Phs-A1 Confers Pre-harvest Sprouting Resistance Independent of Phenology in European Winter Wheat and Genomes Reveal Structural Variation.

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Introduction

Pre-harvest sprouting (PHS), the germination of seeds within heads before harvest, is a major problem in global wheat (*Triticum aestivum L.*) production, manifested by the **loss of bread-making quality** in affected grain. Uncertainties in weather patterns, due to ongoing climate change, are exacerbating this problem and therefore reducing farmers revenue and food availability, as well as increasing price volatility.

Breeding for PHS resistance is one of the most effective measures against this. To find resistant varieties in years lacking pre-harvest rain, **plants need to be challenged** in the field or in the lab (Fig. 1). **Selection** for resistance can not only be based on the phenotype, but also **using genetic markers**, to find such markers genome-wide association (GWA) studies are required.

Recent advances in sequencing technology enabled the assembly of a **wheat reference genomes** [1] as well as **pan-genomes** [2], linking resistance markers to genes and pathways, as well as allowing insights into the **allelic and structural variation** within the gene-pool of wheat.


Figure 1: Pre-harvest sprouting assessment on spikes

Table 1: Markers associated with pre-harvest sprouting grouped by chrom. and strength of evidence

evidence		1A	1B	1D	2A	2B	2D	3A	3B	3D	4A	4B	4D	5A	5B	5D	6A	6B	6D	7A	7B	7D	Un
1 or 2 years	weak	10	6	2	12	11	23	2	5	3	8	9	2	1	12	2	8	3	2	3	9	6	4
3 or 4 years	strong	1	0	0	0	0	0	0	0	0	4	0	0	0	2	0	0	0	0	0	0	0	1

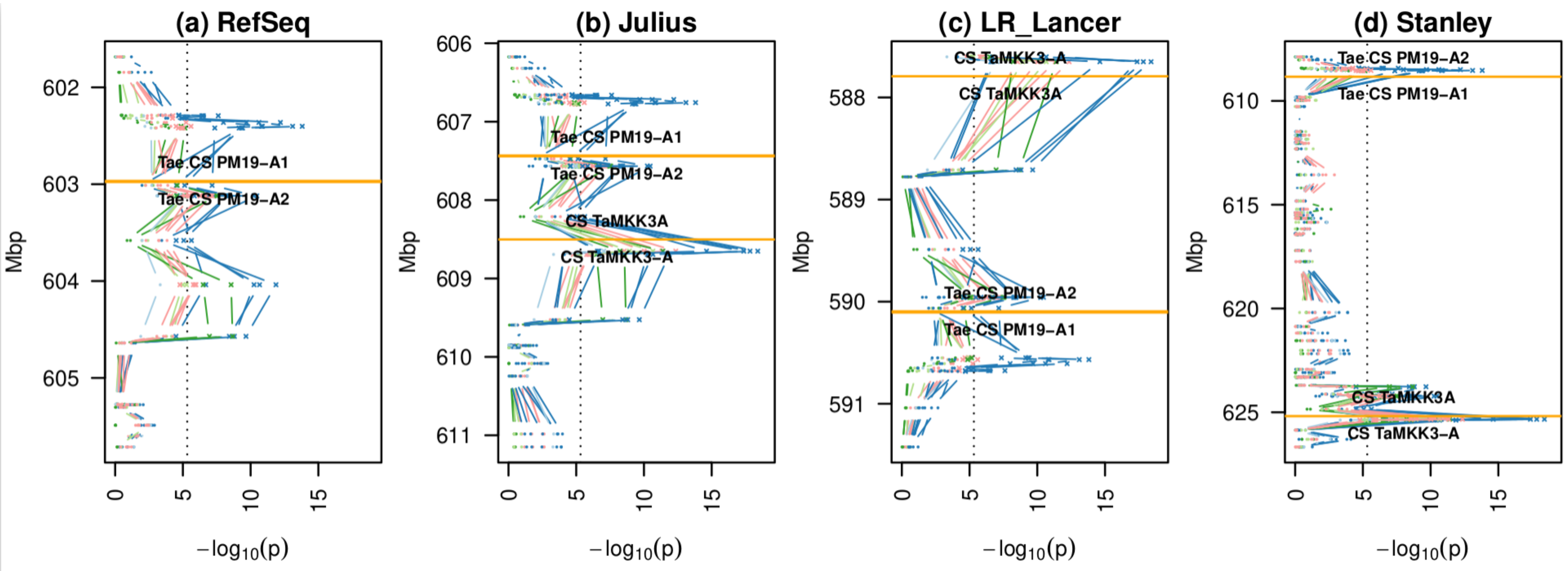

Figure 2: PHS Hotspot on wheat chrom. 4A, mapped to (selected) multiple genomes.

Materials and Methods

- 1.000 European wheat lines tested for PHS resistance in 4 years.
- Field and lab based challenging
- Lines genotyped for 10k SNP markers
- Genome-wide association for each year
- Markers with association in 3 or 4 years
- BLASTn [3] for markers and PHS genes in reference- and pan-genomes
- Pairwise genome alignment, MUMmer [4]

Results and Outlook

GWA found **151 markers with association to PHS**, distributed across all chromosomes (Tab. 1). A **hotspot** of significant markers (Fig. 2) on chrom. **4A** was **only associated with PHS**, not with agronomic traits or phenology and therefore a well suited target for breeding. Up to **three distinct peaks** were found at this hotspot.

