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Influence of Temperature on Appressorial Formation in *M. grisea* in Relation to cAMP-dependent PKA Activity

K. Viswanath*, Taru Sharma, P. Sinha and Mukesh Kumar

Indian Agricultural Research Institute, New Delhi - 110 012, India

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For leaf blast pathogen (*Magnaporthe grisea*), surface features exerted significant influence on conidial germination and appressorium formation. However, external application of cAMP was observed to nullify the difference in germination and appressorium formation due to surface effect as well as temperature influence. PKA activity was significantly higher at 29°C as compared to 22°C and at 34°C no activity detected. MPG gene expressed during appressorium formation was found to be influenced by temperature as expression level of MPG gene was almost 44 fold at 29°C and 34 times at 22°C in comparison to 16°C. Morphogenesis of conidial germ tube tip into appressorium was significantly reduced by external glycerol application (2.5 % or above) and germ tube continued to grow without formation of appressorium. It indicated that cAMP regulated PKA activity was associated with high endogenous glycerol concentration inside the tip cell of the germ tube for influx of water from the host surface through osmosis. Blast pathogenecity is a sequence of events regulated by action of PKA and MAPK and therefore to be modified by temperature and surface influence for free moisture availability. For future management strategy, host surface feature and impact of temperature on blast infection may be vital input as rise in CO_2 and temperature level are thought to alter host-pathogen interaction under changing scenario.

Key words*:* Appressorium formation, *M. grisea*, Pathogenicity, hydrophobicity, PKA activity.

Leaf blast pathogen (*Magnaporthe grisea*) in rice produces specialized structures called appressoria to penetrate the host cell and allow the fungus to enter to underlying tissue [1]. Conidia can germinate at any surface but production of appressoria is unique and depends greatly on ability of fungal hyphae to attach and perceive an appropriate surface [2,3]. Molecular genetic analysis of pathogenic fungi is revealing the presence of conserved signal transduction pathways that regulate infection related development and production of plant disease symptoms [4,5]. The pathogen responds to extracellular cues via various signaling pathways for regulating variety of developmental and differential cellular processes. Extracellular signals are after converted into intracellular signal through secondary messenger such as cAMP. In *S. cerevisiae*, cAMP- mdediated signaling pathway regulates a variety of cellular processes like carbon metabolism [6], response to stress, cell cycle progression [7,8,9] and pseudohyphal growth [10]. A well characterized intracellular target of cAMP in eukaryotic cells is regulatory subunit of PKA [11,12] and binding of cAMP to regulatory subunits release catalytic subunits to phosphorylate more than 250 target proteins involved in appressorium formation and infection [13]. The cAMP response pathway regulates the breakdown of glycogen in the spore during its germination and that glycogen

* To whom all correspondence should be addressed.
E-mail: sinhapath@gmail.com

breakdown in the appressorium is a significant factor in regulating virulence-associated gene expression [14]. In developing appressorium glycogen and triacylglycerol metabolism occurs under control of PMK1 and MAPK pathway [15]. Glycerol accumulation and the time of peak PKA activity appears that PKA is involved in metabolism of fatty acids by beta oxidation, glycerol production through glyoxalate cycle and gluconeogenesis. These processes are likely to be regulated by the action of PKA and stimulated signal transduction pathway involving MPS1 MAPK [16,17]. The turgor generated by the appressorium is due to the glycerol accumulation which is required for cuticle penetration[18]. Lipid, glycogen and trehalose are the major nutrient reserves used by *Magnaporthe grisea* conidia for germination and appressorium differentiation [19] which in turn responsible for turgor generation and plant infection as glycogen reserves in appressoria are completely utilized during appressorium maturation, prior to plant infection [20,18,17]. During appressorium maturation, the rapid changes in primary metabolism are regulated in part by a trehalose-6-phosphate synthase (Tps)-mediating genetic switch based on the levels of glucose-6-phosphate and the NADPH/ NADP balance in cells and in turn interacts with three transcriptional inhibitors which regulate virulence-associated gene expression [21,22,23]. Several pathogenicity related genes involved in infection/growth within host like MPG [24], MPH [25], DES [26], PMK [27], PTH[28] has been reported in infection process for the pathogen and PMK1 mitogen-activated protein (MAP) kinase gene is reported to regulate appressorium formation and infectious growth while its homologs in many other fungi play critical roles in fugal development and pathogenecity[29]. Calcium/calmodulin-dependent signaling for appressorium formation in *Magnaporthe grisea* was reported[30] as several calcium modulators and calmodulin antagonists inhibited appressorium formation while conidia germination remained unaffected. There was an inhibition of appressorium formation by EGTA, a calcium chelator, which was restored by the addition of exogenous $CaCl_2$.

