NILGIRI WHEAT NEWS

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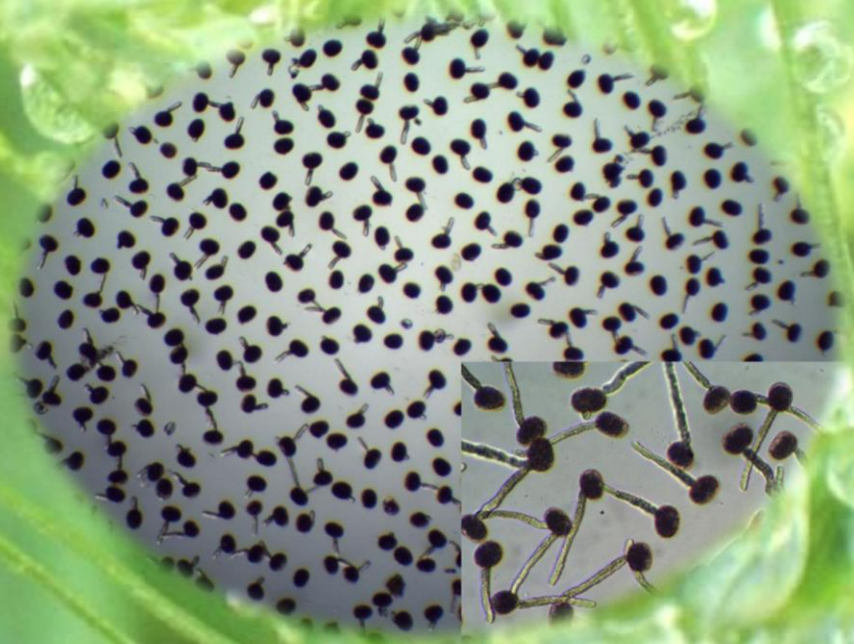

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TAMIL NADU

cDNA-AFLP based expression profiling of isogenic lines of wheat for understanding the molecular basis of host-pathogen interactions in seedling resistance and fitness potential of Race-77 of *Puccinia triticina*

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Leaf rust continued to cause yield loss considerably. Over the lost two decades several wheat cultivars carrying specific rust resistance genes has been released in India and subsequently evoving of new pathotypes has also been reported Therefore understanding basic mechanisms of variability in leaf rust race 77 and analyzing fitness potential of the biotypes present in this race will help in designing suitable rust management strategy in the country. At international level a wheat genomics project is underway in different parts of the world including India. It is expected that the complete genome of wheat would be available in public domain within 2-3 years. Thus parallel analysis of wheat genome and wheat rust genome
would yield useful information on their interaction at molecular level. To checkmate the frequent evolution and reporting of wheat rust (particularly leaf rust) caused by *Puccinia triticina* pathotype 77-5, a serious attempt was made to understand the molecular basis of host-pathogen interactions in seedling resistance and fitness potential of Race-77 of *Puccinia triticina*.

Infection and Seedling growth conditions

Near isogenic lines of wheat namely, WL711+*Lr*57 (resistant to *Puccinia*) and WL711-*Lr*57 (susceptible to *Puccinia*) were employed for the comparative study of proteome and transcriptome during their interaction with the leaf rust fungi, *Puccinia triticina* of race 77-5. Rust inoculum was collected from the rust infected wheat leaf (inoculum source) specially from IARI, RS, Wellington, by using a lancet shaped needle. In order to facilitate the uniform spreading of rust spores, the collected inoculum was evenly applied on both the leaf surfaces of seven day old seedlings. For the NILs, mock inoculations were done with water and were treated as the appropriate controls. After inoculation, the wheat seedlings were incubated in moist chamber and watered to maintain the moisture (RH >95%), temperature (25°C) and photoperiod (16 hours), which are necessary for the development of leaf rust. Upon analyzing the pattern of fungal growth at different time intervals, 96 hpi was chosen for proteomic and transcriptomic analysis.

Proteomic analysis

Two-Dimensional Poly Acrylamide Gel Electrophoresis (2D PAGE)

2D PAGE was employed to dissect out and compare the differentially expressed proteins during wheat-*Puccinia* interaction. Proteins from leaf tissue of both control and 96 hpi susceptible (WL711-*Lr*57) and resistant (WL711+*Lr*57) plants were profiled. Around 400 reproducible protein spots were obtained over a pH range of 4.7 to 5.9 with a molecular weight ranging from 10 to 100 kDa (Figure 1). Eighteen abundant spots were excised from the silver stained gel and mass spectrometric

analysis was performed.

