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In vitro mycelium growth variation among *Fusarium graminearum* isolates causing head blight of wheat in India

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Abstract
Fusarium head blight (FHB) or head scab is one of the most destructive fungal diseases of wheat (*Triticum aestivum* L.) worldwide. One hundred ten diseased samples were collected from naturally infected wheat varieties planted at Indian Agricultural Research Institute, Regional Station, Wellington, the Nilgiris hills and Lahaul Valley (Himachal Pradesh) during 2018 and 2019. Twenty-nine *Fusarium* isolates were obtained, purified through hyphal tip culturing technique and maintained on potato dextrose agar (PDA) media. Morphological characters *viz.*, colour of the mycelia, growth rate and pigmentation wasstudied. Colour of the mycelia produced by different isolates varied from light pink, pink, dark pink to brownish pink. All isolates produced pink pigmentation on PDA. Out of 29 *F. graminearum* isolates used in present study, 23 isolates belonged to Wellington which reached maximum mycelium growth of 85 mm after 144 h of incubation. Maximum mycelium growth in Lahaul valley isolates Fg-L1 and Fg-L2 was recorded 63 and 62.7 mm, respectively after 144 h. Present study revealed variation in mycelium growth rate among *F. graminearum* isolates belonged to Lahaul valley of Himachal Pradesh and Wellington, the Nilgiris, Tamil Nadu.

Keywords: fusarium head blight, wheat, *Fusarium graminearum,* isolates, mycelial growth

Introduction
Bread wheat (*Triticum aestivum* L.) is a major strategic and staple cereal crop consumed by majority of Indians. Northern parts of India are the major wheat cultivating areas, the country's wheat production is 107.19 MT from 30.50 m ha area (Anonymous, 2020) [1]. Fusarium Head blight is more prevalent in regions with hot-temperature (25 °C) and wet climates during kernel formation stage (Caron, 2000) [3]. Fusarium head scab, is one of the most destructive fungal diseases of wheat with cumulative economic losses estimated as$7.7 billion (Nganje *et al*., 2004) [7]. Symptoms starts as Water-soaked lesion occurs onthe glumes and rachis and necrosis spreads within the ear heads gradually leading to partial bleaching followed by complete blighting of wheat ear heads (Wiese, 1987) [12]. Among wheat diseases to be affected by climate change in near future in India is head scab or Fusarium head blight (FHB) caused by different *Fusarium* spp. *Fusarium* species are known to produce mycotoxins which can also cause human and animal diseases (Marasas, 1984) [6]. Fusarium head blight (FHB) inflicted extensive reduction in wheat production in the United States during the 1990s (Windels, 2000) [14]. Disease was first reported in India by Roy (1973) [8] from Siang District of Arunachal Pradesh. The disease was also reported by Brahma and Singh (1985) [2] from Wellington (Tamil Nadu), Chahal *et al.* (1993) [4] from Gurdaspur (Punjab), Saharan *et al.* (2003) [9] from Wellington and During March, 2005, disease appeared in severe form in Dera Baba Nanak area of Gurdaspur (Punjab) on wheat variety PDW 274 and caused significant reduction in yield (Saharan *et al.*, 2007) [11]. Present study was carried out tostudy the variation among *F. graminearum* isolates based on cultural characteristics *in vitro*.

Methodology
Morphological characteristics of *Fusarium* isolates
One hundred ten wheat spikes showing head blight symptoms were collected from the wheat fields from Wellington, Nilgiris, Tamil Nadu (1850m, 11.37°N 76.8°E) and Dalang Maidan, Lahaul valley, Himachal Pradesh (3658m, situated between 31°44'57" and 32°59'57" North Latitude and between 76°46'29" and 78°41'34" East Longitudes) during 2018-2019. Diseased wheat kernels were sterilized in 1% (v/v) sodium hypochlorite for 30 seconds, followed by rinsing in sterilewater for 3 times for 2-3 min, and placed it on the Petri plates poured with

