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Effect of Temperature on Growth and Sporulation of Rice Leaf Blast Pathogen *Magnaporthe oryzae*

Laxman Singh Rajput*, Taru Sharma, Puchakayala Madhusudhan and Parimal Sinha

Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi-110012, India
**Corresponding author*

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A B S T R A C T

Temperature rise due to climate change is expected to change pathogenicity of the pathogen. Temperature has a significant influence on growth and sporulation of rice leaf blast pathogen (*M. oryzae*). Both growth and sporulation were increased up to a temperature (27°C) and declined further in response to increased or decreased with temperature (32°C and 22°C). Various components of rice blast infection observed maximum at 27°C (optimal temperature) compared to suboptimal (22°C) and supra-optimal (32°C) *i.e.* growth and rate of growth in RSEDOMA media, lesion development and rate of lesion development in susceptible variety PRR78, sporulation and rate of sporulation in RSEDOMA media and susceptible variety PRR78. Rise in temperature leads to increase in growth and sporulation of *M. oryzae* in tropical and subtropical regions of the world, it's to be expected to enhance in aggressiveness of *M. oryzae* that results rice blast epidemic in tropical and subtropical regions of the world. Therefore, the impact of temperature on infection components may use for development of crop loss assessment models, rice blast prediction models, evaluation of genotypes for the development of future plant diseases managements strategies.

Introduction

Rice (*Oryza sativa*) is such an important cereal crop, which could provide 20% of total energy intake worldwide and lead staple food for >50% of world's inhabitants (FAO, 2014). Among 36 fungal diseases of rice, rice blast caused by *Magnaporthe oryzae* (Herbert) is very notorious pathogen to decline rice world's production by ~8% per year (Wilson and Talbot, 2009). With this capacity, it shows that rice blast outbreaks are the serious and recurrent problem in all rice-growing regions of the world. In India, rice blast is a serious concern due to favourable climatic condition during the crop season. Weather plays an important role in the appearance, multiplication and spread of blast fungus. Minimum night temperature which needed for blast epidemic is ranges from 20°–26°C, with the association of >90% of relative humidity, dew deposit, extended leaf wetness period (> 10 h) and cloudy drizzling weather during any crop growth stage of susceptible varieties (Padmanabhan, 1965). The interaction among a susceptible host plants, a virulent pathogens and the environment results in plant diseases. Various changes in climatic conditions are always associated with disease levels because the climatic conditions

significantly influence plants, pathogens and their antagonists. Pathogen biology may be directly influenced by climatic conditions in quite a lot of ways. The progressing periods of encouraging temperature, rainfall and relative humidity (RH) those near to the optimal conditions for the pathogen development and dispersal may lead to the incidence of ruthless epidemics. As temperature increases, most of the pathogens will be extend into new geographic areas with new potential hosts and more virulence. Temperature and RH also influence the pathogen survival during over wintering and over summering (Agrios, 2005).

Conidial germination, formation of appressoria and penetration are the important stages of infection process in *M. oryzae*. Various climatic condition influences infection process. *M. oryzae* requires 25-28°C (Sueda, 1928; Suzuki, 1969) and 16-32°C (Liang, 1979) of temperature ranges for conidial germination and while no germination of conidia occurred at 10-15°C (Nishikado, 1927). Spore germination in *M. oryzae* started within 3 hours after host tissues attachment at 18-38°C if it was wet (Kato, 1974) and delayed if dry period exposed (Kingsolver *et al*., 1984). Appressoria formation required a wide range of temperatures (Suzuki, 1969; Yoshino, 1972; Kato 1974; Rahnema, 1978). In *in vitro*, appressoria formation observed at the temperature of 21-30°C but interestingly RH had no impact on appressoria formation (El Refaei, 1977). Colonization of *M. oryzae* increased with increasing temperature up to 28°C and highest sporulation was possible at 20°C (Kato *et al*., 1970; Kato, 1974; Kato and Kozaka, 1974; El Refaei, 1977).

