

# General disease resistance loci against biotrophic pathogens in wheat

Lillemo M<sup>1</sup>, Asalf B<sup>1</sup>, Singh RP<sup>2</sup>, Huerta-Espino J<sup>3</sup>, Chen XM<sup>4</sup>, He ZH<sup>4,5</sup>, Brown JKM<sup>6</sup>, Bjørnstad Å<sup>1</sup>  
<sup>1</sup>Dept. of Plant and Env. Sciences, Norwegian Univ. of Life Sciences, Ås, Norway, <sup>2</sup>CIMMYT, México, <sup>3</sup>Campo Experimental Valle de México-INIFAP, Mexico, <sup>4</sup>Institute of Crop Sciences/National Wheat Improvement Center, CAAS, Beijing, China, <sup>5</sup>CIMMYT China Office, c/o CAAS, Beijing, China, <sup>6</sup>Dept. of Disease and Stress Biology, John Innes Centre, Norwich, England.

## INTRODUCTION

There is increased interest in breeding wheat cultivars for partial and race non-specific resistance against diseases like leaf rust (LR), stripe rust (YR) and powdery mildew (PM) which are caused by biotrophic pathogens with a high evolutionary potential. The CIMMYT bread wheat line Saar was developed in Mexico with a high level of race non-specific resistance to LR and YR based on the adult plant rust resistance loci *Lr34/Yr18* and *Lr46/Yr29* (Navabi et al. 2003, 2004). International testing showed that it also exhibits good partial resistance to PM, and a genetic study indicated that the resistance was governed by at least three genes (Lillemo et al. 2006). The PM resistance of Saar was furthermore shown to be associated with Leaf Tip Necrosis (LTN), which indicated that the resistance to LR, YR and PM in Saar could be under common genetic control (Lillemo et al. 2007). The objectives of the research presented in this paper were: (i) to elucidate the molecular genetic control of LR, YR and PM resistance in Saar through QTL mapping, and (ii) to characterize the PM resistance associated with *Lr34/Yr18* and *Lr46/Yr29* through detached leaf-tests with near-isogenic lines (NILs).

## MATERIALS AND METHODS

A population of 113 RILs from a cross between Avocet and Saar was evaluated for PM resistance over two years at two locations in Norway (Hamar and Ås in 2005 and 2006) and once in Beijing, China, in 2005. LTN and LR were evaluated in Cd. Obregon, Mexico in the 2004-05 cropping season and YR in Toluca, Mexico in 2005. The population was genotyped with SSR markers selected from a bulked-segregant analysis with the five most resistant and susceptible entries based on the PM data, and supplemented with DArT genotyping. Simple and composite interval mapping was conducted with PlabQTL v. 1.2.

NILs with *Lr34/Yr18* and *Lr46/Yr29* in the genetic background of Avocet and *Lr34/Yr18* in the background of Lalbahadur were grown in a spore-free glasshouse compartment and used for sampling leaf segments from plants at different growth stages from the first seedling leaves to flag leaves of adult plants. Leaf segments of 3 cm were laid down in plastic boxes with benzimidazole agar and inoculated with fresh conidia of *Blumeria graminis* f.sp. *tritici* with the aid of a settling tower.

Boxes with inoculated leaf segments were placed in a growth chamber at 15°C and 16 h photoperiod at low light intensity. The number of sporulating colonies on each leaf segment was counted 10 days after inoculation, and divided by the leaf area to calculate the *infection efficiency* (colonies/cm<sup>2</sup>). The *sporulation rate* (spores/colony) was assessed by tapping spores from individual leaf segments into 0.1% NaCl solution and counting the spore density in a Coulter Counter.

## RESULTS

High correlation coefficients among the disease severity scores of the Avocet x Saar population (Table 1) indicated that part of the resistance to LR, YR and PM was under common genetic control, and a likely involvement of *Lr34/Yr18* in the resistance to PM was indicated by a strong association with LTN (Table 1).

**Table 1.** Correlation coefficients among phenotypic traits in the Avocet x Saar population. All correlations were highly significant at the level  $\alpha=0.0001$ .

	PM China	LR Mexico	YR Mexico	LTN Mexico
PM Norway	0.697	0.738	0.762	-0.624
PM China		0.547	0.552	-0.575
LR Mexico			0.762	-0.849
YR Mexico				-0.640

A summary of the QTL mapping is shown in Table 2. Two QTLs were detected with resistance from Saar that had major effects on all three diseases. These correspond to the adult plant rust resistance loci *Lr34/Yr18* and *Lr46/Yr29* on 7DS and 1BL, respectively. One further QTL with resistance from Saar was detected on 3AS that affected both PM and YR. Avocet contributed a major QTL for PM resistance on 4BL, while minor QTLs for PM resistance from Saar were detected on 5AL, 5BL and 7BL. More details about the QTL mapping in Avocet x Saar and information about closely linked molecular markers are given by Lillemo et al. (2008).