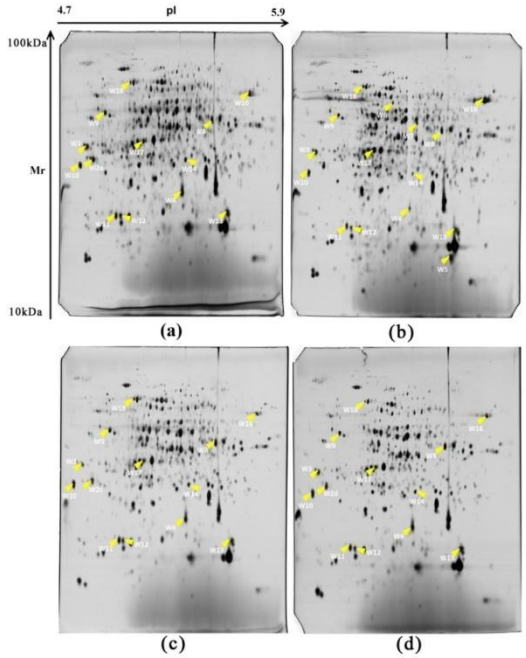

Figure 1. 2D PAGE analysis of proteins extracted from leaf tissues from WL711-*Lr57* (Susceptible) and WL711+*Lr57* (Resistant). a) Susceptible control; b) Susceptible 96hpi; c) Resistant control and d) Resistant 96hpi.

Functional annotation of the identified proteins

The MASCOT analysis was performed and BLASTp analysis was performed for the set of proteins that belonged to organisms other than Wheat/*Puccinia* in order to determine their corresponding matches (Table 1). Based on the predicted functions, the identified differentially expressed proteins were categorized into six classes *viz.*, photosynthesis related, defense related, nucleotide repair related, DNA replication related, protein synthesis related and transposable element related proteins.

Transcriptomic validation of selected potential candidate proteins

Gene specific primers were designed for the selected potential candidate proteins according to their corresponding wheat coding sequences (CDS) through NCBI Primer-BLAST tool. The expression of the candidate genes were analysed through Step One Plus real time PCR detection system (Applied Biosystems, USA). Expression profiling was performed through qRT-PCR for a set of selected proteins identified in this study, namely, MMS19 (W4), Disease resistance protein RPM1 (W10), Ethylene receptor 1 (W12) and PPR domain containing protein (W14). The primers were designed through NCBI Primer-BLAST tool. The specificity and PCR profile of the designed primers were evaluated with wheat genomic DNA and then qRT-PCR was performed by using the normalized cDNA samples. The relative abundance of the selected proteins were observed by qRTPCR (Figure 2.)

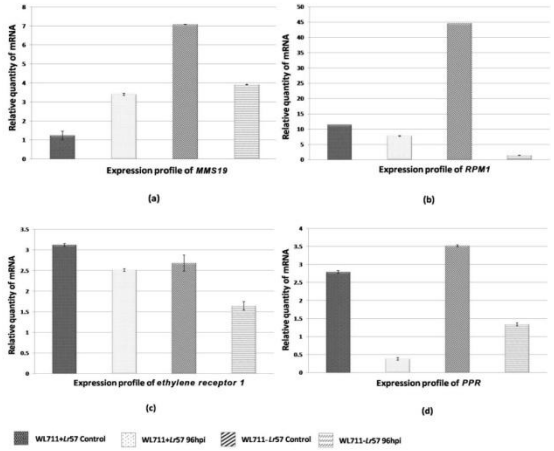

Figure 2. Expression profiles of (a) MMS19, (b) ethylene receptor 1, (c) PPR and (d) RPM1 in control and 96 hpi wheat NILs namely, WL711+*Lr57* and WL711-*Lr57* upon challenging with Puccinia triticina (race. 77-5).

***In-silico* comparative genomic study for the identified proteins**

Seventeen plant species were selected for the genome wide study of the differentially expressed proteins that were obtained from wheat during Wheat-*Puccinia* interaction. The

chosen species include diverse sub groups *viz*., cereals, fruits, tree, legumes, vegetables etc. The genome wide study for the protein obtained from *Puccinia* was performed with 17 fungal species. The chosen fungal species belong to diverse categories *viz*., pathogenic fungus, model organisms in genomic research, beneficial fungus etc. Hence, the genome wide study of the differentially expressed proteins during Wheat-*Puccinia* interaction with the chosen species would pave way for characterizing these proteins in diverse genetic background. Orthologs of the differentially expressed proteins were determined in the chosen representative species through BLASTp analysis. MSA was performed and then phylogenetic trees were constructed using the best fit model determined through MEGA6 (Figure 3).