Global climate change, particularly increased in temperature and CO^2 levels (IPCC, 2007) are the consideration to manipulate all the disease triangle elements *i.e.*, host, pathogen and climate factors and their interactions (Legreve and Duveiller, 2010). Thus, climate change may influence disease incidence and severity and also influence further co-evolution of plants and their pathogens (Chakraborty, 2005; Burdon *et al*., 2006; Eastburn *et al*., 2011). Optimal temperature conditions for surviving of present species is available in tropical regions while pathogens surviving in cooler climates of higher latitudes required lower temperature; therefore, global warming is expected to their fitness enhance and the rise in epidemics risk of the disease with which they are associated (Ghini *et al*., 2011). The role of temperature on growth and development of *M. oryzae* is always under consideration for development of new simulation models; therefore the aim of this study was to determine the effect of temperature on growth and sporulation of *Magnaporthe oryzae* causing rice blast.

Materials and Methods

Source of biological material

To determine the effect of the three different temperatures 22, 27 and 32°C blast susceptible genotype PRR78 was used. Pusa basmati virulent isolate (MJ-24) of *Magnaporthe oryzae* obtained from IARI, PUSA, New Delhi-12.

Radial Growth on rice straw extract dextrose oat meal agar (RSEDOMA) media at three temperatures

The relative growth of *M. oryzae* was measured on RSEDOMA media (Rice Straw 20g; dextrose 20g; oat meal 20g; agar 20g; biotin 25ng; per litre distilled water). In RSEDOMA media at the centre of the Petri-plate a mycelial disk (0.4 cm) was inoculated and incubated in the BOD at temperatures of 22, 27, 32°C respectively. Average relative growth was measured at 10^{th} day after incubation at respective temperature.

Sporulation on RSEDOMA media at three temperatures

For sporulation induction, *M. oryzae* mycelial disk (0.4 cm) were inoculated in RSEDOMA media and kept in a BOD incubator (attached with black fluorescent tube of wavelength range of 350-390 nm) with cycles 14 h Near-UV light and 10 h dark at set of temperatures of 22°, 27°, 32°C respectively (Yaegashi and Herbert, 1976; Talbot *et al*., 1993). For fluorescent light exposure, the slants were kept at 20 cm distance from the light source for induction of sporulation.

Inoculation of plants for sporulation and lesion development

PRR8 a susceptible variety was raised in polypropylene pots (10 ×10 cm) filled with uniform soil mixture with optimum moisture supply. Six pots with three plants were prepared for each treatment. Inoculations were done at when the 6^{th} leaf was half emerged with @ 10^5 conidia/ml of sterile water (Sharma *et al*., 2005) containing 0.02% Tween 20 (Ghatak *et al*., 2013).

Plants were maintained in growth chamber 1 week before inoculation with nearly 70% of RH and day and night temperatures 26°C or 24°C. Plants with mock inoculation (sterile water with 0.025% Tween 20) were used negative control. Plants were covered with black polythene bags and incubate under the dark condition at three different temperatures *i.e.*, 22°, 27° and 32°C with the daily cycle of 14 h light (>95% RH) followed by 10 h of darkness (> 95% RH).

Blast spot or lesion size was measured through software *i.e.*, Assess 2.0 (Lakhdar, 2008). With the same sample, spores number was counted using a haemocytometer under the microscope (Ghatak *et al*., 2013).

Results and Discussion

Selection criteria for selecting of temperatures

Based on infection ability model three temperatures were selected for this study *i.e.*, 22°, 27° and 32°C. Infection ability (*y*) model can explains sporulation and lesion development rate at temperature T (°C) $y = r[T] = 0.24 \times [(34.1-T)/6.6] [(T-7.9)/19.6]^{2.9697}$. These temperatures were designated as suboptimal (22°C), optimal (27°C) and supra-optimal (32°C) temperature for *M. oryzae* (Viswanath, 2015).

