Plant Biotechnology Journal
Open Access

Plant Biotechnology Journal (2025), pp. 1–3 doi: 10.1111/pbi.70038

Brief Communication

An NLR receptor confers broad-spectrum resistance to diversified powdery mildew sublineages in wheat and barley

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Received 17 December 2024;
revised 14 February 2025;
accepted 20 February 2025.
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Keywords: Pm21 alleles, NLR, broad-spectrum resistance, phytohormone signaling crosstalk.

Wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) are the two most important crops in the Triticeae tribe that diverged approximately 11.6 million years ago. Both crops are severely threatened by notorious fungal diseases such as powdery mildew. The pathogens causing powdery mildew disease on cereal crops are *Blumeria graminis* (*syn. Erysiphe graminis*), which have evolved and existed as distinct sublineages (called *formae speciales*, f.sp.). Intriguingly, mildew isolates from each *formae speciales* subfamily can only cause disease on a cereal host species, but normally not on the others, thus referred to as non-host species. Thus far, powdery mildew resistance genes isolated from wheat, barley and relatives encode mostly nucleotide-binding oligomerization domain-like immune receptors (NLRs) and a few non-NLR proteins (Sánchez-Martín and Keller, 2021; Zou *et al*., 2023). While most of the NLR-type *Pm* genes mediate isolate-specific resistance, the *Pm21* gene, originally introgressed from wheat wild relative *Dasypyrum villosum* (*Dv*), confers broad-spectrum resistance (BSR) to all tested isolates of the wheat powdery mildew, *B. graminis* f.sp. *tritici* (*Bgt*) (Zhang *et al*., 2023). All cloned *Pm21* homologous genes encode typical coiled-coil (CC)-subtype NLRs (Han *et al*., 2024; He *et al*., 2018, 2020; Huang *et al*., 2023; Xing *et al*., 2018; Zhu *et al*., 2023). However, whether the *Pm21* genes confer BSR to a diversified *B. graminis* subfamily that colonizes a diverged Triticeae crop, for example, *B. graminis* f.sp. *hordei* (*Bgh*) infecting only barley, remains uninvestigated.

Our previous analysis reveals that the *Pm21* locus in *Dv* accessions harbours at least 38 non-redundant *Pm21* alleles and these alleles were classified into seven clades, representing a valuable NLR gene pool (He *et al*., 2020). To further explore the *Pm21* gene resources, the *Pm21-B1* allele, as a member from the largest clade B and isolated from a resistant *Dv* accession W619414, was selected for further comparative analysis with the *Pm21* allele (i.e. *Pm21-A1* allele from clade A). We aimed to broaden the utilization of *Pm21* allelic gene pool in diverged Triticeae crops.

The *Pm21-B1* allele differs from the *Pm21* allele by several InDels and many SNPs (He *et al*., 2020), with most polymorphisms resided in the LRR domains (Figure 1a, Figure S1). To assess the function of *Pm21* and *Pm21-B1* in diverged crops, we performed single-cell transient gene expression assays in both wheat and barley by particle bombardment. Overexpression of either *Pm21* or *Pm21-B1* significantly reduced fungal haustorium index (HI%) in transformed epidermal cells of wheat KN199 and barley Golden Promise (GP) leaves, inoculated with wheat PM isolate *BgtE18* or barley PM isolate *BghK1*, respectively (Figure 1a). These data suggest that both *Pm21* alleles are functional and confer resistance to diversified PM fungi, importantly in two diverged crop species. Further agro-infiltration assay in *Nicotiana benthamiana* showed that the CC domain of Pm21 and Pm21-B1 induced cell death, while both full-length proteins did not, with the autoactive mutant variants as positive controls (Figure 1b and Figure S2). Interestingly, the Pm21-B1_CC-NB fragment also triggered cell death, whereas the Pm21_CC-NB did not

Please cite this article as: Fan, R., Feng, L., Liu, Y., Tang, Q., Zhao, Y., Li, Y., Gong, S., Lin, R., Huang, S., Qi, T., Förderer, A., Yang, L., Wang, Y., Chai, J., Schulze-Lefert, P., He, H. and Shen, Q.H. (2025) An NLR receptor confers broad-spectrum resistance to diversified powdery mildew sublineages in wheat and barley. *Plant Biotechnol. J*., https://doi.org/10.1111/pbi.70038.

