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Research paper

Effect of abiotic factors on pathotypes causing yellow and brown rust in wheat

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ABSTRACT

An attempt was made to determine the most favourable abiotic factors influencing germination of urediniospores of different pathotypes of *Puccinia* species. The causal organism of rusts in wheat is *Puccinia* spp. Five pathotypes of *Puccinia striiformis* (46S119, 78S84, 110S84, 110S119, 238S119) causal organism of yellow rust and two pathotypes of *Puccinia triticina* (77-5 and 77-9) causal organism of brown rust in wheat were obtained from Department of Plant Pathology, Punjab Agricultural University, Ludhiana. The data related to spore germination on agar slides was analysed and the levels of urediniospores germination at different temperatures (5,10,15 and 20°C) and pH (5,6,7 and 8) for each pathotype was compared using analysis of variance. The most appropriate temperature and pH were later used to conduct an experiment to study effect of different light intensities (500, 750,1000 and 1250 lux) on spore germination of all the pathotypes under study. The data showed that on agar, 15°C was proved as most suitable for urediniospore germination for *Puccinia striiformis*. Mean per cent spore germination was highest over the temperature range 15°C (43.55%) for *Pst* pathoypes and dropped significantly at 10°C (37.97%), 20°C (29.66%) and 5°C (21.04%). Mean urediniospore germination for *Puccinia triticina* was highest at 20°C (43.89%) followed by 15°C (39.44%), 10°C (30.43%) and ***5°C*** (27.39%). Experimental results revealed that per cent spore germination was better under pH 7 followed by 6, 5 and 8 for all the pathotypes considered for study. The highest urediniospore germination was observed for 1250 lux (46.54%) followed by 1000 lux (41.29%), 750 lux (38.42%) and 500 lux (27.60%).

Key words: *Puccinia* spp., Yellow rust, Brown rust, Urediniospore germination, Abiotic factors

Yellow rust is deadly fungal disease of wheat caused by *Puccinia striiformis* which delimits its yield in the north western plain and hill zone (NWPHZ) of country. Since 2008, the disease has been present in mild to severe forms and has resulted in losses up to 68.8% (Pannu *et al.,* 2010), which translated to a monetary loss of Rs 236 crore in Punjab during the epidemic year of 2009-10. In sub-mountainous areas of Himachal Pradesh, Jammu & Kashmir, Punjab, Haryana, and Uttarakhand, yellow rust frequently occurs in higher proportions whereas leaf rust (caused by *P. triticina*) is prevalent wherever wheat is grown. According to Kolmer *et al.,* (2009), yield losses of more than 30% can be attributable to 60-70% infection on the flag leaf at spike emergence. Defoliation during the jointing stage can result in 95% yield losses whereas; defoliation during the dough stage can result in 10% yield losses. Pathotype 46S119 is believed to have evolved from pathotype 46S103 (+vYr9) and was the first pathotype in India to have virulence to Yr9 (Prashar *et al.,* 2007). The samples taken from across Punjab showed that the most common Pst, 78S84 and 46S119, had frequency variations of 75:25, 35:65, and 20:80 in the years 2010-11, 2011-12, and 2012-13, respectively (Pannu *et al.,* 2014). The pathotype 78S84, which predominated from 2005-06 to 2010-11, was not observed in nature during the two growing seasons of wheat (2017-18, 2018-19). It's interesting to note that pathotypes 110S119 and 238S119 have gradually increased in frequency. Samborski (2013) revealed that in the case of more than 50% of the brown rust infected plants, the most important parameters were number of days with fog, relative humidity, low humidity and cloud cover. Sunkad *et al.,* (2023) observed that maximum colony growth of pathogen and the dry root rot disease severity was at 30-35ºC which is considered as optimum temperature range for growth of pathogen and development of disease. Highest severity of dry root rot and lesser plant growth parameters such as root length, shoot length and total biomass were

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observed at 40-60% soil moisture regimes, irrespective of type of soil.

