Identification of Closely Linked Flanking Markers to *Rht8* in a Wheat Recombinant Inbred Line (RIL) Population

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INTRODUCTION

Plant height (PH) is an important quantitative trait of wheat. It affects plant lodging, harvest index, disease resistance and yield. The *Rht8* as a gibberellin (GA) sensitive gene on the short arm of chromosome 2D significantly reduces plant height. Compared with *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*), this semi-dwarfing gene is not associated with the GA pathway, and thus, does not reduce leaf area or coleoptile length. For this reason, *Rht8* has been widely used in breeding programs worldwide, especially in moisture-limited situations where deep planting is essential. To date, a simple sequence repeat (SSR) marker *Gwm261* has been the only one used for screening of *Rht8*, but recombinants are frequently found between *Rht8* and *Gwm261* because they are several map units apart. Therefore, *more* closely linked markers are needed for wide deployment of *Rht8* in new cultivars.

OBJECTIVES

- ★ Identify closely linked flanking markers to *Rht8*
- ★ Validate these markers in a recombination inbred line (RIL) population.

MATERIALS AND METHODS

- ➢A population of 132 recombinant inbred lines (RIL) was developed from G97380A (*Rht8*) X G97252W (*rht8*)
- ➢Genotyping-by-sequencing (GBS) was used to identify single-nucleotide polymorphisms (SNPs).
- ➢Plant height was repeatedly evaluated for the population .
- ➢Simple sequence repeats (SSR) markers based on reference sequence and kompetitive allele specific PCR (KASP) markers based on GBS SNPs were designed.
- ➢JoinMap 4.1 was used to construct a linkage map and WinQTLCart2.5 was used for QTL mapping. R and SAS were used for data analysis.

RESULTS

- ➢ The distribution of plant height followed a normal distribution in both seasons, respectively (Fig. 1).
- ➢A total of 2,514 single nucleotide polymorphism (SNPs) and 3 SSR (Gwm261, Xcfd53, PH2918-11) markers were constructed on linkage map. The map contains 2,238 SNPs and 3 SSRs, covering all 21 chromosomes (Fig. 2, Fig. 3).
- ➢Marker PH2198-11 showed the largest effect on height reduction, therefore is the most closed marker to *Rht8*.
- ➢Xcfd53 and PH2198-11 are the best flanking markers for *Rht8,* which can significantly improved selection accuracy compared to original Xgwm261 marker (Table1).
- ➢For high-throughput screening, flanking KASP markers were developed (Figs. 3 & 4), they are closer to *Rht8* than Xgwm261, even though not as close as the two flanking SSR markers (Fig. 3).

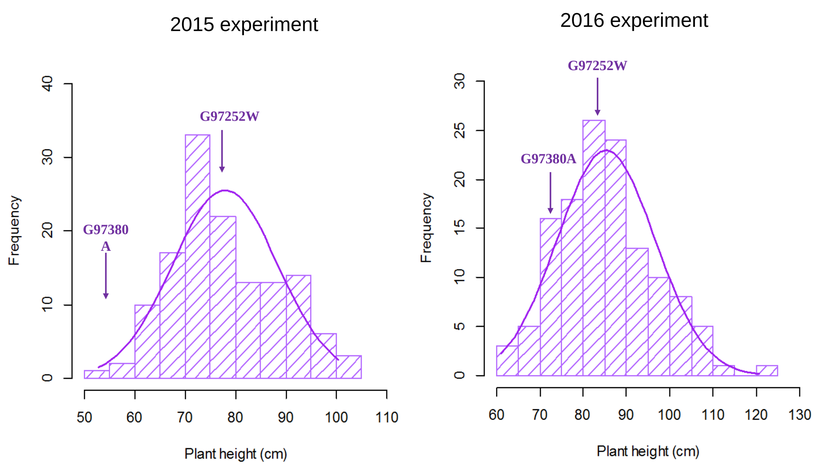


Fig. 1. The distribution of the plant height for the RIL population evaluated in two seasons. The curve line represents the normal distribution.