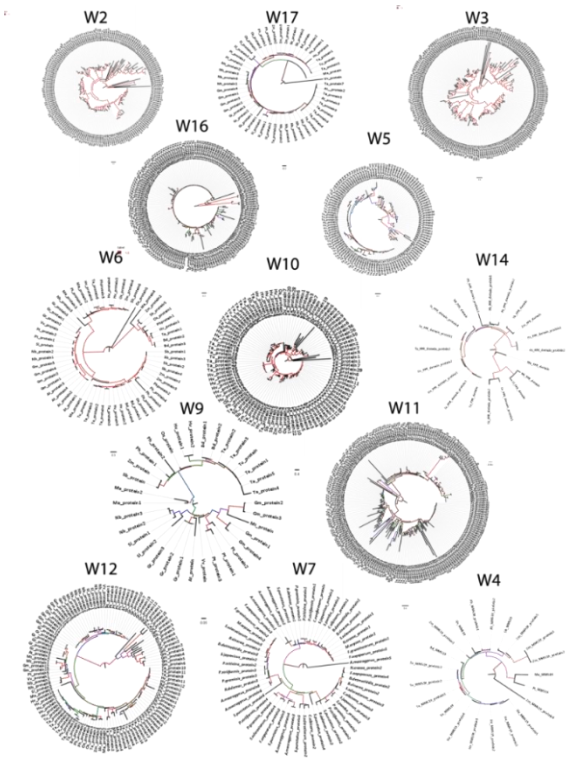

Figure 3. Phylogenetic tree of the selected protein spots. The tree was constructed through MEGA 6 with 1000 bootstrap replications. The nodes were colored based on their bootstrap values. Red and brown colour represents the upper and lower extremes of the bootstrap values respectively.

Summary and Conclusions of the Progress made so far

Resistant (WL711+*Lr*57) and susceptible (WL711-*Lr*57) near isogenic lines of wheat were used to unravel the molecular basis of seedling resistance conferred by *Lr*57 gene during wheat-*Puccinia (*race 77-5) interaction.

- Differentially expressed proteins during incompatible and compatible interactions were identified through 2D-PAGE and their peptide mass fingerprinting analysis was performed through MALDI TOF.
- MASCOT analysis revealed that Mcm protein 6, a DNA replication related protein (only protein from *Puccinia* in this study) was present only in the susceptible 96 hpi plant.
- For the first time, the differential expression of a nucleotide repair related protein, MMS19, during wheat-*Puccinia* interaction is being reported in this study. Besides these key proteins, proteins associated with defense, photosynthesis, protein synthesis and transposable elements have been identified.
- Transcriptomic validation was performed for a set of selected potential candidate proteins through qRT-PCR. In order to characterize the identified proteins in diverse genetic background, *in-silico* genome wide comparative study was performed with the chosen representative species and it was found that the defense related proteins exhibited minimum sequence conservation in comparison with other proteins.

Details of New Leads Obtained

Through 2D-PAGE, key proteins associated with seedling resistance conferred by *Lr*57 gene against leaf rust were identified. Differential expression of MMS19 during wheat-*Puccinia* interaction is being reported for the first-time.

Possible breakdown of durable resistance in wheat varieties with *Lr24* gene against diversified pathotypes of *Puccinia triticina* at Wellington (The Nilgiri hills)

