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Characterization of hyphae growth in developing stripe rust pathogen in IL598, ILT756 and WL711

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Abstract
Bread wheat (*Triticum aestivum* L. Thell, 2n=42) is a widely cultivated crop in India or worldwide and a potent source of nutrients. Loss of the wheat production in different region of India affected due to leaf and stripe rust infection. There was two introgression line (ILT598 and ILT756) and one parental line (WL711NN) were selected for characterization of resistance and susceptible line based on hyphal growth in developing stripe rust pathogen inside the infected wheat leaf. There was collection of leaf sample from wheat at six different time interval at Genetics area of School of Agricultural Biotechnology Punjab Agriculture University Ludhiana. Detection of hyphal growth at 0 hr in all three line ILT598, ILT756 & WL711NN were observed null. Simultaneously at 12, 24, 48, 72, 96 hr observation of hyphal growth in WL711 were observed maximum while in ILT598 minimum. There were in ILT756 observed moderate infection. Use of this assay (WGA-FITC) in ILT598 depicts more apoptotic area while in ILT756 relatively less, in WL711 maximum hyphal colonization due to both leaf and stripe rust as reported breakage of Resistance gene. Collected sample of ILT598, ILT756 & WL711NN at seedling stage depicted all above mentioned discussion.

Keywords: *Puccinia striformis*, *Puccinia triticina*, wheat, plant cell death, yellow rust, leaf rust

Introduction
Bread wheat (*Triticum aestivum* L. Thell, 2n=42) is a staple crop in India. Stripe (yellow) rust (YR) caused by *Puccinia striiformis f.sp. tritici*, is one of the major diseases of wheat in temperate regions also found in the tropics and subtropics (Boyd, 2005). Leaf rust is more destructive disease than stripe rust which have causative agent *Puccinia triticina*. It infects leaves, leaf sheath and spikes of a wheat plant. This can infect barley, rye and more than 50 grass species also (Line 2002). The losses to wheat crop due to YR varies from 10-70 percent, depending upon the weather, races of pathogen, susceptibility of cultivar, and time of infection. In contrast, some wheat cultivars grown extensively for many years retain a good level of resistance, including Cappelle Desprez (Johnson, 1983) [7]. There are resistance type host resistance observed a separation with non-host resistance. Due to intermediate resistance confused to distinguished between host and non-host resistance. With example of *Hordeum vulgare* and Brachipodium species in this scientist were observed there been relation between leaf browning and leaf chlorosis with hyphal colonization (Dowson *et al.* 2016) by *P. striformis f sp. tritici*. This study depicted as there were visualizing fungal structure and percent of colonization among all ILT598 & ILT756 as well WL711. Currently the disease deployment of through genetic mode (R genes) of deployment as well Chemical fungicide application. Many R genes in ILT598, ILT756 & WL711NN were identified against leaf and stripe rust. Lr57 (Kuraparthy 2007a) [3] and Yr 40 respectively in ILT598 & ILT756 have been showed leaf and stripe resistance but after evolving pathotypes there were in ILT756 breakage of resistance for Yr 40 gene. Still IL756 retained Leaf rust resistance report of these two R genes Lr57 and Yr 40 respectively mapped on chromosome of 5Ds (Kuraparthy 2007a) [3]. WGA-FITC stain characterized the hyphal growth with respect to host pathogen interaction. Simultaneously making yellow pustules on to leaf surface (Mares & Cousen, 1977; Garrood, 2001) [6, 5]

Material and method
Sample preparation
After inoculation with stripe rust race 110S119 first leaf of inoculated seedling samples were taken after different time intervals. The rust infection was studied at the microscopic level by using the protocol developed by Ayliffe *et al.* (2011, 2013).

Sample fixation and staining

Wheat germ agglutinin (WGA) is a lectin that interacts with chitin oligomers present in the cell wall of fungi. WGA-FITC is a lectin conjugated with the fluorescein iso-thiocyanate (FITC) fluorophore which gets attached to the fungal hyphae and helps in visualization of intercellular growth and pustule formation on infected leaves when observed under fluorescence microscope. Leaves were harvested at 14 days post inoculation and placed in 15mL centrifuge tubes containing 1.0 M KOH with a droplet of surfactant (TWEEN-20). Chlorophyll pigment was cleared off from the leaves by incubating them in the KOH solution at 37 °C for 12 to16h. Subsequently, the KOH solution was decanted after incubation and neutralization of leaves were done by washing them three times in 50mM Tris at pH 7.5. After decanting of the final wash solution, leaves were dipped in 1.0ml stain solution (20 µg/mL WGA-FITC (L4895- 10MG; Sigma–Aldrich) in 50mM Tris at pH7.5). Leaf tissue was incubated overnight, then washed with water, mounted, and observed under blue light excitation on a fluorescence microscope (NIKON) with a GFP filter. The stain solution was standardized for more than single time usage.