It appears that appressorium formation process in *M. grisea is* influenced externally by surface feature and internally regulated and/ or modulated by several cascading biochemical pathways ultimately leading to turgor pressure development for penetration. Role of temperature and host surface interaction during pathogenecity in terms of appressorium development is largely unknown. Therefore, the aim of this investigation was to assess the role of temperature and surface influence on appressorium formation in relation to cAMP-dependent PKA activity.

MATERIALS AND METHODS

Sporulation on artificial media

M. grisea isolate collected from naturally infected rice field was grown on Petriplates containing PDA at 28^{0}C for 5 days. For sporulation, slants (Rice Leaf Extract Sucrose Agar) were inoculated with mycelial disk (0.4 cm) from 5 days old culture and were kept in a BOD incubator (with black fluorescent tube of wavelength range of 350-390 nm) set at a temperature of 28^{0}C in a 14h light and 10 h dark cycle [24]. For fluorescent light exposure the slants were kept at 20 cm distance from the light source for induction of sporulation.

Surface hydrophobicity and wettability of artificial and natural leaf surfaces

For estimation of surface hydrophobicity and wettability the method given by Lee and Dean (1993)[31] was followed. Cotton blue solution (0.1%) of 50 µl was placed on test surfaces and allowed to dry in shade. Glass slide (Blue Star, microslides, Mumbai, India) and cover slip (Blue Star, microscopic cover glass, Mumbai, India) were used as artificial test surface whereas rice and pearl millet leaf as natural surface. Area of dye was scanned (300 dpi) and measured through Image analysis software (Assess 2.0). Hydrophobicity index was estimated by subtracting the area of the drop (mm^2) from 100. Surface wettability was measured and compared in rice and pearl millet leaves based on water drop and rain water interception.

Induction of appressorium development on artificial and natural leaf surfaces

For artificial surface cleaned glass slide and cover slip (fixed on glass slide with sticking agent) were used for appressorium formation. Conidia were harvested from 10 days old sporulating culture by scraping with a glass rod in sterile distilled water and diluted to a concentration of 10^5 spores/ml. Sterilized Petriplates (14 cm dia.) were used to make moist chamber using autoclaved

three layers of filter papers moistened with sterile water. Slides were set inside the moist chamber and 30 µL spore suspension was applied on the surfaces and kept in BOD incubator set at 29^{0}C for 24 h to form appressoria. Samples were removed at intervals of 9, 15 and 24 h for microscopic observation. Mean of 5 samples each containing about 100 germinating conidia was used for comparison. Standard error for sample size was found to stabilized nearing 92-108 conidia or above. As natural leaf surface, rice and pearl millet leaves were used for appressorium formation. Moist chamber was prepared as done earlier Sterilized glass slides were used to stick detached leaves on double sided stick labels. Forty-five days old leaves both from rice (Pusa basmati) and pearl millet (fertility restorer genotype) was fixed on sticky slides and 50 µl of spore suspension was applied and the plates were incubated at dark for 24 h at 27ºC.

