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EFFECTS OF SUGAR SOLUTIONS, TEMPERATURE AND TIME INTERVALS ON UREDOSPORE GERMINATION OF *Puccinia polysora* UNDERW. : THE CAUSATIVE AGENT OF POLYSORA RUST IN MAIZE (*ZEA MAYS* L.)

Soumen Pati*, Nabakishor Nongmaithem, Ph Sobita Devi and Prajna Samal

Department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal - 795 001, Manipur, India.

*Corresponding author E-mail : soumenpati1617@gmail.com

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ABSTRACT

In vitro experiments were conducted to assess the impact of different sugar solutions, temperature variations, and time intervals on the germination of *Puccinia polysora* uredospores between 2021-22. In the case of glucose, the lowest spore germination rate was observed in the control treatment with sterile distilled water (16.50%). Spore germination increased from 0.5% (23.42%) to 2.0% (59.24%) glucose concentration but decreased above 2%. Similarly, with various sucrose concentrations, spore germination increased from 0.5% (25.08%) to 2.0% (61.82%) sucrose concentration, with the control plate showing the lowest uredospore germination rate (16.50%). The optimum temperature for spore germination was found to be 25°C after 24 hours of incubation, resulting in the highest germination rate (63.17%). Conversely, the lowest spore germination was observed at 5°C (11.40%) and germination steadily decreased from 35°C onward.

Key words : Glucose, Sucrose, Temperature, Time interval, Uredospore.

Introduction

Maize (*Zea mays* L.), commonly known as corn, is a cereal grain first domesticated by indigenous peoples in southern Mexico about 10,000 years ago (Benz, 2001). Maize is widely cultivated throughout the world, and a greater weight of maize is produced each year than any other grain. According to the US Department of Agriculture (USDA, 2020), the United States, China, and Brazil are the world's top corn producers, accounting for 48% corn area. Whereas, India contribute only 3% of total production. Maize is grown in all the hill and valley districts of Manipur. In case of maize, the estimated area, the average yield, and production for the agricultural year 2019-20 was recorded as 2.22 thousand hectares, 2228.35 kgs. per hectare and 4.94 thousand tonnes respectively (ESM, 2020).

On a global scale, maize is infected with up to 112 diseases. We have records of roughly 35 of them in India. Seed rots and seedling blights, foliar diseases, Downy

mildews, Stalk rots, Rusts and Wilts are some of the divisions (GEAC, 2021). Southern rust is a troublesome, sporadic foliar disease of maize caused by *Puccinia polysora* Underw. The disease is restricted to tropical and subtropical regions of the world due to the pathogen's temperature sensitivity. In India, it can be found in the states of Karnataka, Andhra Pradesh and Tamil Nadu. Of the three rusts that occur on corn worldwide, southern rust of corn has been reported as the most destructive among all the reported rusts of maize.

For obligate parasites like this pathogen, *in vitro* determination of sensitivity to a fungicide requires the identification of several other factors such as substrate (Sugars), a particular temperature with a specific time period after inoculation, which promotes the highest rate of spore germination in shortest time. So, our study aimed to select substrate, temperature and time interval for promoting maximum germination of *P. polysora* uredospores.

Materials and Methods

In vitro examinations were conducted at the Department of Plant Pathology, College of Agriculture, C.A.U., Imphal, Manipur, India during 2021-22 to know the effect of different sugar solutions, temperature, and time intervals on germination of uredospores of *Puccinia polysora*.