Yellow rust is a polycyclic disease, therefore if infection occurs; weather parameters like temperature, humidity can downplay the condition or cause epidemics. It has been determined that night temperature between 10 and 12°C are ideal for uredospore germination (Line, 2002). Temperature and light play significant roles in the spore germination and subsequent infection of the host plant by different rust pathogens (Bonde *et al.,* 2007). Exposure of dry or hydrated urediniospores of *P. hemerocallidis* to cool white fluorescent light (600 μmol s^{-1} m^{-2}) or to sunlight (950—1910 μmol s^{-1} m^{-2}) for 2 hour or 4 hour significantly reduced germination and germ tube elongation on detached daylily leaves. A 4-hour exposure to either light source significantly reduced lesion development of *P. hemerocallidis* on detached daylily leaves with fewer lesions developing from hydrated compared to dry urediniospores. Increasing exposure to fluorescent light negatively affected sporulation of *P. hemerocallidis* and *P. pelargonii-zonalis*. Complete suppression of sporulation was not observed for either fungus with up to a 24 hour exposure to fluorescent light. Light exposure negatively affected disease development by *P. hemerocallidis* and *P. pelargonii-zonalis*. Exposure to high light intensities may affect spread of rust diseases on ornamental plants (Dong and Buck 2011).

It is evident from different studies that urediniospore germination of different pathotypes of *Puccinia spp.* is influenced by temperature and light but no such study has been conducted for new pathotypes of *Puccinia spp.* emerging at present in Punjab. So, this in-vitro study was planned to determine conducive abiotic factors for germination of urediniospores of different pathotypes of *Puccinia spp*.

MATERIAL AND METHODS

Pathotypes and their maintenance in growth cabinets

Five pathotypes of *Puccinia striiformis* (46S119, 78S84, 110S84, 110S119, 238S119) and two pathotypes of *Puccinia triticina* (77-5 and 77-9) were obtained from Department of Plant Pathology, Punjab Agricultural University, Ludhiana. These pathotypes were maintained on seedlings of the susceptible cultivar PBW 343 grown in pots at Pot house, Department of Plant Pathology, Punjab Agricultural University, Ludhiana during *rabi* season, 2021-22. Approximately, 10-12 seeds of PBW 343 were sown in 12.5 cm diameter pots having sterilised soil and covered with transparent sheet as shown in Fig. 1.

Fig. 1: Growth cabinets placed at an appropriate distance

The pots were placed in a growth cabinet for 8-10 days. When the first leaf was fully expanded, the seedlings were inoculated with the available pathotypes. Fresh urediniospores were collected, in a clean dry boiling tube, from a stock pathogen culture. These urediniospores were transferred to the surface of the first seedling leaves by means of a paint brush, previously sterilised with ethanol. The pots of seedlings were then transferred to the growth cabinet. The growth cabinets were left open from one side during the day and sprayed with water during evening and kept closed overnight which permitted optimum relative humidity conditions for uredospore germination. All the seven growth chambers were maintained at an appropriate distance from each other.

Spore germination study

Working with agar, agar coated slides were prepared, under aseptic conditions by pipetting on each slide. Three slides for each pathotype, germination temperature (5,10,15 & 20°C) and pH (5,6,7&8) treatment were arranged and inoculated in laminar air flow. The pH was adjusted using 0.5 N sodium hydroxide (NaOH) and 2.0 N hydrochloric acid (HCl). The bottom of each petri plate was lined with a moist filter paper so that a relative humidity of approximately 100% could be rapidly achieved. Following inoculation, the replicated slides were allocated to each of the temperature treatments in incubator in light and dark alternatively after 12 hours. After 48 hours of incubation, the slides were removed from the petri plates and germination was assessed by direct microscopic observation. For each replicate, ten sample spores were counted. All those spores having germ tubes at least as long as their width were considered as germinated. The most appropriate temperature and pH were later used to conduct experiment to study effect of different light intensities (500, 750,1000 and 1250 lux) on spore germination of all the pathotypes which were adjusted using coloured paper and measured by luxmeter.

