www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(7): 110-113

www.thepharmajournal.com
Received: 03-04-2022
Accepted: 09-05-2022

Sanjog Chhetri
Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal, India

Srabani Debnath
Directorate of Research, All India Coordinated Research Project on Maize, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, West Bengal, India

Corresponding Author:
Srabani Debnath
Directorate of Research, All India Coordinated Research Project on Maize, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, West Bengal, India

Effect of different temperature levels and incubation time on germination of urediniospores of *Puccinia polysora* Underw

Sanjog Chhetri and Srabani Debnath

Abstract
The Southern corn rust (SCR), caused by the fungus *Puccinia polysora*, has been one of the major foliar diseases of Maize. In plant disease development, temperature and incubation time is the critical factor. *In vitro* experiments were conducted to assess the effects of temperature and time of exposure on *P. polysora* uredospore germination. The development of the germ tube was assessed for 12 hours. The 0.6g of fresh urediniospores were added to 10ml sterilized distilled water and plated in a 0.1 ml aliquot in water – agar (1.5%), placed in BOD, and regulated at temperatures of 5 ºC, 10 ºC, 15 ºC, 20 ºC, 25 ºC, 30 ºC, 35 ºC and 40 ºC. Readings were performed after 1, 3, 6, 12, 18 and 24 hours. The studies on the effect of temperature and incubation time on germination of urediniospores revealed that maximum percent spore germination was found at 25ºc (25%, 33%, 57.67%, 67.67% and 78% at 2hrs, 3hrs, 6 hrs, 9hrs and 12hrs respectively). The minimum and maximum temperatures where germination percentage were found to be nil at different incubation times are 5 ºC and 40 ºC respectively.

Keywords: Southern corn Rust, SCR, *Puccinia polysora*

1. Introduction
Maize (*Zea mays* L.) is the world's most widely grown and economically valuable cereal crop. Maize yields were 1,148 million tonnes in 2019, whereas rice and wheat yields were just 755 million and 766 million tonnes, respectively (FAO, 2019) [2]. Unsurprisingly, abiotic and biotic stresses are common in maize belts worldwide. There are three types of rust affecting corn plants namely – common rust caused by *Puccinia sorghi*, southern corn rust (SCR) caused by *Puccinia polysora* and tropical rust caused by *Physopella zeae* (Dolezal, 2011; Cummins, 1941; Ramirez-Cabral *et al.*, 2017; Krattiger *et al*., 1998) [11, 9, 20, 16]. Of the three rusts that occur on maize worldwide, southern corn rust (SCR), caused by *Puccinia polysora* Underw, has been reported as the most destructive among all the reported rusts of maize (Ramirez-Cabral *et al*., 2017) [20]. *P. polysora* was first identified from a herbarium specimen of *Tripsacum dactiloides* collected in Alabama, United States of America in 1891 and was named by Underwood (1897) [27]. Cummins (1941) [9] studied over herbarium specimens and revealed that *P. polysora* was common in Central and South America before 1891, as well as in Massachusetts, USA in 1879. (Krattiger *et al*., 1998) [16].
The disease was first noticed in Karnataka (South India) in 1992 on certain maize cultivars in the Mysore district (Payak, 1992) [19]. The presence of *Puccinia polysora* Underw. In India was again confirmed in the Mysore district of Karnataka. Moderate to severe infection of this rust was noticed in October 1999, during Post-Entry Quarantine Inspection (PEQI) of maize crop at Bangalore (Agarwal *et al*., 2001) [1].
The life cycle of *P. polysora* is still not understood because the teliospores are rare in nature and to date, the germination of teliospores is not achieved. (Crouch and Szabo, 2011; *Guo et al*., 2013) [7, 12]. No aecia and pycnia are reported to date and germination of teliospores is still not known (Cammack, 1959) [5]. Yield loss could be as high as 45 percent to 50 percent as a result of this disease onslaught (Rodriguez *et al*., 1980) [21]. The damage caused by southern corn rust is particularly severe in late-planted corn fields (Scott *et al*., 1976) [22]. SCR occurs in all continents; furthermore, future trends might incline toward a general reduction in incidence in the Southern Hemisphere concomitant with a general increase in incidence in the Northern Hemisphere (Ramirez-Cabral *et al*., 2017) [20].
Despite the fact that *P. polysora* and *P. sorghi* have distinct morphologies, the differences between the two diseases and their causal organisms may be subtle or even impossible to detect (Crouch and Szabo, 2011) [7].