Effect of sugars on uredospore germination

The effect of sugar on uredospore germination was observed. Two different sugars, *viz.*, glucose and sucrose, were used in five different concentrations, *i.e.*, 0.5, 1.0, 1.5, 2.0, and 2.5% in water agar (0.5%). Cavity slides were used in this experiment. Mature uredospores of *P. polysora* were collected from naturally infected maize plants. Uredospores suspension was created. The uredospore concentration was adjusted to 2×10^6 spores/ml using a haemocytometer. On the other hand, five different concentrations of each of the above-mentioned sugars were prepared in conical flasks. Then, using a micropipette, one drop of sugar from each concentration was mixed with one drop of uredospore suspension in cavity slides. There were three replications for each treatment. An untreated control was maintained with sterile distilled water. Throughout the experiment, the ambient temperature was kept at 25°C. A piece of moist cotton was kept inside cavity slides containing petri dishes to maintain the moisture level. The observations on spore germination were done under microscope 40X magnification after 24 hours of incubation. Per cent uredospores germination was calculated by following formula:

$$\text{Spore germination \%} = (A/B) \times 100$$

Where,

A = No. of uredospores germinated

B = No. of uredospores observed

Effect of different temperature and time interval

For evaluating the effect of different temperature levels and time intervals on the germination of uredospores, four different temperatures (5, 15, 25 and 35°C) were applied and at each different level of temperature, the germination percentage of uredospores was evaluated under a microscope. At every temperature level, uredospore germination percentage was checked at different time intervals, *i.e.*, 2 hours, 4 hours, 8 hours, 16 hours, and 24 hours. The same time intervals were followed at all the temperature levels.

Results and Discussion

Effect of sugars on uredospore germination

The results presented in Table 1 and Fig. 1 revealed that for various glucose concentrations, maximum uredospore germination was recorded at 2% concentration (59.24 per cent) followed by 1.5% (52.70 per cent), 2.5% (48.28 per cent), 1.0% (46.59 per cent), and 0.5% (23.42 per cent) concentration, respectively. However, in control plate, uredospore germination percentage was very low (16.50 per cent). Spore germination percentage increased from 0.5% to 2.0% glucose concentration and dropped above 2% glucose level. Similar experiment on *Cerotelium fici* by Anusha *et al.* (2018) revealed that dextrose at 3.0 per cent has recorded the least germination (24.18%) followed by sucrose at 0.5 per cent (27.00%) and dextrose at 0.5 per cent concentration (28.86%). Whereas maximum germination was found in dextrose at 2.0 per cent (43.05%) and sucrose at 2.0 per cent (43.41%).

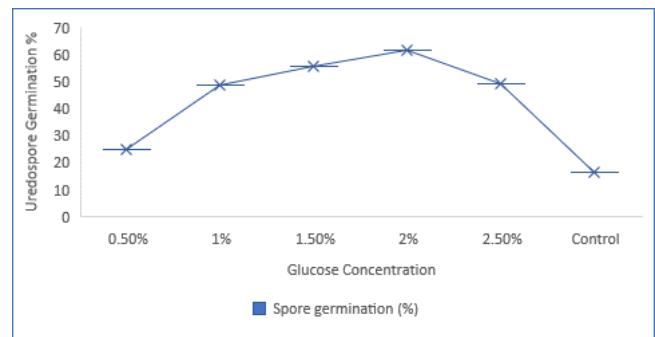


Fig. 1: Effect of different concentration of glucose on uredospore germination of *P. polysora*.

Table 1: Effect of different glucose concentration on uredospore germination of *P. polysora*.

S. no.	Glucose concentration (%)	Spore germination (%)
1	0.5	23.42 (28.94)
2	1.0	46.59 (43.05)
3	1.5	52.70 (46.56)
4	2.0	59.24 (50.33)
5	2.5	48.28 (44.01)
6	Control	16.50 (23.96)
	S.Em ±	2.31
	CD @ 0.01	9.96

*Values in parenthesis are arc sine transformed.

In the case of different sucrose concentrations, the results presented in Table 2 and Fig. 2 revealed that maximum uredospore germination was recorded at 2% (61.82 per cent) followed by 1.5% (55.66 per cent), 2.5% (49.08 per cent), 1.0% (48.74 per cent) and 0.5% (25.08

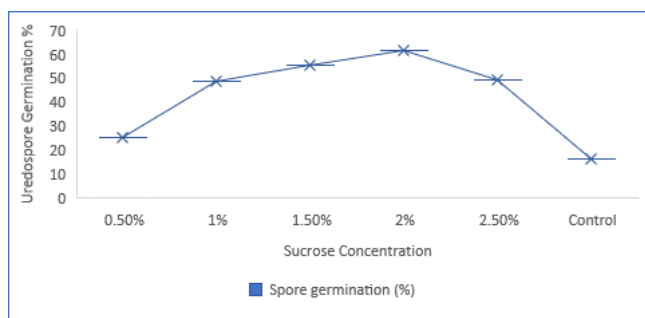


Fig. 2 : Effect of different concentration of sucrose on uredospore germination of *P. polysora*.