Statistical analysis

The mean per cent spore germination (MPSG) for each sample was then calculated and the mean value for each replicate and treatment was calculated. The data was analysed and the levels of urediniospore germination at different temperature, pH and light for each pathotype were compared using analysis of variance using trial version of SPSS software.

RESULTS AND DISCUSSION

Effect of temperature

The first stage of infection is urediniospore germination, which is influenced by environmental factors like temperature (Buck *et al.,* 2010). In this experiment, spore germination was first observed visually at different temperature levels. MPSG of five pathotypes of *Pst* (46S119, 78S84, 110S119, 110S84, 238S119) and two pathotypes of *Pt* (77-5 and 77-9) was calculated. The data showed that on agar, 15°C proved as most suitable for urediniospore germination for *Pst* and 20°C for *Pt*. The MPSG of *Pst* pathoypes was statistically different from each other at all temperature levels (Table 1). MPSG was more over the temperature range 15°C for *Pst* pathoypes 238S119 (49.68%) followed by 110S119 (46.54%),

Table 1: Spore germination (%) of *Puccinia striiformis* f.sp. *tritici* and *Puccinia triticina* pathotypes under different temperature, pH levels and light intensity

Pathotype	Spore germination (%)											
	Temperature (°C)				pH Levels				Light intensity (lux)			
	5	10	15	20	5	6	7	8	500	750	1000	1250
Pst 238S119	31.34	43.17	49.68	38.47	37.08	49.23	57.00	19.36	37.97	48.80	53.50	62.83
Pst 110S119	26.59	41.64	46.54	33.79	33.26	43.57	53.40	18.33	31.43	44.87	46.20	53.53
Pst 110S84	18.06	37.14	42.46	27.23	28.50	37.74	42.33	16.31	31.50	40.9	42.43	43.13
Pst 46S119	15.53	34.86	41.18	26.94	27.12	35.27	39.95	16.17	25.53	31.43	31.60	42.43
Pst 78S84	13.70	33.04	37.88	21.86	24.37	32.39	36.94	12.78	21.46	28.30	31.40	35.10
Pt 77-5	24.35	26.68	37.43	42.95	31.46	36.45	48.37	15.15	25.53	41.10	43.73	44.90
Pt 77-9	30.42	34.17	41.45	44.42	38.55	44.84	51.21	18.61	19.76	33.57	40.17	43.77
	CD (p=0.05) = 1.09				CD (p=0.05) = 1.09				CD (p=0.05) = 2.82			

110S84 (42.46%), 46S119 (41.18%) and 78S84 (37.88%), but dropped significantly at 5°C for pathotype 238S119 (31.34%) followed by 110S119 (26.59%), 110S84 (18.06%), 46S119 (15.53%) and 78S84 (13.70%). MPSG for both *Pt* pathotypes was statistically different at different temperature levels except in case of 77-5 pathotype for which it was statistically similar at 5 and 10°C (Table 1). MPSG for *Pt* was highest at 20°C where pathotype 77-9 achieved approximately 44.42% and 77-5 achieved 42.95% germination. At higher temperatures, the rate of germination dropped prominently to 30.42% in pathotype 77-9 and 24.35% in case of 77-5 pathotype. The specific temperatures can alter the urediniospores germination and proliferation of their germ tubes, temperature plays a crucial role in the development of plant diseases (Bonde *et al.,* 2007). Yellow rust is a polycyclic disease, therefore if infection happens; climatic factors like temperature can downplay the condition or cause epidemics. It has been determined that night time temperatures between 10 and 12°C are ideal for urediniospore germination (Line, 2002). The rate of germination responds substantially to even small temperature changes, with a wide temperature range acting as the optimal for germination (Dey *et al.,* 2015). The minimum temperature in range of 7-13°C was observed as most favourable for yellow rust advancement under Punjab conditions in field (Sandhu *et al.,* 2021). Chhetri and Debnath (2022) conducted studies on the effect of temperature and incubation time on germination of urediniospores and revealed that maximum per cent 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 minimum and maximum temperatures where germination percentage was found to be nil were 5°C and 40°C, respectively.