Effect of temperature on appressorium development under external cAMP and glycerol application

To determine the effect of cAMP on appressorial formation, 10 per cent of 5mM cAMP was mixed directly to the conidial suspension and 30 µL of the suspension was placed on glass slide and cover slip surface fixed on the glass slide. For incubation at temperature of 22, 29 and 34^{0}C slides were placed inside moist chamber and kept for 24 h in set BOD incubators. Slides were removed at intervals of 9, 15 and 24 h for microscopic observation. Mean of 5 samples each containing about 100 germinating conidia was used for comparison. In another set of glass slides and cover slip along with cAMP, 30 µL glycerol solutions (0, 1.0, 2.5, 5.0 and 10.0 %) were applied.

Effect of temperature on appressorium development and PKA activity

Spore suspension was prepared from 8-10 days old culture grown on rice leaf extract agar slants and mixed with exogenous cAMP (0 and 5 mM). For conidial germination and appressorial formation, spore suspension was transferred to a 140 mm Petriplates and incubated for 24 h at temperatures 22, 29, and 34^{0}C. PKA estimation was done by using protocol assay (Pep Tag assay for Non-Radioactive detection of cAMP dependent Protein kinase A from Promega). Germinated conidia with appressoria were collected and centrifuged at 10000 rpm for 15 min at 4°C. The pellet was collected and subjected to grinding in a mortar pestle using liquid nitrogen in PKA extraction buffer (25 mM Tris Hcl at pH 7, 0.5 mM EDTA, 0.5 mM EGTA, 10 mM Beta Mercaptoethanol, 1 µg/ml Leupeptin and 1 µg/ml Aprotinin as per the manufacturer's instructions from Promega). The lysate was centrifuged (14000 X g) for 5 min and the supernatant stored at 4^{0}C.

Reaction mixture containing 5 µl PKA reaction 5X Buffer , 5 µl A1 Peptide (Pep-tag), 5 µl PKA activator 5X solution and 1 µl peptide protection solution was prepared in a 0.5 ml tube as per the protocol and kept on ice until addition of sample. The reaction mixtures prepared were incubated at 30^{0}C in water bath for 1 minute. For negative control, in addition to reaction mixture water was added to make final volume 25 µl. For positive control along with reaction mixture dilutions (using PKA dilution buffer- 380 mM K_3PO_4 and mM DTT) of cAMP-dependent protein kinase in variable concentrations (0.5, 2.0, 4, 6.0 and 8.0 µl) were added accordingly to make final volume of 25 µl. For assay of PKA activity in samples 10 µl of supernatants collected from the lysate were added to the reaction mixtures and followed by incubation at room temperature for 30 minutes. After incubation the reaction was stopped by placing the tubes on a dry bath at 95^{0}C for 10 minutes and stored at 4^{0}C until ready to load onto the gel.

Gel was prepared with 0.8 per cent agarose solution in 50 mM Tris-HCL (pH 8.0) and samples were loaded into the wells and run at 100 V for 15-18 in 50 mM Tris HCL buffer (pH 8.0). After separation the gel was removed and viewed under UV light.

The gel with negatively charged (phosphorylated) bands and positively charged (non-phosphorylated) bands were excised with a razor blade and transferred to 75 µl of gel solubilization solution and 50 µl of acetic acid and absorbance was read at 570 nm .

Total protein from samples (containing appresorium) was estimated through Bradford method indicated about 0.6-0.8 µg of total protein required for PKA activity which could be approximated to 5x10^5 conidia in 5 ml.

Pathogenicity gene expression under temperature influence

Mycelia were harvested from suspension culture of *M. grisea* grown in rice extract liquid medium at different temperatures (16, 20, 24, 26, 29°, 32° ± 0.5°C). RNA was isolated from each sample by standard Trizol method [32] from 5 days old cultures. For pathogenicity gene MPG1 cDNA was synthesized by kit (SV total RNA isolation system, Promega)> Real time PCR was performed in 20ìl volume containing 10ìl of each MPG primer (Forward Primer-AACAGCAAGGAGCTTAAGAA, Reverse Primer-CGAGCAGAAGTTGTTGATG), 10 ìl of SYBR green buffer, 2 ìl template cDNA for 95°C 3 min, 52°C 45 sec and 65 °C 60 sec for 40 cycles (Kim et al. 2005). No template control (to monitor the presence of primer dimer), non RT control and GAPDH as reference gene were maintained for above reactions. GAPDH (GAP) was used as reference gene as it was found to have minimum variation in expression in comparison to α–tubulin and α-EF. Average Ct values was normalized by using reference gene GAP for each conditions or treatments as 2Δ Ct where ΔCt= CtMPG – CtGAP.