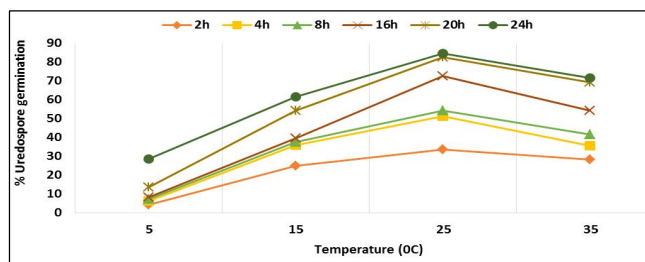


Fig. 3 : Effect of different temperature levels and time intervals on germination of uredospores of *P. polysora*.

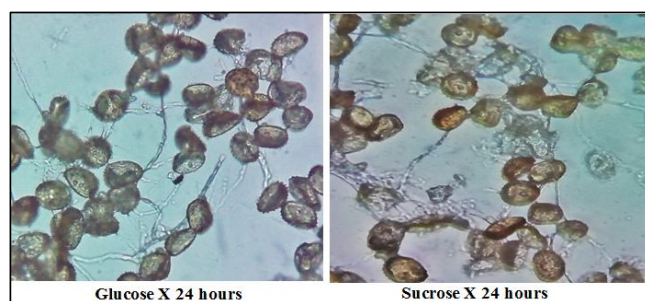


Plate 1 : Effect of different sugar concentrations on uredospore germination of *P. polysora*.

percent) concentration, respectively. However, in control plate, uredospore germination percentage was very low (16.50 per cent). Spore germination percentage increased from 0.5% to 2.0% sucrose concentration and dropped above 2% sucrose level. Similar results have been observed by Sumangala *et al.* (2014), who found that uredospore germination of *Puccinia melanocephala* was optimum at 2.0 per cent in sucrose and least in 0.5 per cent sucrose. Kochman and Brown (2008) observed higher rates of spore germination for *Puccinia graminis avenae* Erikss. & Henn. and *Puccinia coronate avenae* Fraser & Led. by using the substrate water-agar *in vitro*, compared to germination on oat leaves.

Effect of temperature and time interval

From the results presented in Table 3 and Fig. 3, it can be concluded that uredospore germination of *P. polysora* varies both in rate and percentage depending on different temperatures and time intervals. Least spore germination was observed in 5 °C with a mean germination

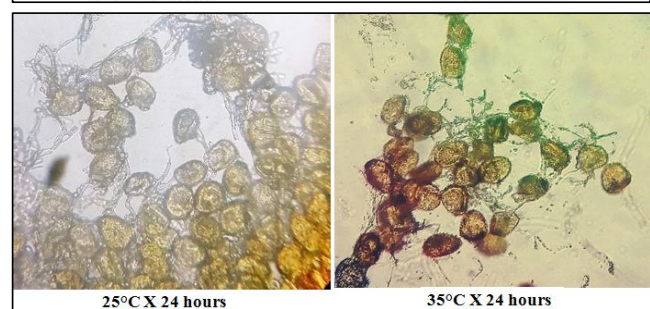
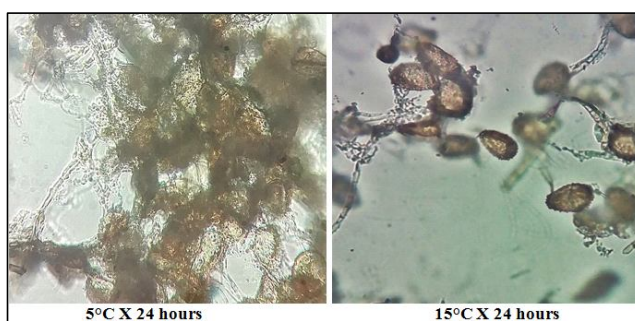


Plate 2 : Effect of different temperature and time intervals on germination of uredospores of *P. polysora*.