Effect of pH

The spore germination was maximum under pH 7 followed by 6, 5 and 8 which were statistically different for all the pathotypes considered for study. It was unsurprising to find that the MPSG was also more at 15°C for *Pst* (46S119, 78S84, 110S119, 110S84, 238S119) and 20°C for *Pt* (77-5 and 77-9) under all the pH treatments. This experiment was performed as a preliminary investigation to find the most appropriate temperature. MPSG of all the pathotypes is compared in Table 1. The data showed that on agar, pH 7 proved most suitable for urediniospore germination for *Pst* as well as *Pt*. MPSG of 238S119 was higher at pH 7 (57.00%) followed by 6 (49.23%), 5 (37.08%) but dropped significantly at pH 8 (19.36%). The MPSG for *Pt* was also highest at pH 7 where pathotype 77-9 achieved approximately 51.21% and pathotype 77-5 achieved 48.37% germination. Deng and Suzuki (2008) conducted study on germination of basidiospores at different temperature and pH levels. They observed that the basidiospores germinated at 10°-35°C with an optimum at 25°-30°C. The highest germination percentage (83.0%) was observed in aqueous solution at pH 8.0.

Effect of light

The spore germination of different pathotypes was observed vigilantly. By carefully examining the data, it was observed that MPSG of *Pst* (46S119, 78S84, 110S119, 110S84, 238S119) and of Pt (77-5 and 77-9) was highest at 15°C and 20°C, respectively at pH 7. So, this information was used to conduct experiment to study the effect of different light intensities (500, 750,1000 and 1250 lux) on spore germination of all the pathotypes under study. This is schematically presented in Table 1. The most intriguing finding was that all the pathotypes showed an increasing trend in MPSG with increase in light intensity. The type of affect and the degree to which light affects germination, however, differ depending on the light source and the species of rust.

At specific temperatures, *Pst* pathotypes MPSG was accelerated by fluorescent light. MPSG under all the light intensity levels was statistically different from each other (Table 1). The average highest urediniospore germination was observed for 1250 lux (46.54%) followed by 1000 lux (41.29%), 750 lux (38.42%) and 500 lux (27.60%). Among the pathotypes, the average highest urediniospore germination was observed for 238S119 (50.78%) followed by 110S119 (44.01%), 110S84 (39.49%), 46S119 (32.75%) and 78S84 (29.08%). Likewise, pathotype 77-9 showed higher (38.82%) MPSG with increase in light intensity as compared to 77-5 pathotype i.e. 34.32%. It is known that light has an impact on germination of urediniospores from various species of rust fungi. Similarly, Buck *et al.,* (2010) studied the effect of light on different species and germination. When other conditions are favourable (such as free water is present and the temperature is favourable), light suppresses germination in several rust fungus. Increased exposure to fluorescent light negatively affected some *Puccinia spp*., relatively unaffected in 10 h incubation. Exposure of *Puccinia triticina* urediniospores to sunlight rapidly reduced germination and germ tube elongation. Germ tube elongation but not germination of hydrated urediniospores of *P. triticina* was significantly reduced compared to dry urediniospores exposed to 10 h fluorescent light followed by 24 h dark incubation. Exposure to fluorescent light (all fungi) or sunlight (two fungi) negatively affected urediniospore germ tube elongation.

CONCLUSIONS

From this in vitro study, it can be concluded that urediniospore germination of pathotypes of *Pst* and *Pt* was maximum at 15°C and 20°C, respectively. pH 7.0 favoured urediniospore maximum germination. Light play significant role in the urediniospore germination. It has been observed that in Punjab temperatures are rising during *rabi* season and from this experiment, it is evident that urediniospore germination is favoured by more light intesity and temperature in range of 15 to 20^{o}C so if high temperatures along with sunny days prevail rust can flourish in fields.

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