potato dextrose agar (PDA) medium. The plates were kept in incubator at 25 ±2 °C for 5 days. Fungus (*Fusarium* spp.) were subcultured till pure culture is obtained using hyphal tip method and cultures were maintained by subculturing for every 15 days once. Following this, *Fusarium* spp./isolates were also transferred onto a carnation leaf agar (CLA) media and plates were placed under UV light for 24 h and then incubated at 25±2 °C to induce conidia formation (Leslie and Summerell, 2006) [5]. Twenty-nine isolates of *F. graminearum* were used in present study. Twenty-seven of these were obtained from Wellington (Tamil Nadu) and two isolates were from Lahaul valley (Himachal Pradesh). Morphological characters such as colour of mycelium, growth ratei.e., at every 24-hr interval up to 144h was recorded and presence of macroconidia of all 29 *Fusarium* isolates were measured using Phase contrast microscope. Data on mycelium growth was analyzed by following CRD design using OPSTAT software. (Sheoran *et al*., 1998) [13].

Results and Discussion

Mycelium colour of all *Fusarium* isolates grown on PDA media at 25˚C was observed till seven days. All isolates produced mycelia of pinkish white (Fig. 1). Pink pigmentation was also observed in all isolates (Table 1). Mycelium growth was measured at an equal interval of 24 h till all the isolates covered the Petri plate. There was difference in the mycelium growth with all the isolates. After 24 h of incubation, in mycelium growth was recorded with 20 *Fusarium* isolates *viz*., Fg-L1, Fg-W2, Fg-W3, Fg-W4, Fg-W5, Fg-W7, Fg-W8, Fg-W10, Fg-W11, Fg-W13, Fg-W14, Fg-W16, Fg-W17, Fg-W18, Fg-W19, Fg-W22, Fg-W23, Fg-W24, Fg-W26 and Fg-W27. There was no initiation in mycelium growth with nine isolates *viz*., Fg-L2, Fg-W1, Fg-W6, Fg-W9, Fg-W12, Fg-W12, Fg-W20, Fg-W21 and Fg-W25 after 24 h of incubation. Maximum mycelium growth (diameter) of 15.3mm was recorded with isolate Fg-W18 after 24 h of incubation which differed significantly from other isolates. After 48 h of incubation, maximum mycelium growth of 36 mm was recorded with isolate Fg-W11 followed by isolate Fg-W18. Maximum mycelium growth of 65 mm was observed with isolate Fg-W18 followed by Fg-W8 after 72 h of incubation.

After 120 h of incubation, mycelium growth of 85 mm was recorded with isolates, Fg-W8, Fg-W11, Fg-W12, Fg-W16, Fg-W17, Fg-W18, Fg-W20 and Fg-W23. After 144 h of incubation, all isolates growth was 85 mm except isolates isolates, Fg-L1, Fg-L2, Fg-W1, Fg-W10, Fg-W26 and Fg-W27. Out of 29 *F. graminearum* isolates used in present study, maximum mycelium growth of 85 mm was recorded in 23 isolates belonged to Wellington after 144 h of incubation. Maximum mycelium growth in Dalang Maidan, Lahaul valley isolates Fg-L1 and Fg-L2 was recorded 63 and 62.7 mm, respectively after 144 h (Table 1). Results indicated significant difference in mycelium growth among Dalang Maidan and wellington isolates (Table 1). All the isolates produced Pink pigmentation on the PDA media.

Macro conidia were observed in all isolates as shown in Fig. 1.

Present study has shown that there is variation among mycelial growth among *Fusarium graminearum* isolates of Lahaul Valley, Himachal Pradesh and Wellington, the Nilgiris, Tamil Nadu. In earlier study, Saharan *et al.* (2002) [10] also reported cultural growth variation among Fusarium graminearum isolates belonged to Narkanda and Lahaul valley of Himachal Pradesh. This variation in mycelial growth rate and colony color may be due to different weather conditions and farm practices in these areas.

