conidial surface nor any damage to spores occurred during the washing process. Washing x substrate interaction were significant, which indicates that germination was significantly different at some levels of washings on the two substrates.

CONCLUSIONS

1. Spore germination was maximum at 30°C, and no germination occurred at 7°C.
2. The fungus is very sensitive to pH changes in respect of conidial germination. Maximum spore germination occurred at pH 6.5.

3. Spores need high humidity for germination. Maximum germination occurred at 100% RH.

4. Washing spores with water does not damage spores. Probably no germination inhibitor exists on conidial surface.

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