The PM resistance associated with the *Lr34/Yr18* and *Lr46/Yr29* loci have recently been named *Pm38* and *Pm39*, respectively (Lillemo et al. 2008).

**Table 2.** Results of composite interval mapping of PM, LR and YR resistance and simple interval mapping of LTN in the Avocet x Saar population. The table shows R<sup>2</sup> values obtained from multiple regression.

Chrom. arm	Marker interval	Source <sup>1</sup>	PM Norway	PM China	LR Mexico	YR Mexico	LTN Mexico
1BL	<i>Xwmc719-Xhbe248</i>	S	31.1	9.5	49.1	17.4	12.1
3AS	<i>Xstm844tcac-Xbarc310</i>	S	19.5			8.7	
4BL	<i>XwPt-1505-Xgwm149</i>	A	40.2	21.0			
5AL	<i>Xgwm617b-Xwmc327</i>	S	10.2				
5BS	<i>Xbarc4-Xgwm274b</i>	S	9.0	9.7			
6AL	<i>Xbarc3-XwPt-7063</i>	A				14.4	
7BL	<i>Xwmc581-XwPt-8007</i>	S		4.9			
7DS	<i>Xgwm1220-Xswm10</i>	S	46.3	27.9	73.1	40.0	76.6
1BL x 7DS					43.1		
% of the phenotypic variance explained			72.0	48.8	81.2	52.8	
% of the genotypic variance explained			80.4	96.0	82.5	55.3	

<sup>1</sup> S = Saar, A = Avocet

Leaf segments of first seedling leaves of the NILs showed a susceptible infection type to 18 differential isolates (data not shown), which indicated that the resistance genes *Pm38* and *Pm39* confer race non-specific resistance to PM in a similar manner as the rust resistance genes at the same loci.

A significant reduction in infection efficiency associated with the presence of *Pm38* or *Pm39* in the genetic background of Avocet was only detected in flag leaves while the effect of *Pm38* was also significant in penultimate leaves (Table 3). No significant reduction in sporulation rate was detected at any growth stage, but there was a tendency toward less sporulation associated with *Pm38* in penultimate leaves (data not shown). No reliable measurement of sporulation rate was possible on flag leaves due to the very low numbers of colonies.

In the more susceptible genetic background of Lalbahadur there was no reduction in infection efficiency (Table 4) or sporulation rate (Table 5) associated with *Pm38* even in the flag leaves. On the contrary, the presence of *Pm38* seemed to increase the infection efficiency (Table 4).

To determine whether the leaf age was important for the expression of *Pm38* resistance in Lalbahadur, the infection efficiency was measured on flag leaves at three different growth stages. Although there was a tendency toward increased resistance in older flag leaves, there was no overall effect of *Pm38* (Table 6).

LTN was expressed in some of the flag leaves and a different grouping of the data according to this parameter revealed a clear effect of *Pm38* in the presence of LTN (Table 7).

**Table 3.** Infection efficiency (colonies/cm<sup>2</sup>) measured on detached leaves of Avocet NILs at different growth stages.

	1st leaves	3rd leaves	5th leaves	F-1 leaves	Flag leaves
Avocet	11.0	10.2	2.34	2.43	0.66
Avocet+ <i>Pm38</i>	10.1	11.8	3.73	<b>1.04</b>	<b>0.08</b>
Avocet+ <i>Pm39</i>	12.6	10.8	1.40	1.82	<b>0.06</b>

**Table 4.** Infection efficiency (colonies/cm<sup>2</sup>) measured on detached leaves of Lalbahadur NILs at different growth stages.

	1st leaves	3rd leaves	5th leaves	F-1 leaves	Flag leaves
Lalbahadur	31.8	21.0	17.4	15.0	9.1
Lalb+ <i>Pm38</i>	25.3	20.5	26.3	36.3	16.0

**Table 5.** Sporulation rate (spores/colony) measured on detached leaves of Lalbahadur NILs at different growth stages.

	1st leaves	3rd leaves	5th leaves	F-1 leaves	Flag leaves
Lalbahadur	60.3	46.8	81.3	31.6	20.9
Lalb+ <i>Pm38</i>	64.6	40.7	69.2	40.7	20.4

**Table 6.** Infection efficiency (colonies/cm<sup>2</sup>) measured on flag leaves of Lalbahadur NILs at different growth stages.

	GS 55	+5d	+16d	+23d
Lalbahadur	6.39	1.25	1.08	0.95
Lalb+ <i>Pm38</i>	10.09	3.95	2.76	1.59

**Table 7.** The effect of LTN on infection efficiency (colonies/cm<sup>2</sup>) measured on flag leaves of Lalbahadur NILs at different growth stages.