RESULTS

Surface response on appressorium formation in relation to hydrophobicity and wettability

Surfaces like glass slide, cover slip and rice leaf surface were highly hydrophobic as compared to pearl millet leaf measured in terms of water retention (Table 1). Lower ratio of number of appressorium to conidia was found to correspond with higher degree of hydrophobicity in case of glass slide and rice leaf surface. However cover slip surface although having higher degree of hydrophobicity had shown appressorium formation four times higher than glass slide (Fig 1A). Surface wettability as a measure of continuous film of water and its retention, was estimated 8.1 in rice and in pearl millet surface 40.2, indicated leaf surface of the latter was more retentive and conducive for infection process than on rice (Table 1 and Fig 1B). Higher degree of hydrophobicity on rice leaf surface corresponded with low wettability and thus low water retention after rainfall whereas on pearl millet surface water retention was shown to be remarkably higher (Fig 2).

Comparable level of hydrophobicity between cover slip and glass slide but large variation in ratio of appressorium formation indicated only hydrophobicity was not the reason for difference. Difference in hydrophobicity between rice and pearl millet leaf appeared to be associated with moisture retention property that made rice leaf less conducive to pathogen than pearl millet leaf surface. Pearl millet surface probably favoured appresorium formation because of moisture retention and spore attachment. Thus leaf surface characteristics of the two hosts did play significant role in making difference in spore attachment, germination and appressorium formation.

Temperature response on conidia germination and appressorium development under cAMP

Temperature response was higher on glass slide as 79.6 to 88.4 % germination was observed in 22-29°C as compared to 83.8-97.1% in 34°C (Table 2). Interestingly influence of temperature was non-significant when the germination process was observed on cover slip as 90.3-100.0 % was observed irrespective of temperature. Therefore, it indicated that surface feature might be playing some role in conidia germination for the pathogen. However, addition of cAMP (5 mM) was shown to increase

Table 1. Wettability and hydrophobicity of artificial and host leaf surfaces (45 days old leaves of rice and pearl millet)

Surface type	Mean area of retention, mm2	Measure of wettability (A)	Hydrophobicity (100-A)	Ratio, number of appressorium /number of conidia
Glass slide	13.6	13.6	86.4 (± 4.2)	0.15
Cover slip	19.6	19.6	80.4 (± 6.3)	0.45
Rice	8.1	8.1	91.9 (± 3.2)	0.14
Pearl millet	40.2	40.2	59.8 (± 4.8)	0.43

Table 2. Effect of temperature on conidial germination and appressorium formation (*M. grisea*) over glass slide and cover slip under the influence of cAMP