Effect of temperature on growth of *M. oryzae* in RSEDOMA media

Temperature effect significantly *M. oryzae* growth in media. Maximum growth was observed at 27°C (41.7 mm), compare to 32°C (26.5 mm) and 22°C (24.5 mm). At optimal temperature (27°C) growth was nearly double in media compare to 22°C and 32°C. Similarly, maximum rate observed at 27°C (4.2 mm/day), compare to 32°C (2.6 mm) and 22°C (2.4 mm) (Fig. 1 and Table 1).

Effect of temperature on lesion size of *M. oryzae* on PRR 78 variety

Lesion size developed on PRR 78 variety by *M. oryzae* were significantly influenced by temperature. As temperature increased lesion size developed up to a temperature and later on decreases. Maximum lesion size was observed at 27°C (45 mm^2), compare to 22°C (7.5 mm^2) and 32°C (5.5 mm^2). Lesion size developed at optimal temperature (27°C) were nearly 6 times higher compare 22°C and 9 time higher compare 32°C. As results showed higher temperature 32°C was not suitable for blast symptom development and growth. Similar, maximum lesion development rate was observed at 27°C (3.21 mm^2/day),

compare to 22°C (0.54 mm^2/day) and 32°C (0.39 mm^2/day) (Fig. 1and Table 1).

Effect of temperature on sporulation of *M. oryzae* in RSEDOMA media

Temperatures were affected tremendously sporulation of *M. oryzae* in RSEDOMA media. Maximum sporulation was observed at 27°C (2.22×10^5spores/ml of sterilize water) compare to 22°C (1.67×10^5 spores/ml of sterilize water) and 32°C (1.47×10^5 spores/ml of sterilize water). Sporulation at optimal temperature was 1.51 times more compare to 32°C temperature and 1.33 times more compare to 22°C. That mean at higher temperature sporulation efficiency decreased significantly. Maximum sporulation rate was observed at 27°C (0.22×10^5 spores/ml of sterilize water/day) compare to 22°C (0.17×10^5 spores/ml of sterilize water/day) of sterilize water) and 32°C (0.15×10^5 spores/ml of sterilize water/day) (Fig. 2 and Table 2).

Table.1 Effects of temperature on *M. oryzae* growth

Temperatures (°C)	Growth and growth rate of *M. oryzae*			
	RSEDOMA media		PRR78 variety	
	Growth in RSEDOMA media (mm)	Growth rate (mm/day)	Lesion size (mm^2)	Lesion development rate (mm^2/Day)
22	24.5 ± 4.8^c	2.4	7.5 ± 0.8^b	0.54
27	41.7 ± 5.8^a	4.2	45 ± 6.8^a	3.21
32	26.5 ± 4.2^b	2.6	5.5 ± 0.6^b	0.39
CD at 1%	1.12		3.23	

Table.2 Effects of temperature on *M. oryzae* sporulation

Temperatures (°C)	Sporulation and rate of sporulation of *M. oryzae*			
	RSEDOMA media		PRR78 variety	
	Sporulation (spores/ml of sterilize water)	Rate of sporulation (spores/ml of sterilize water/day)	Sporulation (spores/mm^2 of lesion)	Rate of sporulation (spores/mm^2 of lesion/day)
22	1.67×10^{5b}	0.17×10^5	1.12×10^{5b}	0.813×10^5
27	2.22×10^{5a}	0.22×10^5	24.1×10^{5a}	1.721×10^5
32	1.47×10^{5c}	0.15×10^5	0.66×10^{5c}	0.004×10^5
CD (P =0.01)	1322.6		862.5	