Table 2 : Effect of different sucrose concentration on uredospore germination of *P. polysora*.

S. no.	Sucrose concentration (%)	Spore germination (%)
1	0.5	25.08(30.03)
2	1.0	48.74(44.28)
3	1.5	55.66(48.26)
4	2.0	61.82(51.84)
5	2.5	49.08(44.47)
6	Control	16.50(23.96)
	S.Em±	1.64
	CD @ 0.01	7.10

*Values in parenthesis are arc sine transformed.

of 11.40 per cent and maximum spore germination was observed in 25°C with a mean germination of 63.17 per cent. (%). From 35°C onward, it showed decreasing trend of germination (50.06%). In each temperature level, uredospore germination was minimum after 2 hours and germination reached maximum after 24 hours of incubation.

Similar experiment on *P. polysora* by Hollier and king (1985) revealed that that germination of uredospores on water agar was similar for 4, 8, 12 and 16 hrs incubation periods. Percentage germination increased sharply between 12-16°C and decreased sharply at temperatures higher than 26°C. No germination occurred at 36°C. Dey *et al.* (2015) investigated the influence of temperature on uredospore germination of *Puccinia sorghi* on water agar and discovered that germination varies in rate and

Table 3 : Effect of different temperature levels and time intervals on germination of uredospores of *Puccinia polysora*.

Temperature (°C)	Uredospore germination (%)						Mean
	2h	4h	8h	16h	20h	24h	
5	11.77(4.16)	14.41(6.19)	15.76(7.38)	16.71(8.27)	21.68(13.65)	32.41(28.72)	18.79* (11.40)**
15	30.09(25.13)	36.81 (35.90)	37.79 (37.55)	38.96(39.53)	47.37 (54.13)	51.64 (61.49)	40.44(42.29)
25	35.39(33.54)	45.83 (51.45)	47.37 (54.13)	58.51 (72.72)	65.28 (82.51)	66.95 (84.67)	53.22(63.17)
35	32.10 (28.24)	36.60 (35.55)	40.15 (41.58)	47.41 (54.20)	56.28 (69.19)	57.80 (71.61)	45.06(50.06)
Mean	27.34(22.77)	33.41(32.27)	35.27(35.16)	40.40(43.68)	47.65(54.87)	52.20(61.62)	
Comparing the mean of	S. Em± CD at 1%						
Treatment (A)	0.59 1.70						
Concentration (B)	0.48 1.39						

*Arcsine transformed values; **Data in parenthesis are original values.

percentage depending on temperature and time interval. Spore germination was highest in the temperature range of 20-30°C after 24 hours of incubation. The mean maximum spore germination temperature was recorded at 25°C (66.16% germination), which is statistically comparable to the temperature of 30°C (optimum range). There was no germination below 5°C and only a small fraction of seeds germinated after 8 and 4 hours, respectively, at those temperatures.

Conclusion

It can be inferred that a 2 per cent sucrose concentration provided the most favourable conditions for maximum uredospore germination of the *Puccinia polysora* pathogen under in vitro conditions, with a 2 per cent glucose concentration ranking as the second most conducive medium for maximum germination. Likewise, maximum uredospore germination was observed in 25°C temperature. So, these are the most favourable parameters that are necessary for pathogen growth and spread.

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Conflict of interest

The authors declare no known conflict of interest that could have appeared to influence that could have appeared to influence the work reported in this paper.

Authors contribution

S.P., executed the experiment, collected and analyzed the data; N.N, planned the research work; P.S. and Ph. S.D., edited and reviewed the manuscript.

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