Temp	*Germination % on glass slide (on cover slip)						*Appressorium % on glass slide (on cover slip)					
	9 h		18 h		24 h		9 h		18 h		24 h	
	No cAMP	cAMP	No cAMP	cAMP	No cAMP	cAMP	No cAMP	cAMP	No cAMP	cAMP	No cAMP	cAMP
22°C	79.6 ± 4.0 (90.3 ±9.9)	84.8±4.1 (84.0±5.0)	82.9±5.1 (92.6 ±9.4)	86.0±7.1 (95.1±6.8)	85.4±3.6 (93.8 ±6.1)	92.8±8.2 (96.9 ±4.1)	0.0±0.0 (14.8 ±8.6)	10.2±7.1 (41.4 ±6.3)	3.3±7.4 (20.8 ±3.6)	14.5±9.8 (80.7 ±6.3)	3.3±7.4 (23.9 ±6.2)	19.5±4.0 (81.8 ±6.0)
29°C	81.4 ±1.8 (92.9 ±6.7)	87.6±7.5 (90.6 ±5.7)	84.8±3.9 (96.6 ±7.4)	90.9±8.0 (96.6 ±7.4)	88.4±6.6 (97.1 ±6.3)	94.4±7.8 (98.1 ±3.6)	8.3±5.1 (37.6 ±4.1)	19.6±6.3 (44.7 ±4.5)	18.6±3.1 (52.3 ±7.7)	33.1±7.5 (83.6 ±5.9)	22.3±8.1 (58.3 ±8.5)	57.1±7.5 (85.1 ±9.4)
34°C	83.8±8.3 (94.1 ±8.1)	80.1±7.5 (94.5 ±8.2)	88.3±7.3 (95.4 ±6.5)	87.1±2.4 (100 ±0.0)	97.1±6.3 (96.0 ±8.9)	91.0±5.7 (100 ±0.0)	0.0±0.0 (0.0 ±0.00)	0.0±0.0 (0.0 ±0.0)	0.0±0.0 (0.0 ±0.0)	0.0±0.0 (0.0 ±0.0)	0.0±0.0 (0.0 ±0.0)	0.0±0.0 (0.0 ±0.0)

*Mean of five microscopic views (in percentage) with ±SD

Table 3. Temperature response on germination and appressorial formation (*M. grisea*) on coverslip surface in presence of glycerol

Temp °C	Glycerol%	*Germination%						*Appressorium%					
		9		15		24		9		15		24	
22		Without cAMP	cAMP	Without cAMP	cAMP	Without cAMP	cAMP	Without cAMP	cAMP	Without cAMP	cAMP	Without cAMP	cAMP
	0	89.1	89.8	82.6	91.2	93.8	96.9±	8.2	35.8	20.8	80.7	23.9	81.8
	0.1	85.5	86.0	87.7	88.5	89.8±6.7	93.2±5.0	0.0	0.0	11.6	66.6	14.9±5.3	82.1±8.2
	0.5	84.4	83.8	85.6	85.6	86.8±3.1	90.4±3.6	0.0	0.0	8.16	59.4	11.7±6.2	71.8±6.4
	2.5	75.3	79.9	81.2	82.6	84.5±5.1	86.6±3.8	0.0	0.0	4.4	56.4	5.9±1.8	67.6±5.4
	5	77.2	77.6	79.4	81.6	82.1±6.0	84.5±5.9	0.0	0.0	2.8	42.4	4.8±2.6	58.1±6.0
	10	70.3	72.0	78.3	79.9	80.2±9.2	83.3±7.8	0.0	0.0	2.5	28.2	4.6±2.7	33.1±7.5
CD (p=0.05)						8.26	7.16					5.18	8.98
29	0	76.5	86.7	79.6	89.5	97.1	98.1	35.2	48.7	52.3	83.6	58.3	85.1
	0.1	69.0	89.3	84.8	92.6	89.8±6.8	96.0±8.9	0.0	46.5	6.5	87.7	8.0±5.0	92.6±4.5
	0.5	66.9	88.7	81.8	90.3	88.1±9.8	91.3±8.3	0.0	12.5	6.2	83.2	7.4±4.2	85.3±6.7
	2.5	54.3	86.7	78.9	87.3	86.1±5.4	89.0±4.1	0.0	6.7	2.5	34.5	5.3±3.0	75.1±9.6
	5	27.7	85.6	75.4	80.5	84.9±3.8	82.8±8.7	0.0	1.7	1.6	27.7	4.1±3.8	42.6±7.7
	10	21.1	42.8	41.3	50.4	55.8±10.6	68.1±4.2	0.0	0.0	1.8	6.7	4.0±3.7	13.1±3.7
CD (p=0.05)						9.07	10.57		5.11	8.35			