Table 1: Cultural characteristics of different *Fusarium graminearum* isolates recorded at an interval of 24 hours

Sr. No.	Isolate	Colony Color	Mycelial Growth (mm)					
			24 h	48 h	72 h	96 h	120 h	144 h
1	Fg-L1	Dark Pink	3.7	22.3	36.7	50	57.3	63
2	Fg-L2	Light Pink	0	10.7	26.7	37.3	49.3	62.7
3	Fg-W1	Pink	0	6.7	18.7	32.3	45	59.7
4	Fg-W2	Pink	1.7	22.7	41.7	66.7	81	85
5	Fg-W3	Light Pink	2.7	15.3	37.7	65	76.7	85
6	Fg-W4	Dark Pink	1	11.3	26.7	48.3	70	85
7	Fg-W5	Pink	2	11.7	31.7	58.3	80.3	85
8	Fg-W6	Pink	0	21.3	45	61.3	77.3	85
9	Fg-W7	Brownish Pink	3	12.3	31	53.3	78.3	85
10	Fg-W8	Dark Pink	4.3	30.3	60	85	85	85
11	Fg-W9	Light Pink	0	16	39	61.7	75.7	85
12	Fg-W10	Dark Pink	3	11.3	27.3	42.7	63.3	81.7
13	Fg-W11	Pink	0.7	36	51	73.3	85	85
14	Fg-W12	Dark Pink	0	21	49.3	80.7	85	85
15	Fg-W13	Light Pink	6.3	25.7	46.3	66	82	85
16	Fg-W14	Dark Pink	3.3	12.7	32.7	55	78.3	85
17	Fg-W15	Pink	0	1	26.7	64.3	76.7	85
18	Fg-W16	Pink	2.3	10.7	36.3	65.3	85	85
19	Fg-W17	Brownish Pink	6.7	18	42.7	66.7	85	85
20	Fg-W18	Light Pink	15.3	35	65	80.7	85	85
21	Fg-W19	Light Pink	4.3	23	40.7	66.7	81.7	85
22	Fg-W20	Dark Pink	0	19.7	5	80.7	85	85
23	Fg-W21	Dark Pink	0	13.3	39.3	67.7	82	85
24	Fg-W22	Brownish Pink	2	11.3	24.7	46.7	73.3	85
25	Fg-W23	Light Pink	2.3	27.3	55.3	80	85	85
26	Fg-W24	Dark Pink	5	16.7	34.3	56.7	78.3	85
27	Fg-W25	Dark Pink	0	8.7	29.3	54.3	71	85
28	Fg-W26	Dark Pink	6.7	20	28.3	48.3	62.7	74.3

29	Fg-W27	Light pink	9.7	24.7	30.7	41.7	46.7	55.7
CD (0.05)	0.37	0.83	1.27	1.61	1.01	0.61	CD (0.05)	0.37

Fg: *Fusarium graminearum*

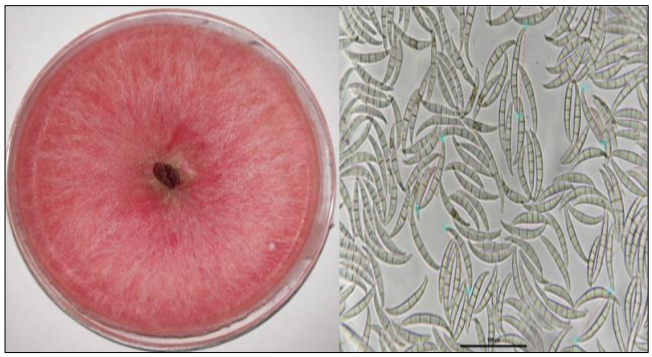

Fig 1: *Fusarium graminearum* culture W1 on potato dextrose agar media (left) and its macroconidia (right) produced on carnation leaf agar (40X Magnification)

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