2014 Technical Workshop

22-25 March

Universidad La Salle Noroeste
Cd. Obregón, Mexico

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**Lead author is a graduate student.*

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PNPi, *Puccinia NPR1 interactor*, a rust effector that supresses NPR1-mediated resistance by competing with TGA2

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NPR1 (nonexpresser of PR genes) is a key transcriptional co-regulator of plant defense responses. Upon pathogen challenge in *Arabidopsis*, NPR1 translocates from cytoplasm to nucleus, where it interacts with TGA-bZIP transcription factors to activate the expression of several pathogenesis-related genes. We identified a secreted protein from *Puccinia striiformis f. sp. tritici* interacting with wheat NPR1 in an established yeast two-hybrid library. Bio-informatic analysis shows that *PNPi*, *Puccinia NPR1 interactor*, is conserved among *Puccinia* species. Further yeast two hybrid tests showed that PNPi specifically interacted with an NPR1-like domain in wheat NPR1 via its C'-terminal domain. Using yeast three hybrid assays we found that PNPi can compete with interactions between NPR1 and TGA2. The *in planta* interaction between this effector and its target was further validated using bimolecular fluorescence complementation (BiFC) assays in tobacco leaves. Expression profiling by qRT-PCR assays showed that the expression level of *PNPi* was sharply up-regulated during the late stages of *Pst* infection (8 days post inoculation). In the adjacent region of barley leaves inoculated with *Pseudomonas syringae* DC3000, suppression of an *NPR1* downstream gene occurred in transgenic barley lines expressing *PNPi* but not in the wildtype.

A comparative transcriptome analysis to dissect host-pathogen interactions

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Leaf rust is the most prevalent rust of wheat, annually causing significant yield losses worldwide. More than 60 leaf rust resistance genes are genetically identified and characterized primarily as race-specific genes defined by hypersensitive responses (HR). A few of these genes that confer only partial resistance without the classical HR have been classified as race non-specific resistance genes. To avoid the "boom-and-bust" of cultivar replacement due to rapidly evolving variants of the pathogen, cultivars with durable resistance are desirable. Pyramiding multiple resistance genes with different defense mechanisms has been proposed as a promising breeding strategy to achieve durable resistance; however, limited information about the molecular mechanism underlying host-pathogen interactions has hindered the development of durable rust resistant cultivars via this approach. Previously histological studies on a panel of Thatcher NILs suggested different mechanisms in these NILs to combat infection. To identify the molecular mechanisms underlying compatible and incompatible host-pathogen interactions, we performed a comparative transcriptome analysis via RNA-seq on the NILs challenged with multiple *P. triticina* races across a time series during the infection process. With the advantage of RNA-seq, we were able to capture the dynamic interactome of the host-pathogen interactions, bringing us a more comprehensive understanding of the relationship. Moreover, the key determinants that defined the race and host-specific response were revealed by this comparative approach. This information will be invaluable for future breeding strategies to develop durably resistant varieties.

Quantification of fungal colonization in wheat lines with adult plant stem rust resistance

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The discovery of *Pgt* race Ug99, and subsequent appearance of variants within this race group, emphasized the need for durable stem rust resistance in wheat. One strategy to counteract the breakdown of single resistance genes is to use adult plant resistance (APR) in commercial varieties. In addition to mapping chromosome regions involved in APR, accurate phenotyping is necessary to understand the nature of this resistance type. In this study different techniques were used to track stem rust development in adult plants of two John Innes Centre lines displaying APR. Lines JIC218, JIC542 and 37-07 (susceptible control) were grown in a greenhouse and infected with *Pgt* race PTKST after anthesis. Uvitex-2B staining showed haustorial mother cell formation in all lines at 72 hours post-infection (hpi). The lines were clearly distinguished by quantifying fungal biomass in the leaf sheath of the last internode using WGA-TITC binding fluorescence and RT-qPCR at 120 hpi. The expression a *rust transporter protein 1* gene indicated that APR lines could be clearly recognized at 120 hpi based on haustorium formation. These methods proved useful in describing *Pgt* colonization events after controlled infection of lines with APR to stem rust.

Understanding durable rust resistance in barley

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Barley, an important crop worldwide, is affected by three rusts: leaf rust (caused by *Puccinia hordei*), stem rust (*P. graminis*) and stripe rust (*P. striiformis*). This paper provides an overview of the global importance and current situation with barley rusts, and outlines our long-term research on barley rust pathosystems. In Australia, leaf rust is economically the most important rust of barley, causing yield losses of about $21 million annually. We initiated research on durable adult plant resistance (APR) to this disease in 1998 and discovered and characterised the first gene conferring APR to this disease, *Rph20*. Several other APR genes that interact with *Rph20* in an additive manner, including *Rph23*, have been found. Three specialised forms of *P. graminis* infect barley in Australia: *P. g.* f. sp. *tritici* (wheat and triticale), *P. g.* f. sp. *secalis* (rye), and a somatic hybrid between these, the 'Scabrum' rust. While we have not been able to demonstrate any effect of the durable stem rust *Rpg1* gene against any of these forms, we have characterised several new sources of stem rust resistance. SSR markers revealed multiple genotypes among isolates of the Scabrum rust pathogen. These studies have illustrated how different forms of rust fungi can re-assort virulence genes and evolve, with implications for transgenic approaches in resistance breeding. While *P. striiformis* f. sp. *hordei* (*Psh*) has not been detected in Australia, a form of stripe rust, 'barley grass yellow rust' (BGYR), first found on weedy *Hordeum* spp. in 1998 and now widespread, is virulent on some barley cultivars. Research on the genetic basis of resistance to BGYR in Australian barley germplasm has characterised 7 seedling-effective and one APR genes that are completely ineffective against *Psh*. Annual off-shore testing of Australian barley germplasm against *Psh* aims to incorporate resistance to *Psh* as a pre-emptive measure in case it enters Australia.

Lr34 is the key to durable leaf rust resistance in the Canadian cv. Pasqua

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Wheat cv. Pasqua, released in Canada in 1991, has maintained a high level of leaf rust resistance while being grown on a total area of over 2 m ha. Pasqua was developed as a deliberate effort to stack resistance genes into a single cultivar, and it contains *Lr11*, *Lr13*, *Lr14b, Lr30* and *Lr34*. These were all very effective resistance genes at the time they were incorporated, however *Lr11*, *Lr13* and *Lr14b* became ineffective due to the emergence of virulence in the pathogen population and *Lr30* currently only provides partial resistance. To understand the key to the durable nature of resistance in Pasqua we analyzed the level of resistance conditioned by each of these genes individually and their various combinations. Pasqua was previously crossed and backcrossed with Thatcher by the late P.L. Dyck to determine its genetic basis of resistance. The backcross lines were screened with *P. triticina* races to identify lines containing each of the possible gene combinations from this cross. These lines were then evaluated in inoculated field nurseries during years 2009-2012 to estimate the effectiveness of each gene combination. While *Lr34* alone was partially effective, gene combinations involving *Lr34* were more resistant. Gene combinations without *Lr34* were much more susceptible than those involving *Lr34*. The key to the durable leaf rust resistance in Pasqua appears to be *Lr34* and its interaction with other genes. Pyramids of resistance genes can condition durable resistance, but key resistance genes such as *Lr34* act in a synergistic manner with other genes in these pyramids to impart higher levels of durable resistance.