*Mean of five microscopic views (in percentage) with ±SD

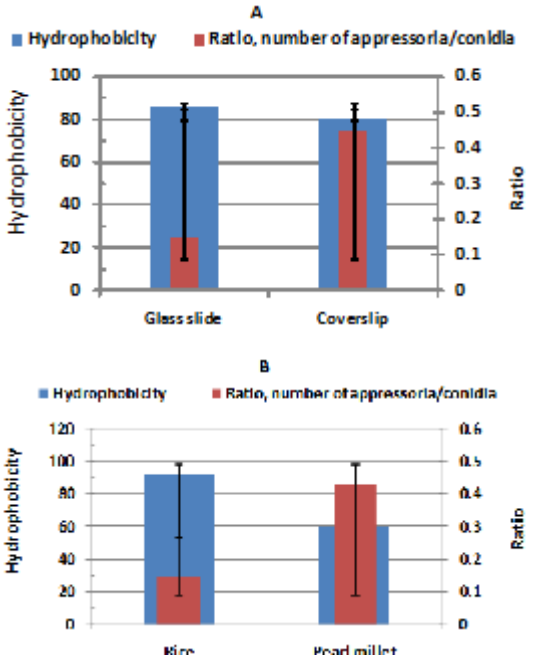

Fig. 1. Hydrophobicity and wettability on artificial surfaces (A) and host surfaces (B) used for appressorium formation (*M. grisea*)

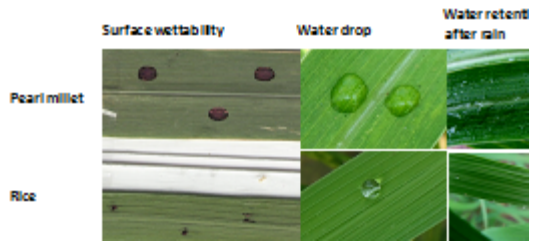

Fig. 2. Surface wettability, hydrophobicity and water retention on rice and pearl millet leaf surfaces in relation to drop application and drain.

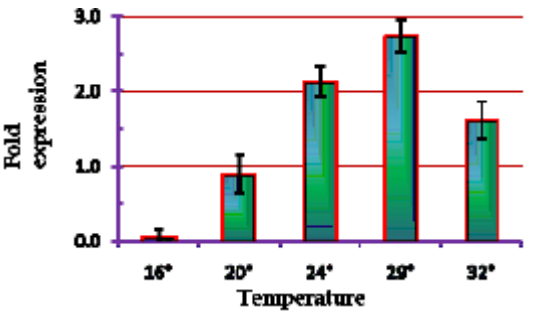

Fig. 3. Expression fold for pathogenicity gene MPG (*M. grisea*) in relation to temperature.

germination percentage in both type of surfaces as 80.1-96.6% germination was observed within 18-24 h and there was hardly any variation in germination percentage between the surfaces. Similarly, differential temperature influence on germination was also found to be non-significant irrespective of surfaces under the influence of cAMP. Therefore, cAMP was observed to nullify the difference in germination percentage due to surface effect as well as differential temperature influence.

Temperature influence on appressorium formation was noted to be significantly higher at 29°C observed between 9-24 h as 8.3-22.3% appressorium formation recorded as compared to 0-3% at 22°C on glass slide. Similarly, formation of appressorium was in higher quantity on cover slip surface at 29°C as 37.6-58.3% appressorium noted as compared to and 14.8-23.9% at 22°C. In both surfaces appressorium formation was 2.5 times higher at 29°C than at 22°C. Although significantly higher percentage of appressorium was noted at temperature 29°C as compared to 22°C but addition of cAMP was found to make no difference as 80.7-85.1% appressorium formation was recorded on the cover slip surface after 18-24h. But at 34°C no appressorium was formed irrespective of surface or addition of cAMP. Therefore, appressorium formation was noted to be influenced by temperature which could be supplemented by addition of cAMP but in not at 34°C.

Impact of external glycerol to stop pathogenicity on cover slip

Conidia germination on cover slip was not profoundly affected by glycerol. However, appressorium formation was found to be significantly reduced by the addition of glycerol (Table 3). Glycerol concentration 2.5% or above found to affect appressorium formation severely as only 2.5-5.9% of the conidia shown to form appressoria. However, the inhibitory effect of glycerol was shown to be nullified to some extent due to cAMP influence. Therefore it indicated that cAMP had influence on appressorium formation.