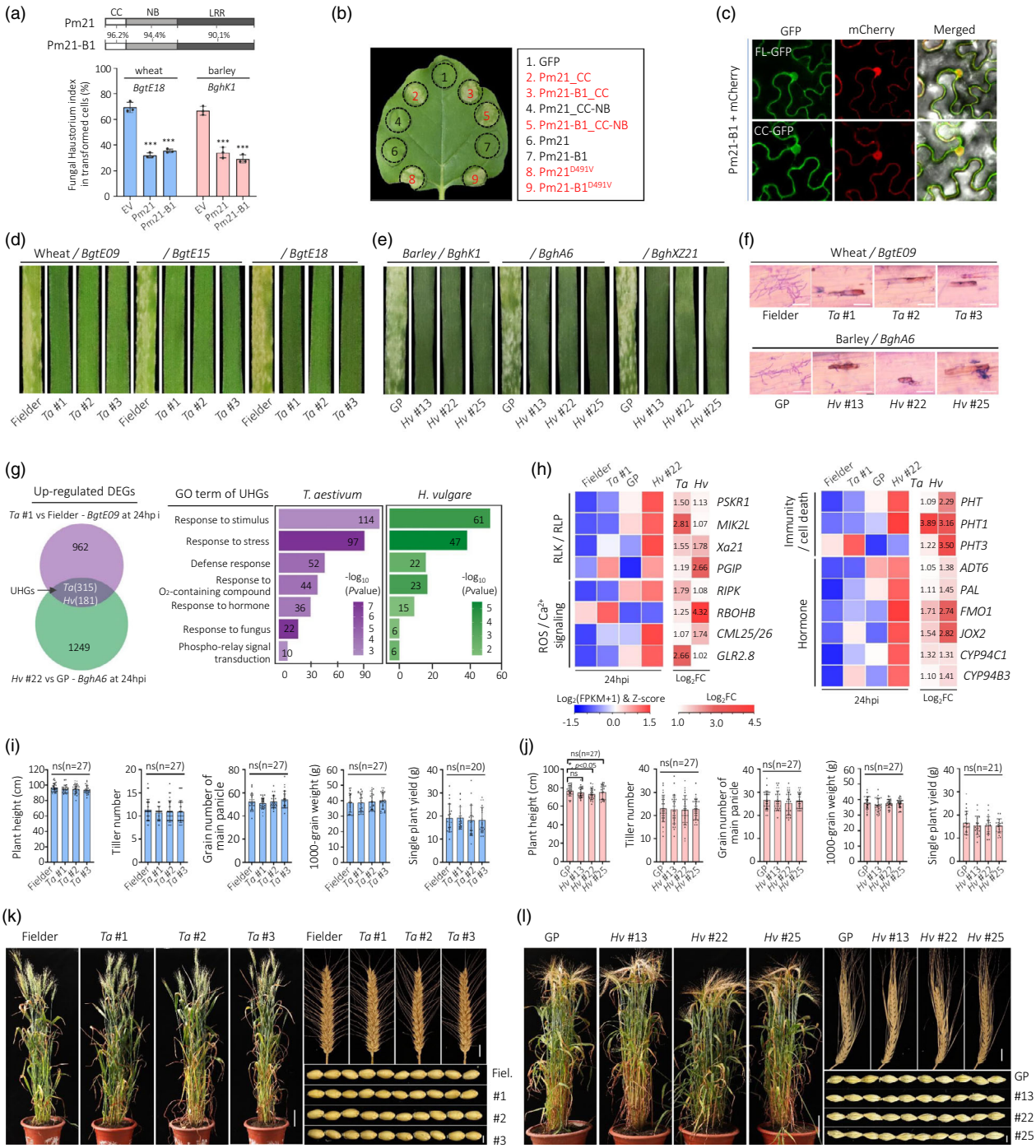

Figure 1 Functional dissection of *Pm21* alleles in broad-spectrum resistance against diversified powdery mildew subspecies. (a) Schematic illustration of the domain structure and sequence similarity between Pm21 and Pm21-B1 (upper panel), and transient gene expression of the two alleles in leaf epidermal cells of wheat KN199 and barley GP (lower panel). Two-tailed student's *t*-test indicates a significant difference with asterisks ($P < 0.001$). (b, c) Agro-infiltration assay in *N. benthamiana* indicates the cell-death inducing activity of the CC domain of both Pm21 and Pm21-B1 and the CC-NB domain of Pm21-B1, highlighted in red (b), and subcellular localization of the full-length (FL) and CC domain of Pm21-B1 (c). (d, e) Immune phenotype of the *Pm21-B1* transgenic wheat (d) and barley lines (e), at 7 dpi of *B. graminis* isolates. (f) DAB staining indicates H_2O_2 accumulation in *Pm21-B1* transgenic plants at 24 hpi. (g, h) RNA-seq analysis for transgenic plants vs recipients at 24 hpi. The Venn diagram shows the numbers of up-regulated DEGs and the up-regulated homologous genes (UHGs), and related GO terms (g) and heatmap annotation (h). (i–l) Measurement of five key agronomic traits of the wheat (i) or barley (j) transgenic lines and the recipient cv. Values are means and standard errors with sample sizes (*n*) above the columns. Representative photos of the plants, mature spikes and seeds of the wheat (k) or barley (l) transgenic lines and the recipient, bar = 10, 1.0 and 0.5 cm, respectively. Trait measurements have been repeated three times over three crop growth cycles with similar results (i, j). "*$P < 0.05$" marks a significant difference in plant height between GP and Hv#22 (j).