This locus is already described as **Phs-A1** [5], at which **two genes** (PM19 [6] and TaMKK3 [7]) have been identified that show variation linked to PHS. Surprisingly, some association peaks matched gene locations only loosely and **marker mapping varied substantially across genomes**, suggesting the presence of structural variation.

Pair-wise alignment of pan- to reference-genomes, revealed patterns of **structural variation at Phs-A1** (Fig. 3) across pan-genomes, suggesting that PHS is not only influenced by allelic, but also by structural variation at Phs-A1. However, to rule out the presence of assembly artefacts **independent long-read assemblies** of genomes would be required, those are not available and beyond the scope of this study.

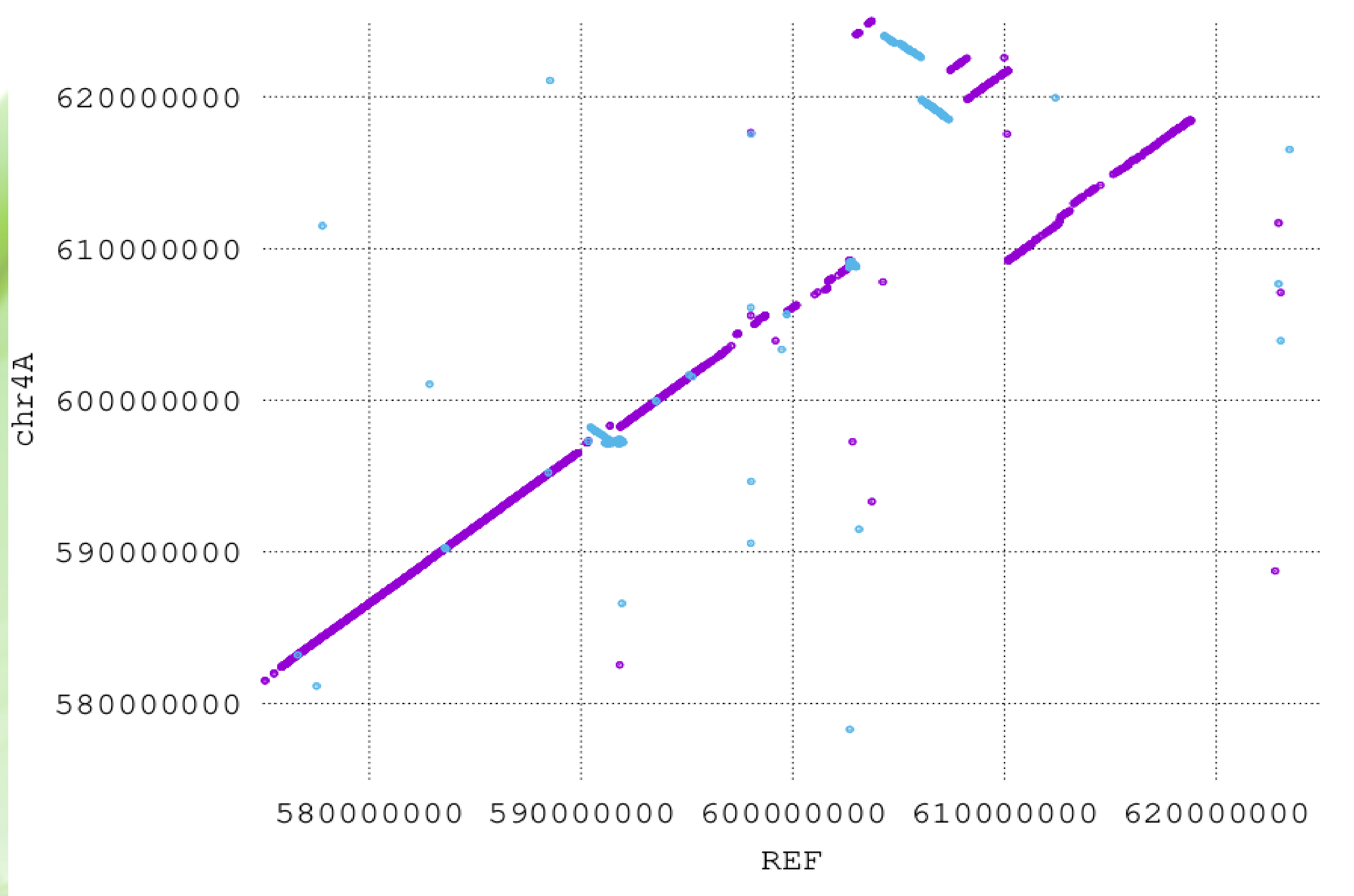

Figure 3: Structural variation within the pre-harvest sprouting hotspot on 4A, cv. Stanley aligned to RefSeq.

Authors' Contributions
HGD performed the association analysis and wrote the manuscript for this poster, NA and FL conducted the sprouting provocation and phenotyping, SM, FL and HB designed experiments and supervised this study.

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