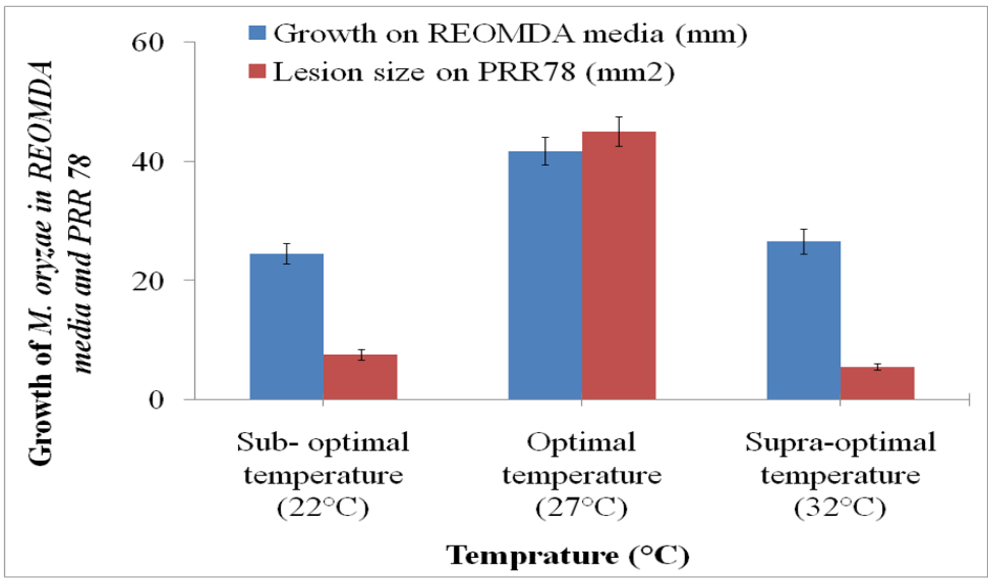

Fig. 1. Effects of temperature on *M. oryzae* growth

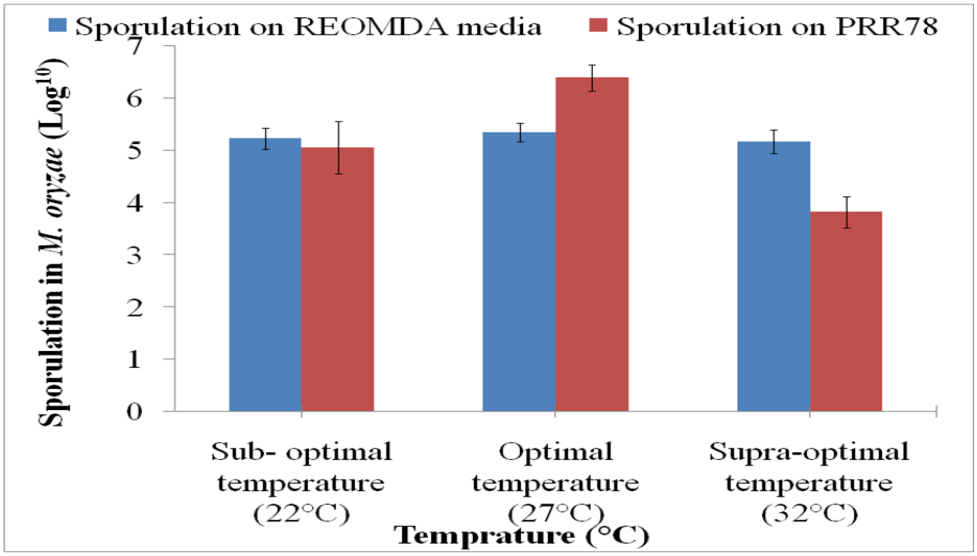

Fig. 2 Effect of temperature on *M. oryzae* sporulation

Effect of temperature on sporulation of *M. oryzae* in PRR78 variety

In susceptible variety PRR78, *M. oryzae* was highly sporulated at optimal temperature (27°C). Sporulation was affected significantly by temperature. Maximum sporulation was observed at 27°C (24.1×10^5 spores/ mm^2 of lesion) of compare to 22°C (1.12×10^5 spores/ mm^2 of lesion) and 32°C (0.66×10^5 spores/ mm^2 of lesion). At 27°C sporulation was nearly 365 times higher than 22°C and 21.5 times higher than 22°C. At 27°C sporulation rate was observed maximum (1.721×10^5 spores/ mm^2 of lesion/day) compare to 22°C (0.813×10^5 spores/ mm^2 of lesion/day) and 22°C (0.004×10^5 spores/ mm^2 of lesion/day). It was observed that at the higher temperature, sporulation of *M. oryzae* was inhibited (Fig. 2 and Table 2).

Temperature influence on growth and sporulation of *M. oryzae* was observed at three temperatures *i.e.,* 22°C, 27°C and 32°C, where they were unknown. *M. oryzae* showed a typical kind of growth pattern. It observed that growth and sporulation of *M. oryzae* effected by temperature on RSEDOMA media as well as susceptible rice plants. The growth of fungus in RSEDOMA media and lesion development in susceptible plant both were observed maximum at optimal temperature (27°C) and compare to sub optimal (22°C) and supra-optimal temperatures (32°C). Interestingly less growth was observed in susceptible plant compare to media in both 22°C and 32°C temperatures, that indicate it may be by operating PTI in the plant (Boyd *et al*., 2013) or temperature effects on pathogen (Luo *et al*., 1998) or cumulative of both. Leaf blast infection and host evasion profoundly affected by temperature (Luo *et al*., 1998) and that play a key role in the epidemic of leaf blast (Kato and Kozaka, 1974; Teng *et al*., 1990). Similarly, at 16°C and 20°C lesions developed slowly than at 25°C and 32 °C (Kato and Kozaka, 1974) and higher temperature restricted lesion development (Yoshino and Yamaguchi, 1970). For successful infection, *M. oryzae* produce appressorium, that also tremendously inhibited at the higher temperature (34°C) which indicated