Discovery and fine mapping of *Rph28*: a new gene conferring resistance to *Puccinia hordei* from wild barley

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Leaf rust is a highly destructive disease of barley caused by the fungal pathogen *Puccinia hordei.* Genetic resistance is considered to be the most effective, economical and eco-friendly approach to minimize losses caused by this disease. A study was undertaken to characterize and fine map a seedling resistance gene identified in a *Hordeum vulgare* ssp. *spon- taneum*-derived barley line, HEB-04-101, that is broadly effective against a diverse set of Australian *P. hordei* pathotypes. Genetic analysis of an F3 population derived from a cross between HEB-04-101 and the *H. vulgare* cultivar Flagship (seedling susceptible) confirmed the presence of a single dominant gene for resistance in HEB-04-101. Selective genotyping was per- formed on representative plants from non-segregating homozygous resistant and homozygous susceptible F3 families using the targeted genotyping-by-sequencing (tGBS) assay. Putatively linked SNP markers with complete fixation were identified on the long arm of chromosome 5H spanning a physical interval between 622 and 669 Mb based on the 2017 Morex barley reference genome assembly. Several CAPS (cleaved amplified polymorphic sequences) markers were designed from the pseudomolecule sequence of the Morex assembly (v1.0 and v2.0), and 16 polymorphic markers were able to delineate the *RphHEB* locus to a 0.05 cM genetic interval spanning 98.6 kb. Based on its effectiveness and wild origin, *RphHEB* is distinct from all other designated *Rph* genes located on chromosome 5H and therefore the new locus symbol *Rph28* is recommended for *RphHEB* in accordance with the rules and cataloguing system of barley gene nomenclature.

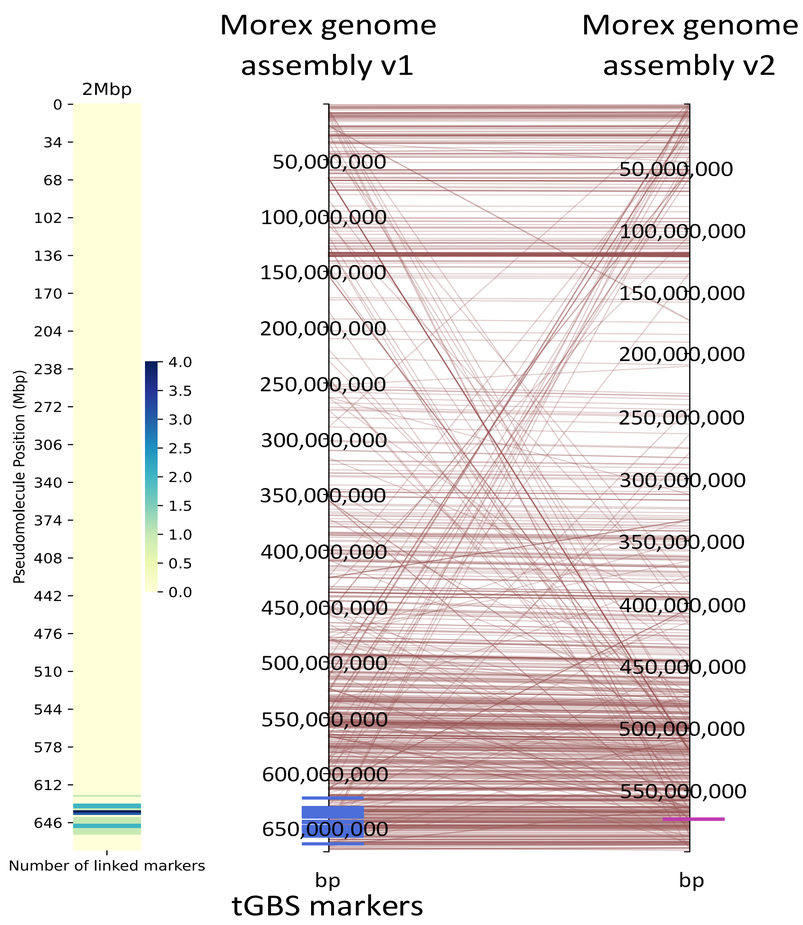

Fig. 1 Putatively linked markers on chromosome 5H identified through targeted genotyping by sequencing in v1 and v2 Morex genome assembly. Blue high- lighted region indicated linked region from 622 to 669 Mbp in v1. Diagram generated using the software Pretzel (https://plant informatics.io/; Keeble-Gagnere et al. 2019)