We carefully examined both species (Fig.1) as in the field, the colouration of uredinial sori may be an informative diagnostic character, with *P. polysora* pustules appearing orange to tan form on the upper surface of the leaves, and *P. sorghi* pustules appearing dark red to brown, sporadically scattered, and appeared only on upper leaf surface (Halvorson *et al.*, 2021 and Crouch and Szabo, 2011) [7, 12]. The urediniospores of common rust (Fig.1g) are roughly subglobose, and echinulate with three or four equatorial germ pores (Deadman *et al*., 2006) [8] whereas urediniospores of SCR (Fig.1d) are one-celled, yellowish to golden, echinulate, with 4–5 equatorial pores, hyaline towards the outer member, and had an ellipsoid shape appearing both on the lower and upper surface of the leaves (Halvorson *et al*., 2021; Crouch and Szabo, 2011) [7, 13].

The key determinants of grain rust spore germination, penetration, establishment, and spread are temperature and humidity (Pavgi, 1972) [18]. Temperature is a critical factor in plant disease development because the specific temperatures can affect the urediniospores germination and germ tube growth (Bonde *et al*., 2007) [3]. In this regard, our goal is to determine the optimum and cardinal temperature for urediniospore germination in relation to various incubation durations.

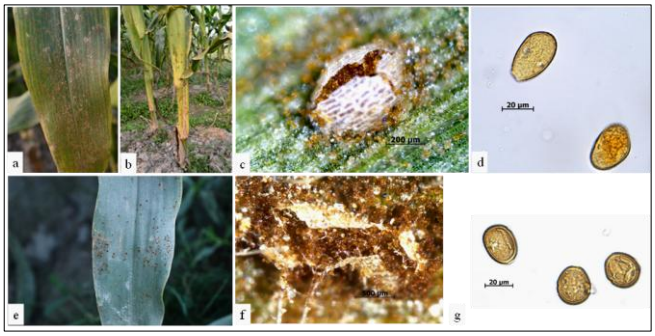

Fig 1: Symptoms and morphology of Southern corn Rust and Common rust. a-d, *Puccinia polysora*: a-b, field symptoms; c, uredial sorus; d, urediniospore. *Puccinia sorghi:* e, field symptom; f, uredial sorus; g, urediniospore.

2. Materials and Method

The fresh urediniospores with distinct pustules were collected from the maize field infected by the *Puccinia polysora* fungus, from the variety Kaveri 50, located at the AB Block farm, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal. The experimental design was a complete randomized design in an 8x5 factorial scheme, with an eight-level temperature factor (5, 10,15, 20, 25, 30, 35 & 40 °C), and the incubation time factor (2, 3, 6, 9 & 12 hours). Each treatment included 3 replications, with one plate per replication. Under in vitro conditions, fresh urediniospores were sprayed on cleaned and sterilized glass slides. Slides were covered with a thin smear of 1.5% water agar and were placed at sterilized Petri-dishes with moistened filter papers. The spore suspension was made by adding, 6g of fresh urediniospores to 10ml sterilized distilled water and 0.1 ml aliquot was poured in slides paced in Petri-dishes which were placed in B.O.D in dark conditions (Subrahmanyam *et al*., 1988) [25]. Spores were considered germinated when the germination reached a greater or similar diameter of the urediniospore (Menzies and Belanger, 1996) [17]. At the end of each incubation period, germination was interrupted through the addition of 0.1 ml of lactophenol on the surface of the water-agar medium. The observation on spore germination was recorded under the microscope at 40 X magnification. Percentages of germination were calculated for 100 spores on a slide. Percent urediniospores germination was calculated by the following formula:

$$\text{Percentage Germination} = \frac{A}{B} \times 100$$

Where A = No. of urediniospores germinated; B = No. of urediniospores observed.

Diseased symptoms were studied visually on the standing plants in the field and the picture was taken by the DSLR camera (Nikon D5600). Further, for a detailed study of the symptoms, a stereomicroscope [Zeiss Stereo (Model-Discovery V8) and Nikon (Model – SMZ 25)] were used. For Morphological observation, Zeiss light microscope (Axio Scope. A1) was used for the study of the morphology structure of the pathogen and the measurements were taken by using Carl Zeiss Vision (Axio Vision LE Rel.4.3) software.