	GS 55 +16d	GS 55 +23d
Lalbahadur	1.08	0.95
Lalb+ <i>Pm38</i> , - LTN	6.13	4.86
Lalb+ <i>Pm38</i> , + LTN	<b>0.51</b>	<b>0.31</b>

It was observed in the detached leaf experiments with both Avocet and Lalbahadur NILs that lines with *Pm38* and *Pm39* had a more frequent occurrence of necrosis associated with PM colonies. Preliminary results from microscopy studies show that both genes are associated with a higher frequency of hypersensitive cell death at the seedling stage (data not shown).

## DISCUSSION

The most significant finding of the QTL study was the major contribution to PM resistance of the LTN-associated rust resistance loci on 7DS and 1BL. The PM resistance of these loci was further confirmed in the detached leaf experiments with NILs, which also showed that the PM resistance of both loci is race non-specific and only expressed in adult plants. These are also common features of the LR and YR resistances of these loci and indicate that the underlying genes most likely work through physiological processes that affect all three diseases in a similar manner. Interestingly, the *Lr34/Yr18/Pm38* locus is also associated with resistance to stem rust, but the expression of the resistance depends on the genetic background (Spielmeyer et al. 2008; Vanegas et al. 2008).

While the resistance of *Pm38* and *Pm39* was expressed in all flag leaves in the moderately susceptible genetic background of Avocet, the resistance of *Pm38* could only be detected in the highly susceptible background of Lalbahadur when LTN was clearly expressed. This could be an indication of a direct physiological link between LTN and the disease resistance at this locus.

Further insight into the resistance mechanisms of *Lr34/Yr18/Pm38* has come from a recent microarray study that found the resistance gene to be associated with an up-regulation of many genes commonly associated with abiotic stress responses in both rust inoculated and mock inoculated flag leaves (Hulbert et al. 2007). The up-regulation of most of these transcripts was much stronger in the leaf tips than the leaf bases, which corresponds well with the general disease resistance gradient commonly found in adult plant leaves of lines with *Lr34/Yr18/Pm38*.

The general disease resistance loci *Lr34/Yr18/Pm38* and *Lr46/Yr29/Pm39* are of major advantage to wheat breeding as they allow for simultaneous selection of race non-specific and potentially durable resistance against

three of the most important biotrophic pathogens in wheat. Furthermore, the selection can be aided by closely linked molecular markers (Lillemo et al. 2008).

## REFERENCES

- Hulbert SH, Bai J, Fellers JP, Pacheco MG, Bowden RL (2007) Gene expression patterns in near isogenic lines for wheat rust resistance gene *Lr34/Yr18*. *Phytopathology* 97:1083-1093
- Lillemo M, Asalf B, Singh RP, Huerta-Espino J, Chen XM, He ZH, Bjørnstad Å (2008) The adult plant rust resistance loci *Lr34/Yr18* and *Lr46/Yr29* are important determinants of partial resistance to powdery mildew in bread wheat line Saar. *Theor Appl Genet* 116:1155-1166
- Lillemo M, Singh RP, Huerta-Espino J, Chen XM, He ZH, Brown JKM (2007) Leaf rust resistance gene *Lr34* is involved in powdery mildew resistance of CIMMYT bread wheat line Saar. In: Buck HT, Nisi JE, Salomon N (eds) *Developments in Plant Breeding Vol 12 Wheat productions in stressed environments Proceedings of the 7th International Wheat Conference Mar del Plata, Argentina Nov 27-Dec 2, 2005*, pp 97-102
- Lillemo M, Skinnies H, Singh RP, van Ginkel M (2006) Genetic analysis of partial resistance to powdery mildew in bread wheat line Saar. *Plant Dis* 90:225-228
- Navabi A, Singh RP, Tewari JP, Briggs KG (2003) Genetic analysis of adult-plant resistance to leaf rust in five spring wheat genotypes. *Plant Dis* 87:1522-1529
- Navabi A, Singh RP, Tewari JP, Briggs KG (2004) Inheritance of high levels of adult-plant resistance to stripe rust in five spring wheat genotypes. *Crop Sci* 44:1156-1162
- Spielmeyer W, Singh RP, McFadden H, Wellings CR, Huerta-Espino J, Kong X, Appels R, Lagudah ES (2008) Fine scale genetic and physical mapping using interstitial deletion mutants of *Lr34/Yr18*: a disease resistance locus effective against multiple pathogens in wheat. *Theor Appl Genet* 116:481-490
- Vanegas CDG, Garvin DF, Kolmer JA (2008) Genetics of stem rust resistance in the spring wheat cultivar Thatcher and the enhancement of stem rust resistance by *Lr34*. *Euphytica* 159:391-401