Pathogenicity gene expression under temperature influence

MPG gene expression analysis in *M. grisea* indicated significant temperature influence as expression level increased with the increase in temperature upto 29°C and then declined on the

temperature went above 29°C. Expression level was almost 44 fold at 29°C and 34 times at 24°C in comparison to 16°C. Comparatively low level of expression below 24°C and at 32°C indicated that the pathogenicity gene expression followed a typical unimodal type of pattern as there was an increase in value to a maximum level at 29°C and decrease at lower and higher temperatures (Fig 3).Therefore, pathogenicity gene is expected to get regulated by temperature but to follow typical biological response. It became evident that leaf blast pathogen required optimal temperature range for the synthesis of factors for virulence essential for attachment, penetration and /or to surpass normal defense mechanism of the host.

PKA activity in relation to appressorium development under temperature influence

PKA activity was detected and quantified as number of nmols of phosphate transferred to a substrate per minute per ml. Higher degree of PKA activity was realized at 29°C than at 22°C (Fig 4). However, PKA activity was distinctly absent at 34°C which was the maximum limit of temperature for the growth of the pathogen. Therefore, PKA activity was looked to be influenced by temperature.

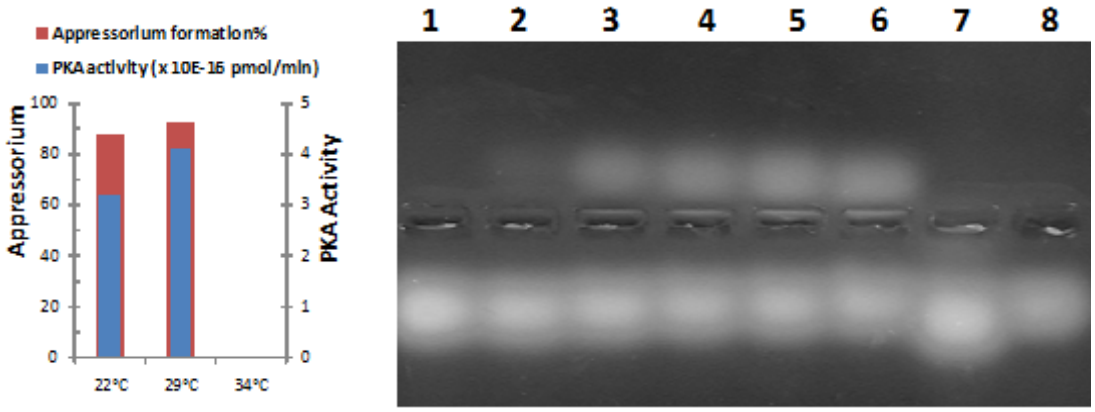

Fig. 4. Temperature influence on cAMP dependent protien kinase activity (µU) in *M. grisea* with reference to appressorium formation on petriplates surface: Electrophoretic separation of phosphorylated (upper lane) and non-phosphorylated (lane below) protiens, Positive control lanes 1: 0.5, 2: 3: 4, 4:6 µl, Lanes 5, 6 and 7: samples exposed at 22°, 29° and 34°C respectively and 8: negative control).

DISCUSSION

Appressorium formation is a critical event in pathogenicity and virulence expression for fungal pathogens. In the current finding role of host surface on appressorium formation has been explained in a perspective to answer many unresolved questions on host-pathogen interaction as far as *M. grisea* is concerned. Appressorium formation pattern on artificial surface indicates surface feature plays important role in cAMP induction as its application on the surface has been observed to nullify the difference in appressorium formation due to surface effect. By now it is established fact that cAMP signaling pathway involving cell surface receptor responds to a specific extracellular signals stimulus and activates adenylyl cyclase (membrane bound enzyme that converts ATP to cAMP) via GTP binding proteins [33,34]. The secondary messenger cAMP mediates extracellular signals to PKA and thereby activates catalytic subunits to phosphorylate downstream targets that control many phenotypic or morphogenetic changes [19]. Appressorium formation process is mediated internally by regulatory biochemical events or networks [35,19,36,16]. However, for manifestation of appressorium there are early events (attachment of germ tube) and ultimate biophysical events like influx of free water from the host surface. Indeed, for any fungal plant pathogen infection process is pre-mediated by sufficient level and period of high