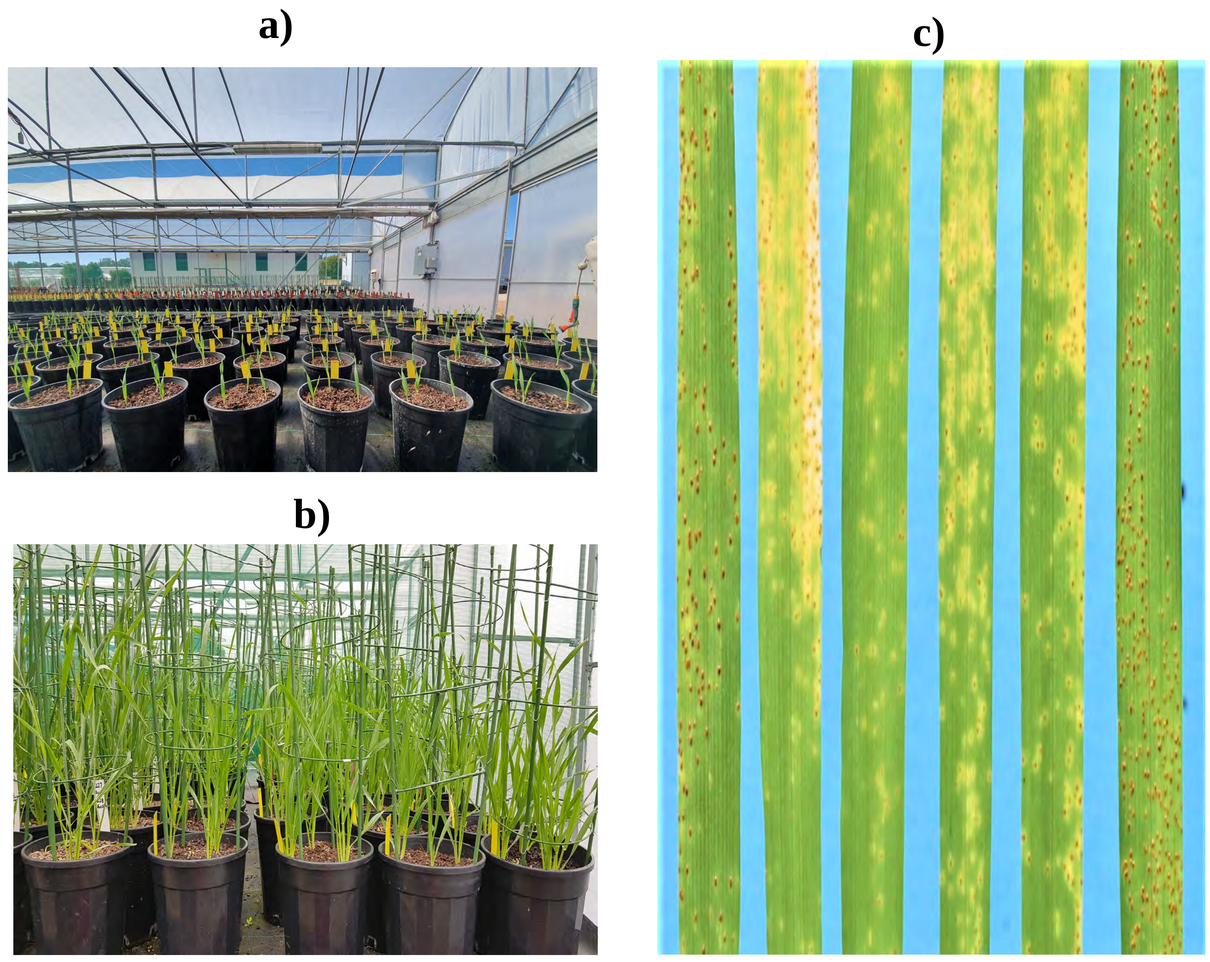

Fig. 2 a) & b) Generation advancement of Flagship/HEB 04-101 population. **c)** Segregation of F3 families in Flagship/HEB 04-101 population (left to right): Flagship (3+), 04-101 (;1+C), Progeny 1 (;1+N), Progeny 2 (;12C), Progeny 3 (23C) and Progeny 4 (3+).