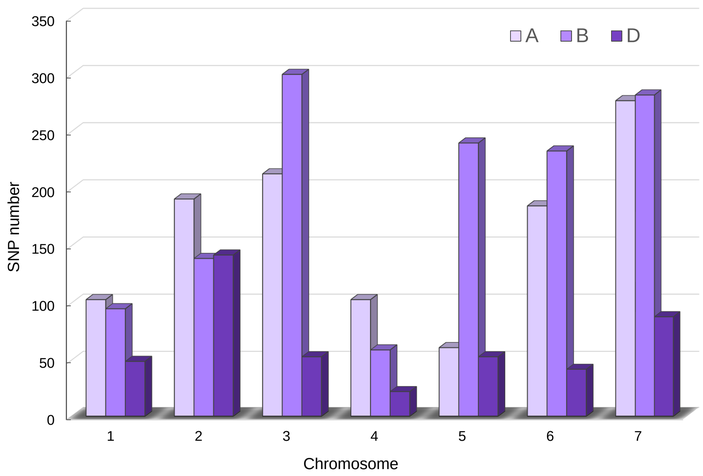
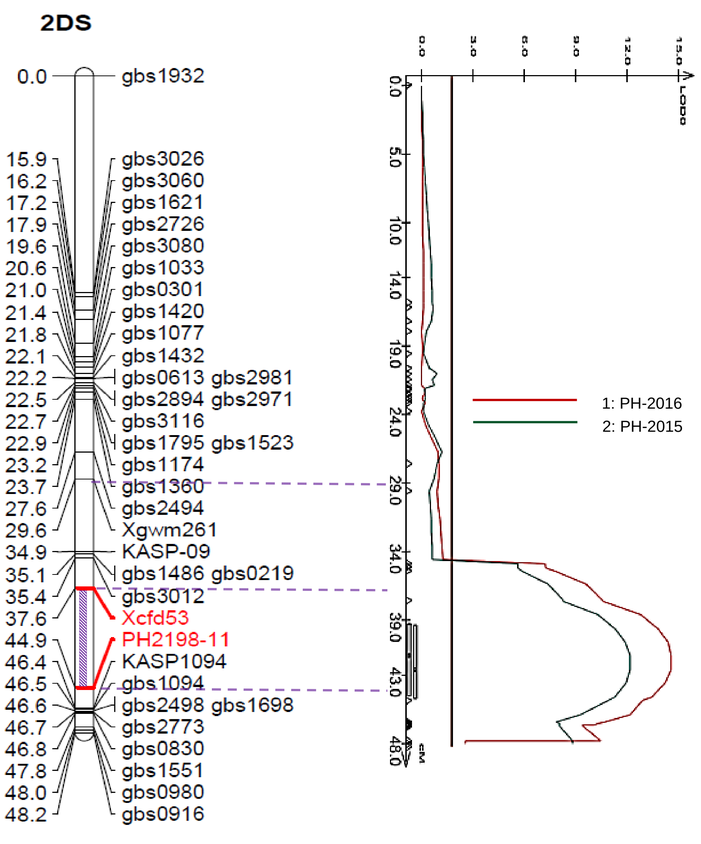


Fig. 2. Genome distribution of mapped SNPs



★ Fig. 3. Linkage mapping results and QTL mapping result on chromosome 2D.

Table.1. Effects of different marker(s) on plant height reduction(cm) evaluated in 2015 and 2016 seasons.

Marker	2015 season	2016 season
Xgwm261	**-9.45 (0.21)**	**-7.60 (0.11)**
Xcfd53	-13.12 (0.40)	-10.21 (0.20)
PH2198-11	-14.15 (0.46)	-12.20 (0.28)
KASP1094	-12.01 (0.34)	-8.62 (0.14)
Xcfd53+PH2198-11	**-16.15 (0.56)**	**-13.04 (0.32)**
Xcfd53+KASP1094	-14.69 (0.50)	-11.07 (0.23)

*Number in bracket means the R^2 or plant height variances explained by the marker.

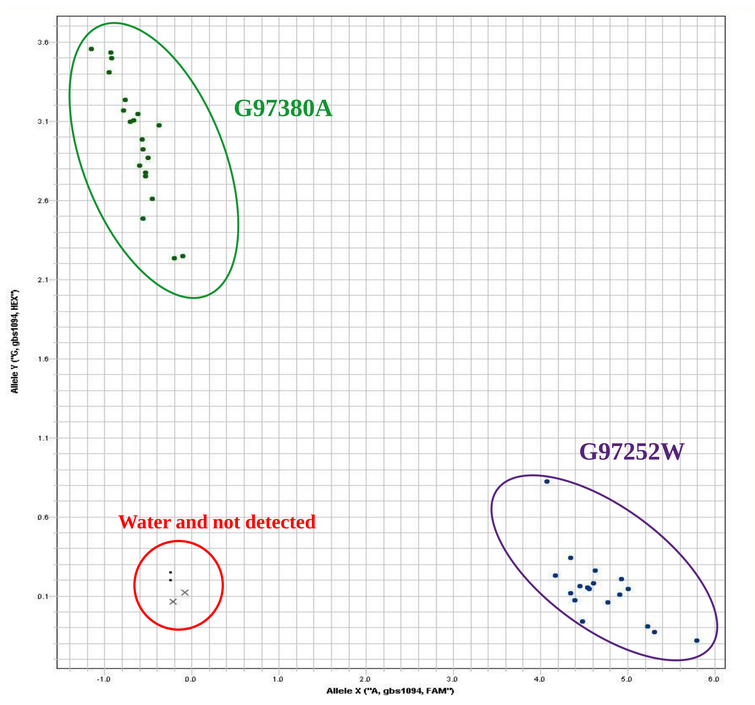


Fig. 4. Segregation of KASP marker KASP1094 in the RIL population of G97380A (*Rht8*) X G97252W (*rht8*).

CONCLUTION

- ➢Two SSR markers, Xcfd53 and PH2918-11, were found to flank *Rht8* at 7.3 cM apart, which are closer to *Rht8* than the original marker Xgwm261. Use of these markers in breeding will significantly improve selection accuracy in developing new cultivars with *Rht8*.
- ➢Several SNPs closely linked to *Rht8* were converted into KASP markers, which can be used for high-throughput screening of *Rht8*.