The photographs were taken with the fitted camera with [Axio Cam ERc5s with Carl Zeiss Vision (Axio Vision LE Rel.4.3) Image Analyzing Software and Nikon Digital Cmos Camera (model- DSFi3) with compatible Image Analyzing Software]

3. Results and Discussion

The studies on the effect of temperature and incubation time on germination of urediniospores revealed (Table1) that maximum percent spore germination was found at 25 °C (25%, 33%, 57.67%, 67.67% and 78% at 2hrs, 3hrs, 6 hrs, 9hrs and 12hrs respectively). The optimum temperature range where the maximum germination of urediniospores was observed is 20 °C -30 °C. The data revealed that below 15°C and above 30 ºC greatly reduce the germination percentage. The cardinal temperatures where no germination was observed were 5 ºC and 40 ºC. The data represents that the time interval affects uredospore germination. Lower urediniospore germination was seen at 2-3 hours and maximal germination from 6 hours onwards, regardless of temperature. This suggests that for the study of better urediniospore germination, a minimum time interval of 6hrs is required. Because of the nature of the rust pathogen, which has the ability to germinate and infect under optimal environmental conditions soon after release, urediniospore germination commenced immediately after attachment to the substratum with the development of a germ tube (Chen *et al*., 2014) [6]. The cytoplasm inside of the urediniospores comes outside of the cell through the germ pore and it moved along with the germ tube (Shaw *et al*., 1998) [23]. In natural conditions, after the formation of germ tubes, usually appressorium is formed but in our research, germ tubes didn't produce any appressoria. It may be due to the even surface of the substrate because it was reported that the germ tube produces appressorium in natural conditions when it contacts with a topographic signal of the correct magnitude. (Hoch and Staples, 1987; Hoch *et al*., 1987) [14, 15]. Yang *et al.* (2015) [28] from China reported that the optimal temperatures for the germination of urediniospores of Southern corn rust and disease development are 26–28 °C and 24–27 °C, respectively and the temperature range for disease occurrence and development is 15–31 °C. The germination percentage increases with an increase in time though up to 6 hours the rate of increase of germination is comparatively high after

that the rate of increase of germination percentage slows down (Sunkad and Kulkarni, 2007) [26]. Our findings reveal that, while there is a wide temperature optimum for germination, the speed of germination responds strongly to even minor temperature changes (Dey *et al*., 2015) [8].

Table 1: Germination percentage mean values of Urediniospores of *Puccinia polysora* in function of temperature (^{O}C) and incubation period (hours)

Temperature ^{O}C	Time (Hours)				
	2h	3h	6h	9h	12h
5	0.00 * (0.00)**	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
10	7.67 (16.07)	15.00 (22.79)	17.00 (24.35)	20.67 (27.04)	21.67 (27.74)
15	15.33 (23.05)	24.00 (29.33)	34.67 (36.07)	38.33 (38.25)	44.33 (41.75)
20	20.67 (27.04)	30.67 (33.63)	37.00 (37.46)	43.33 (41.17)	56.33 (48.64)
25	25.00 (30.00)	33.00 (35.06)	57.67 (49.41)	67.67 (55.35)	78.00 (62.03)
30	19.33 (26.08)	18.67 (25.60)	35.00 (36.27)	42.67 (40.78)	61.00 (51.35)
35	2.67 (9.40)	3.00 (9.97)	13.33 (21.41)	18.33 (25.35)	21.00 (27.27)
40	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0)	0.00 (0.00)
Factors	C.D.	S.E(d)	S.E(m)		
Factor(A)	1.975	0.991	0.701		
Factor(B)	1.562	0.783	0.554		
Factor(A X B)	4.417	2.215	1.566		

*Original values; **Data in parenthesis are Arcsine transformed values; Factor (A) is the temperature (^{O}C) and Factor (B) is Time (Hours)