that pathogen growth was significantly reduced at the higher temperature (Viwsanath *et al*., 2015). Sporulation in *M. oryzae* also showed the same kind of trend like the growth of *M. oryzae*. Extremely decline in sporulation at the higher temperature (32°C) indicated that pathogen unable to infect host plant at the higher temperature. The cardinal temperatures for sporulation are about 9-12°C, 25-28°C and 34-35°C (Henry and Anderson, 1948) and range of temperature for sporulation was reported as 18-32°C (Madden and Ellis, 1988). Sporulation and lesion development are the component of infection process and they are the key determinants of rice blast epidemics. Results of this experiment showed that temperature has the huge impact on both sporulation and lesion development. According to infection model, each degree changed in temperature lead to change nearly 0.20 unit of sporulation and lesion development rate and results of this studies showed that each degree changed in temperature changed growth in media (0.36 mm), lesion development (0.53 mm^2), sporulation in media (1000 spores) and plant (17800 spores). As we know, the temperature will be rise by 1.5-4.8°C by the end of this century, globally (IPCC 2014) and presently rice is cultivated in regions where temperatures are optimal for growth (28°C/22°C). Sporulation and lesion development are the main component of rice leaf blast infection process. As the rise in temperature leads to increase in growth and sporulation of *M. oryzae*, it's to be expected to enhance in aggressiveness of *M. oryzae* that leads to create rice blast epidemic in the tropical and subtropical regions of the world. Therefore, due to climate change or enhancement in temperature could have a huge impact on rice blast epidemics. Specially, in South India, *rabi* rice is growing with temperature range 18° to 24°C, so there slight increases in temperature lead to enhancement in aggressiveness of fungus that may have a huge impact on rice harvest of South India. Therefore, the impact of temperature on infection components may use for development of crop loss assessment models, rice blast prediction models, evaluation of genotypes for the development of future plant diseases managements strategies.

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