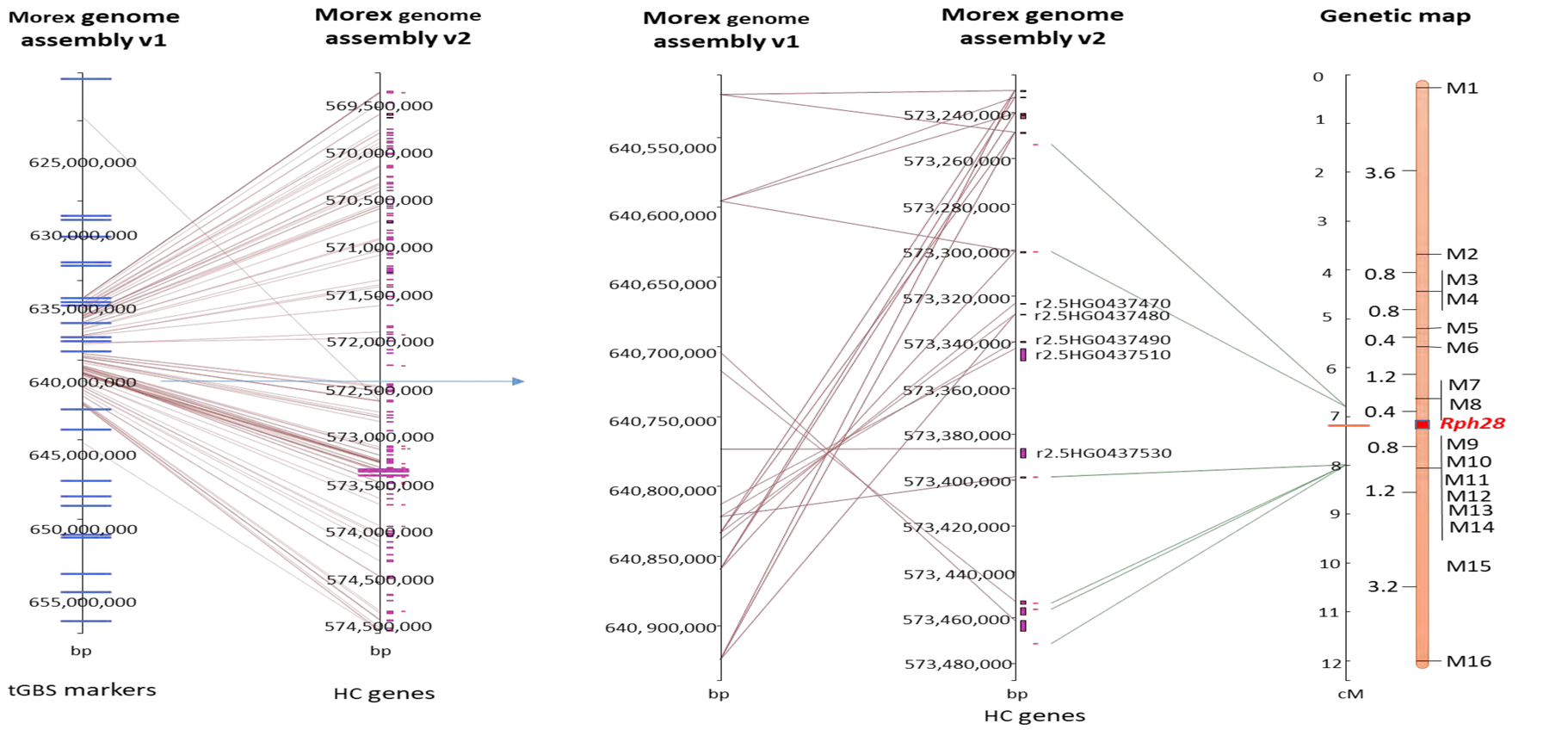

Fig. 3 Physical and genetic map for *Rph28* based on Morex genome assembly v1 and v2. Figure shows *Rph28* is fine-mapped in a physical region of 98.6 kb between M8 (0.4 cM) and M9 (0.8 cM) at 573.30 and 573.39 Mb, respectively. Five HC genes including two disease resistant genes highlighted in purple are shown between flanking markers.