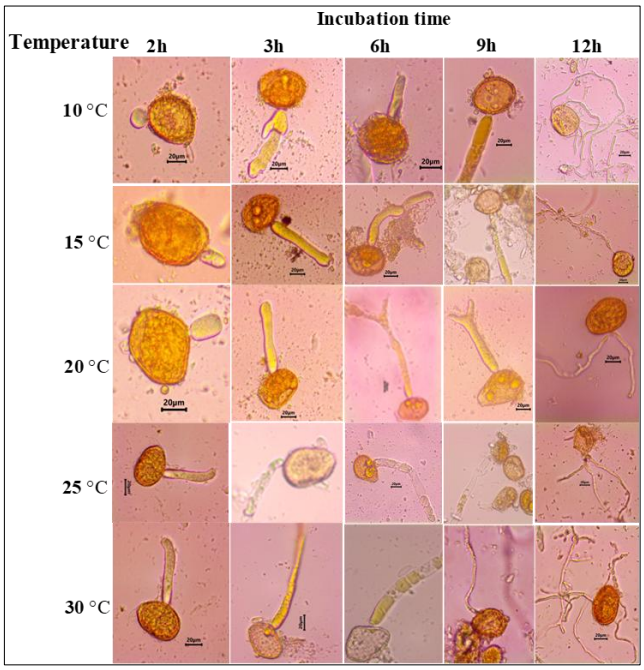

Fig 2: Growth of germ tube in different temperatures effect (°C) and incubation periods (h)

4. Conclusion

Urediniospore germination is the initial step in infection, and it is controlled by environmental conditions such as temperature and the duration of leaf surface wetness (Buck *et al*., 2010) [4]. The maximum percent spore germination was found at 25°C which makes it more vulnerable because the average temperature of India in the years 2017, 2018, 2019 and 2020 were 26.04 °C, 25.9 °C and 25.86 °C respectively (Statista, 2022) [23]. As a result, urediniospores, which play a critical role in disease development in the field, can germinate at extremely low temperatures (10 °C) during the rabi season and extremely high temperatures (35 °C) during the summer season, making them more vulnerable due to their ability to germinate in a wide range of temperatures.

5. Acknowledgement

The authors appreciate the financial support, laboratory facilities, and field facilities provided by All India Coordinated Research Project (AICRP) on Maize, Directorate of Research, Bidhan Chandra Krishi Viswavidyalaya. The authors appreciate the laboratory facilities provided by the All India Coordinated Research Project on Medicinal and Aromatic Plants and Betelvine (AICRP), Directorate of Research, Bidhan Chandra Krishi Viswavidyalaya.

6. References

1. Agarwal PC, Khetarpal R, Payak MM. Polysora rust of maize caused by *Puccinia polysora* and its spread in Karnataka. 2001;71:275-276.
2. Bonde MR, Berner DK, Nester SE, Frederick RD. Effects of temperature on urediniospore germination, germ tube growth, and initiation of infection in soybean by Phakopsora isolates. Phytopathology. 2007;97:997-1003.
3. Buck JW, Dong W, Mueller DS. Effect of light exposure on in vitro germination and germ tube growth of eight species of rust fungi. Mycologia. 2010;102(5):1134-40. DOI: 10.3852/09-283. PMID: 20943512.
4. Cammack RH. Studies on *Puccinia polysora* Underw: III. Description and life cycle of *P. polysora* in West Africa. Trans Br Mycol Soc. 1959;42(1):55-8. https://doi.org/10.1016/S0007-1536(59)80067-X.
5. Chen WQ, Wellings C, Chen XM, Kang ZS, Liu TG. Wheat stripe (yellow) rust caused by *Puccinia striiformis* f. sp. *tritici*. Molecular Plant Pathology. 2014;15(5):433-446. DOI: 10.1111/mpp.12116
6. Crouch JA, Szabo LJ. Real-time PCR detection and discrimination of the southern and common corn rust pathogens *Puccinia polysora* and *Puccinia sorghi*. Plant Dis. 2011;95(6):624-32. https://doi.org/10.1094/PDIS-10-10-0745
7. Cummins GB. Identity and distribution of three rusts of corn. Phytopathology. 1941;31:856-857.
8. Deadman ML, Al Sa'di A, Maqbali Y, Livingston S, Aime MC. First Report of *Puccinia sorghi* on Maize in Oman. Plant Dis. 2006;90(6):826. DOI: 10.1094/PD-90-0826C. PMID: 30781249
9. Dey U, Harlapur SI, Dhutraj DN, Pal D, Pawar DV. Effect of different temperature levels and time intervals on germination of uredospores of *Puccinia sorghi.*

