Identification and functional characterization of a *Xa21-Receptor Like kinase* gene required for *Mla13*-mediated resistance to *Blumeria graminis* f. sp. *hordei* in barley

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Introduction

One of the most studied plant-pathogen interaction in cereals is the association between obligate biotrophic powdery mildew fungus *Blumeria graminis* f. sp. *hordei (Bgh)* and its natural host barley *(Hordeum vulgare).* Genetic resistance in barley to *Bgh* could be either race specific or nonrace specific. Race specific resistance requires the interaction of resistance (R) gene in the host (such as the gene products of the complex *Mla* locus on chromosome 1H) and the products of cognate *Avr* genes of *Bgh* ('gene-for-gene' theory) (Jones, 2001).
One of the R gene classes is the Leucine Rich Repeat (LRR) kinases. This family involves the Xa21 Receptor-like kinases (*RLK*) of rice which confers resistance to *Xanthomonas oryzae* pv *oryzae* (bacterial-blight resistance) (Song et al., 1997). These proteins contain a transmembrane domain, an extracellular LRRs and a cytoplasmic protein kinase domain.
A gene from barley was identified and its function characterized to gain a better insight of its role during barley-*Bgh* interactions and *Mla*-mediated resistance.

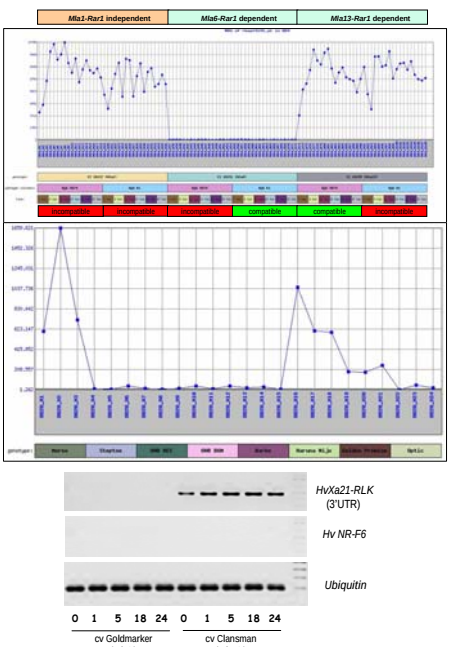

Fig. 2. *Mla* specific expression of *HvXa21RLK* during *Blumeria graminis* infection and its genotype specific expression