Fluorescence microscopy

Slide mounted over the stage of Fluorescence microscope and observed under blue light excitation using GFP filter and, quantitative analysis of % of leaf colonization (P^{col}) and % of leaf harboring Pustules (P^{Pust}) was performed using fluorescence microscopy. The microscopic assay was developed for the quick assessment of the disjoint field of view covered the leaf surface area by scanning the mounted leaf over Fluorescence microscope (Jagger *et al.* 2011) [2].

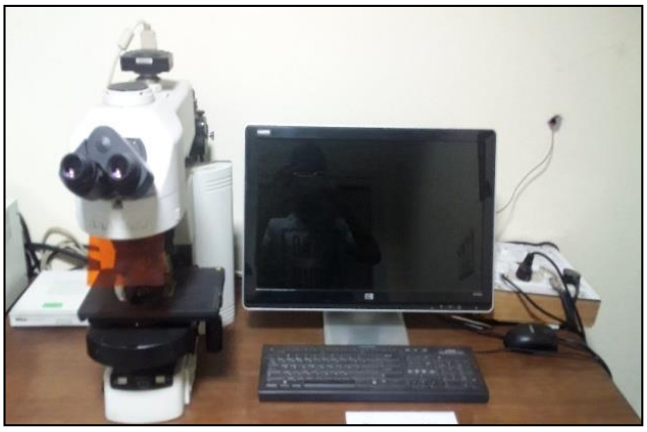

Fig 1: Fluorescence Microscopy

Result and Discussion

Fungal Biomass characterization

Growth of stripe rust hyphae was studied in at seedling stage in seedling leaf samples taken at four different intervals (0hr, 12hr, 24hr & 48hr) after inoculation with stripe rust pathotype 110S119. In all stages the hyphal growth is less in IL T598 as compared to IL T756 where more hyphae are stained at each stage of growth of stripe rust pathogen. Similarly when compared with hyphal growth in WL711, hyphae stained are more than ILT598 and ILT756. This differential pathogen growth in the ILT598, ILT756 and WL711 indicate a different mechanism of resistance in the three lines. WL711 being susceptible allow the stripe rust hyphae to grow fully while ILT756 with stripe rust score of 40MS at adult plant stage and infection type of 1+2 at seedling stage showed comparative less hyphal growth. But ILT598 having stripe rust severity of TR at adult plant stage and infection type: (fleck) at seedling stage had least hyphal growth, indicating the resistance mechanism of ILT598 is different from ILT756.

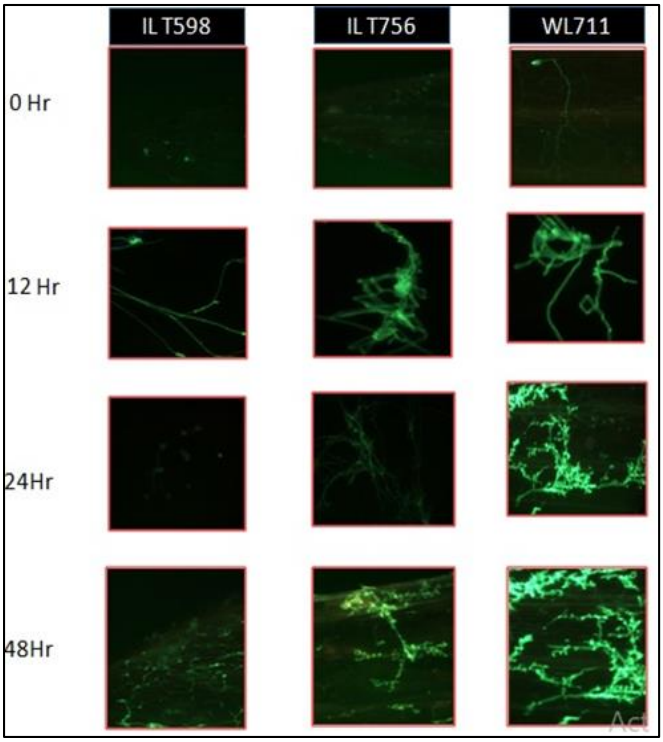

Fig 2: Representation at four different time intervals

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