relative humidity or leaf wetness. Surface hydrophobicity is important may be for binding of germ tube and sensing the surface by secretion of hydrophobin protein encoded by MPG1gene [2,37]. A strong binding on host is required for application of hydrostatic pressure to penetrate host surface. A hydrophobic surface or otherwise a moisture less surface provides a stronger binding than a hydrophilic surface. However, after surface sensing or otherwise cAMP signaling, that leads to ultimate glycerol accumulation, free moisture is required and for that hydrophilic surface (tends to keep moisture) becomes conducive as appressorium formation needs water from the surface. The statement is supported by the finding [38] where higher proportion of PKA activity was observed on hydrophobic surface than on hydrophilic surface. However, for induction of biochemical events, chemical constituents on the host surface which come in contact with germ tube surface receptor is important and the surface molecules played important role in appressorium formation[39]. Comparison of surface wettability as a measure of continuous film of water and retention on rice and pearl millet leaf has indicated leaf of the latter was more conducive for spore attachment, germination and appressorium formation than on rice leaf. Higher degree of hydrophobicity on rice leaf surface otherwise low wettability and thus low water retention even after rain might be the reason of low ratio of appressorium formation as compared to pearl millet. Variation in surface hydrophobicity between cereals and grasses may be the reason that makes differential attachment and water retention properties and resulting perplexed cross-infectivity between cereals and other of grasses as reported for *M. grisea* isolate[40,41]. Therefore, appressorium formation is to be influenced by surface hydrophobicity in relation to binding and surface sensing for cAMP signaling.

For appressorium formation and penetration in *M. grisea*, well conserved biochemical signaling pathways are known including the cAMP signaling, PMK1 and MPS1 MAP kinase pathways[35,19,36,16] as well as carbohydrate mobilization and metabolism [42]. For *M. grisea*, PMK1 mitogen-activated protein (MAP) kinase gene is also reported to play critical roles in mycelial development and pathogenicity[29]. Biochemical events are influenced by temperature as enzyme activity is dependent on temperature and there are well-defined physiological optima. Maximum proportion of appressorium formation and MPG gene expression in *M. grisea* at 29°C indicated a pattern of similar temperature influence as observed in sporulation and lesion development[43]. No appressorium formation at 34°C irrespective of surface or addition of cAMP indicates the role of common biochemical processes. Under natural field condition, blast symptoms could be seen only below 32°C as infection sharply declines above this temperature. It became evident that leaf blast pathogen requires optimal temperature range essential for attachment, penetration and /or to surpass normal defense mechanism of the host. As common biochemical processes are involved in appressorium formation, therefore, diseases are limited to those areas where temperature is simultaneously favourable for a sufficient time period for both pathogen and host.

Surface binding and sensing by germ tube is an important step for activation of membrane bound adenyl cyclase to form cAMP as external application of cAMP could nullify surface influence as well as temperature influence. The cAMP-dependent PKA activity in connection with MAPK cascades of biochemical pathways associated with glycerol accumulation are expected to be modified by temperature as happens for normal growth and sporulation. Glycerol accumulation leads to water influx through osmotic process for turgor pressure necessary for host penetration. Surface hydrophobicity has more to do with surface wettability for water retention required appressorium development than signaling process. Therefore, pathogenicity in blast is to be influenced by surface features and modified by temperature and penultimate dependency on free external surface moisture for hydrostatic pressure generation. Host-pathogen interaction explained in the finding could be a basis for testing signal transduction specific targets for new generation fungicide development. For future management strategy, host surface feature and impact of temperature on blast infection may be vital input as rise in CO_2 and temperature level are thought to alter host-pathogen interaction under changing scenario.

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