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Results & Discussion

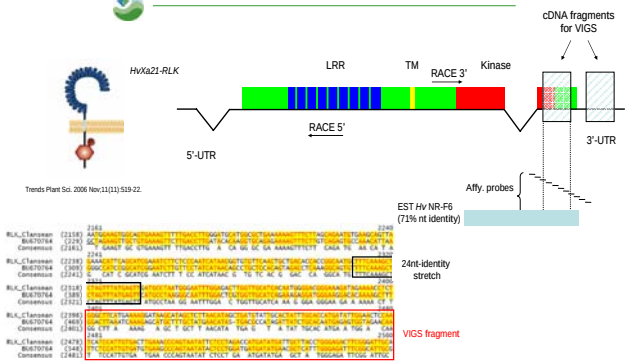


Fig.1. The structure of *Hordeum vulgare Xa21 Receptor-like Kinase*

•*HvXa21RLK* was identified by SSH during *Bgh* infection on Pallas *Mla13* cultivar (Hein et al., 2004). A full length cDNA and genomic clone were isolated from barley cultivar Clansman (*Mla13* genotype). Sequence analysis identified as a *Xa21* Receptor Like Kinase with the following domains: leucine-rich repeat, transmembrane and serine/threonine kinase domains (Fig. 1.).
•*HvXa21RLK* expression increases during the first hours of the incompatible interaction between *Bgh* and barley *Mla1* and *Mla13* cultivars (Fig.2. microarray and semi-quantitative RT-PCR data using a gene specific probe). However, a comparable pattern was observed in a compatible interaction using a susceptible *Bgh* isolate on an *Mla13* cultivar (Fig. 2. microarray data). Interestingly, there is no expression of *HvXa21RLK* on *Mla6* background.
•The closest homologs of *HvXa21RLK* are *Hv NR-F6* (Neu et al., 2003) and Barley1_04666 (Fig.3.). Those related Xa21-RLKs display as well a cultivar-dependant expression as described in figure 3 and 4. Contig Barley1_04666 is expressed in *Mla6* background but not in *Mla1 or Mla13. Hv NR-F6* is not expressed in *Mla13* background according to the semi-quantitative RT-PCR (Fig. 3. and 4.). Taken together, these data suggest that those closely related RLKs are not expressed in our plant-pathogen system.
• We analysed further the role of *HvXa21RLK* with respect to *Mla13* resistance using viral induced gene silencing (VIGS). We engineered VIGS construct by cloning cDNA segments of the kinase domain and of the 3'untranslated region (antisense and short hairpins) were cloned into the previously described *Barley stripe mosaic virus* (BSMV) VIGS vector (Lacomme et al., 2003; Hein et al., 2005). Both fragments were selected on the basis of divergence at the nucleotidic level and having less than 11 nucleotide of uninterrupted identity stretches to the closest RLK homolog, in order to prevent off-target silencing effects due to the incorporation of 21-nt small interfering (si)RNAs within the RNA-Induced Silencing Complex (RISC).
• As observed with SGT1, RAR1 or HSP90 (all components of the ubiquitination machinery required for the *Mla13*-mediated response), silencing of *HvXa21RLK* trigger a resistance-breaking phenotype on *Mla13* cultivar. Significant level of spore germination and mycelium growth result in an increased amount of *Bgh* microcolony by 3 days post inoculation in comparison to control constructs (BSMV.GFP, BSMV.PDS silencing a phytoene desaturase gene not required for *Mla* resistance).

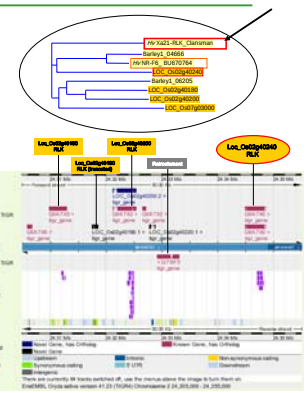


Fig. 3. The closest homologs of *HvXa21RLK* gene from barley are clustered in chromosome 6H, syntenic to rice chromosome 2 harbouring a similar small cluster of *Xa21-RLK* (www.gramene.org)

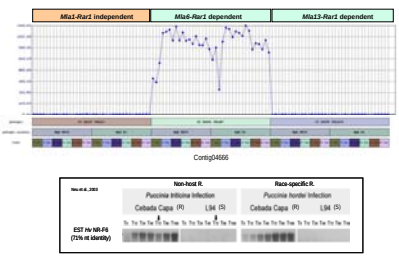
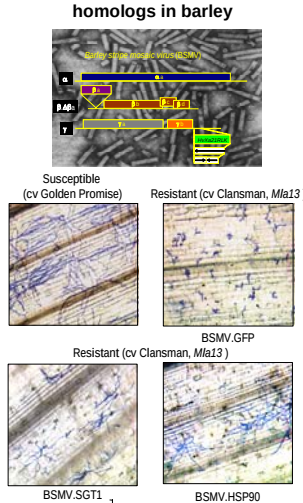


Fig. 4. Expression pattern of closest homologs in barley



Conclusion

•Our findings report the first evidence in monocots that, in addition to the previously described Sgt1, Rar1 and Hsp90 genes, a *Xa21-RLK* is a required component of the *Mla13*-mediated race specific resistance.
• The contrasting cultivar specific expression patterns observed with *HvXa21RLK* and others closely related *RLKs* during compatible and incompatible interactions suggest differential requirement for specific *Mla*-mediated resistance.
• Our data demonstrate that BSMV-induced VIGS is a powerful tool to characterize genes belonging to complex gene families involved in pathogen resistance in barley.

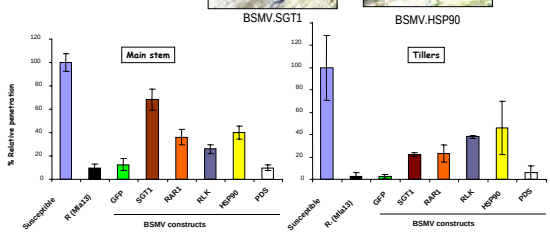


Fig. 5. Resistance breaking phenotype of *Bgh* in *HvXa21RLK* silenced Clansman (*Mla13*) leaves