(Figure 1b). Further, expression of GFP-fused Pm21-B1 protein or CC domain revealed their subcellular localization in both the nucleus and cytoplasm in *N. benthamiana* cells (Figure 1c).

To investigate the function of the *Pm21-B1* allele in Triticeae crops, we generated wheat and barley transgenic lines overexpressing *Pm21-B1*, and selected three positive lines from each crop species for further study (Figure S2). We evaluated the resistance of the transgenic plants using 103 *Bgt* and 70 *Bgh* isolates, respectively. Significantly, all the wheat transgenic lines (*Ta*#1, *Ta*#2, and *Ta*#3) and barley transgenic lines (*Hv*#13, #22 *Hv*#22 and *Hv*#25) showed complete immunity to all tested *Bgt* or *Bgh* isolates, respectively (Figure 1d,e). Compared with the recipients, highly induced expression of defence marker genes *PR1* and *PR2*, as well as enhanced H_2O_2 accumulation and localized cell death were observed in the transgenic lines (Figure 1f, Figure S3). Together, these data suggest *Pm21-B1* confers broad-spectrum resistance to distinct powdery mildew sublineages in both wheat and barley.

To understand the molecular basis underlying the resistance, we conducted RNA-Seq analysis by comparing the transgenic lines to the wild-type recipients after inoculation, respectively, that is, *Ta*#1 vs Fielder (with *BgtE09*), and *Hv*#22 vs GP (with *BghA6*). At 24 hpi, we identified a total of 2271 and 2338 DEGs from wheat and barley, of which 1277 and 1430 DEGs were up-regulated, respectively. Among these, 315 wheat and 181 barley DEGs being homologous genes were designated as up-regulated homologous genes (UHGs) (Figure 1g). GO analysis indicated these UHGs were mainly enriched in pathways such as responses to stimuli, stresses, fungi, etc. (Figure 1g). A heatmap shows that key immune components/pathways were up-regulated significantly in both species, such as RLKs sensing tyrosine-sulfated peptide or phytocytokine, ROS/cell-death/Ca^{2+} signalling and TFs related to immunity (Figure 1h). Notably, the defence hormones JA and SA signalling crosstalk may play a role in *Pm21-B1* triggered BSR in both wheat and barley, as indicated by the induced expression of *JOX2/CYP94C1/CYP94C3* and *ADT6/PAL* (Figure 1h).

We evaluated five major yield-related traits for the transgenic lines over three crop growth cycles and found no obvious differences between the transgenic lines and the respective recipients (Figure 1i,j), except only a markedly reduced plant height for the barley transgenic line *Hv*#22, as compared with GP plants (Figure 1j). In consistent, the transgenic lines displayed similar morphological traits to the WT recipient (Figure 1k,y). These results indicate that *Pm21-B1* overexpressed has no obvious fitness costs for most transgenic wheat and barley lines.

Here, we demonstrated that the NLR-type *Pm21* alleles confer broad-spectrum resistance to mildew fungi from diversified *B. graminis* subfamilies in diverged Triticeae crops. We speculate that the Pm21 receptor may recognize conserved AVR effector(s) that likely exist in many diverged *B. graminis* sublineages. Moreover, overexpression of *Pm21*-B1 allele poses no obvious yield penalty in wheat and barley, indicating the potential value of the *Pm21* alleles in improving resistance to powdery mildew in cereal crops.

Author contributions

QHS, HH and RF designed the project and wrote the manuscript. RF, LF, YLL, QT, YZ, YNL, SG, RL, SH and TQ performed experiments. LF, RF, HH and QHS analysed RNA-seq data. LY and YW contributed new reagents and analysis tools. PSL, JC and AF contributed ideas and discussion.

Funding

This study was supported by the National Key R&D Program of China (2022YFF1001500/2022YFF1001503), the National Natural Science Foundation of China (32171990), the Natural Science Foundation of Jiangsu Province (BK20231321) and State Key Laboratory of PCCE (PCCE-KF-2021-05/2022–07).

Conflict of interest

The authors declare no conflict of interests.

Data availability statement

The data that support the findings of this study are openly available in RNA-seq analysis of wheat and barley Pm21-B1 transgenic line at http://www.ncbi.nlm.nih.gov/bioproject/PRJNA1161191/.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 Comparison of the Pm21 and Pm21-B1 receptors.
Figure S2 Protein accumulation of Pm21 and Pm21-B1 in *N. benthamiana* and verification of *Pm21-B1* wheat and barley transgenic lines.
Figure S3 Induced *PR* gene expression, H_2O_2 accumulation and localized cell death in *Pm21-B1* transgenic plants.
Appendix S1 Materials and methods.