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Based on the recommendation of IRC, 2015, separate experiments were conducted to unfold the status of consistency in maintenance of resistance by different *Lr* genes with special reference to *Lr24* incorporated in different wheat cultivars. To initiate this study, a set of wheat (*Triticum aestivum* L.) varieties having *Lr24* gene were evaluated during 2014-15 onwards both under glass house and open field conditions for assessing their resistance level at seedling and adult plant respectively. Original source seeds of test varieties with *Lr24* gene were procured from different sources i.e., IARI Regional stations, Wellington and Indore and Division of genetics, IARI, New Delhi. A total of 147 isolates of leaf rust (*Puccinia triticina*) which have been purified from different genetic background of respective host are also maintained at Wellington. Initially, 10 varieties ie., Thatcher + *Lr24*, 3Ag#, Agent, Amrita, Harshita, Purna, Abha, Vadisha, MP 4010 and Tr 380-14 x 7/3 Ag Lr*24* were repeatedly tested for their seedling reactions against five isolates of *P. triticina*, which have been purified from different varieties with leaf rust resistant genes including *Lr24*. These varieties have been grown at different altitude as well to collect the diversified population of test pathogen. After purification, primary leaves from 7 days old seedlings were inoculated with uredospores of *P. triticina* under glass house conditions. All 5 isolates of *P. triticina* representing different altitudes of Nilgiri hills and a dominant and reference isolate (race 77-5) were inoculated separately on each genotype and critically observed for their infection, colonization and pattern/severity of infection. Out of these, var. Agent expressed some degree of resistance/susceptibility (2, 2+) against *P. triticina* isolate purified from wheat having *Lr24* and other liked genes. The same isolate also expressed some degree of virulence over few resistant varieties with *Lr24* gene. Formation of necrotic spots was very common in almost all the test varieties. However, one or two minute and invisible pustules were found with plenty of uredospores of *P. triticina*. This was confirmed by both simple and advanced microscopy. Based on these positive results, another two set of evaluation were carried out a total of 36 and 42 varieties/lines having *Lr24* and other linked genes. The overall results depicted that few genotypes namely, Agent, HW 2001, HW 2004, HW 2005 TPN 7 (*Lr*24/*Sr24*) and TPN 20 (*Lr*24/*Sr*24) expressed high susceptibility both under glass house and field conditions and breakdown of resistance was confirmed with repeated experiments. However, this important findings need further confirmation through molecular methods. The field reactions even at different locations at Wellington also confirmed the breakdown of resistance in above said varieties with *Lr24* gene. The comprehensive analysis data from glass house and field experiments demonstrated the presence of virulent pathotypes/races of *P. triticina* and therefore, they could form virulence over the durable resistance genes like *Lr24* at Wellington. Further works are under progress to ascertain

these results with molecular analysis of both host and pathogen and their interactions.

Cereal Rye pollen is not recalcitrant anymore

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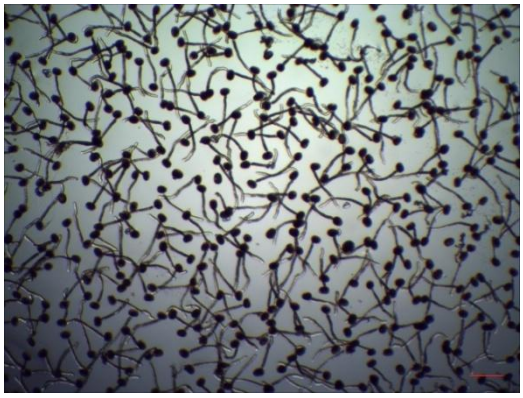
It is the first report on *in vitro* pollen germination of Cereal rye. Rye pollen is classified as recalcitrant pollen as it is very difficult to germinate on nutrient medium. So far, there is no standardized protocol to test viability of rye pollen available. This study addresses the possibility of testing the viability of rye pollen within short time (15-20 min) using pollen germination medium (PGM) reported here. The viability of rye pollen was tested with FDA staining also. The results of both staining and in vitro pollen germination were comparable. In this study, with necessary sugar and inorganic salts, tryptone a nitrogen source was used as one of the media constituents for the first time. A complete PGM for rye consists of maltose, poly ethylene glycol, boric acid, tryptone and agar. During the flowering period, a pollen sterility of 3-12% was recorded. The PGM showed >93% pollen germination with intact pollen tubes after addition of tryptone.



Pollen germination after 15 min after culture**:**

Scale bar: 42 µm

Nematode profiling in I.A.R.I. farm and adjoining area

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The I.A.R.I., Regional Station, Wellington farm area was surveyed for the first time to have preliminary understanding on the nematode fauna. Initial survey indicated the presence of several parasitic nematodes such as *Helicotylenchus, Trichodorus, Hemicycliophora, Rhabditis* and *Dorylaimids.* Among them, in wheat fields *Helicotylenchus* spp. dominated in numbers. Fields adjoining to the institute were also sampled and we found the presence of *Meloidogyne* spp. The exact species of the Root-Knot nematode (RKN) yet to be identified however, we suspect the presence of *M. incognita* and *M. hapla* in mixed population. The typical forking symptom (Fig 1) of RKN was observed in carrot along with small galls. This symptom reduces the market value drastically because of poor consumer preference. Establishment of nematode sick plots had been initiated to proceed further systemic research

studies.

Figure 1: Forking symptom in carrot as a result of root-knot nematode infestation

News

Dr. M. Sivasamy, Principal Scientist and Head, nominated as Zonal Councillor (Peninsular and Southern Hill Zone) by the Central Executive Council of the Society for Advancement of Wheat research (SAWR), Karnal