African Journal of Microbiology Research. 2015;9(19):1299-1303.
10. Dolezal WE. Field Crops Rust Symposium, San Ontonio, TX, Dec, APS. 2015, 14.
11. FAO. FAOSTAT-Agriculture, Food and Agricultural Organizations of the United Nations, 2019. http://faostat3.fao.org/brpwse/Q/QC/E
12. Guo YY, Chen MG, Sun SL, Wu XF, Jiang K, Zhu ZD, *et al*. Genetic diversity of *Puccinia polysora* Underw. in China. Sci Agric Sin. 2013;46(21):4523-33. https://doi.org/10.3864/j.issn.0578-1752.2013.21.015 .
13. Halvorson J, Kim Y, Gill U, Friskop A. First report of the southern corn rust pathogen *Puccinia polysora* on *Zea mays* in North Dakota, Canadian Journal of Plant Pathology. 2021;43(2):S352-S357. DOI: 10.1080/07060661.2021.1982012
14. Hoch HC, Staples RC. Structural and chemical changes among the rust fungi during appressorium development. Annu Rev Phytopathol. 1987;25:231-247.
15. Hoch HC, Staples RC, Whitehead B, Comeau J, Wolf ED. Signaling for growth orientation and cell differentiation by surface topography in Uromyces. Science. 1987;235:1659-1662.
16. Krattiger A, Kulisek E, Casela C. Diagnosis maize diseases with propretary biotechnology applications transfered from Pioneer Hi-bred International to Brazil and Latin America. Embrapa Milho e Sorgo-Capítulo em livro científico (ALICE), 1998.
17. Menzies JG, Belanger RR. Recent advances in cultural management of diseases of greenhouse crops. Can J Plant Pathol. 1996;18(2):186-193.
18. Pavgi M. Morphology and taxonomy of the *Puccinia* species on corn and sorghum. Mycopathologia. 1972;47:207-20. https://doi.org/10.1007/ BF02051659
19. Payak MM. Interception of *Puccina polysora* southern rust of maize in India, New Delhi: NBPGR, IARI, 1992, 1-5.
20. Ramirez-Cabral NYZ, Kumar L, Shabani F. Global risk levels for corn rusts (*Puccinia sorghi* and *Puccinia polysora*) under climate change projections. J Phytopathol. 2017;165:563-74. https://doi.org/10.1111/jph.12593
21. Rodriguez-Ardon R, Scott GE, King SB. Maize yield losses caused by southern corn rust. Crop Sci. 1980;20:812-814.
22. Scott GE, Futrell MC. Southern corn rust. Crop. Soil. Mag. 1976 April-May, 16-18.
23. Shaw BD, Kuo K, Hoch HC. Germination and appressorium development of *Phyllosticta ampelicida* pycnidiospores. Mycologia. 1998;90(2):258-268. https://doi.org/10.2307/3761301
24. Statista. https://www.statista.com/statistics/831763/india-annual-mean-temperature/. 2022.
25. Subrahmanyam P, Reddy PM, McDonald D. Photosensitivity of urediniospore germination in Puccinia arachidis. Transactions of the British Mycological Society. 1988;90(2):229-232.
26. Sunkad G, Kulkarni S. Studies on Perpetuation and Carry Over of Groundnut Rust (*Puccinia arachidis* Speg.) in Northern Karnataka. Karnataka J Agric. Sci. 2007;20(2):297-300.
27. Underwood LM. Some new fungi chiefy from Alabama. Bull Torrey Bot Club. 1897;24(2):81-6. https://doi.org/10.2307/2477799.
28. Yang X, Ding XL, He ZL, Ma ZH. Determination of temperature required by southern corn rust. Plant Prot. 2015;41(5):145-7.
29. Yin S, Gao Z, Wang C, Kang Z, Zhang H. Nitric Oxide and Reactive Oxygen Species Coordinately Regulate the Germination of *Puccinia striiformis* f. sp. *tritici* urediniospores. Frontiers in microbiology. 2016;7:178. 10.3389/fmicb.2016.00178.