

# Identification of the Major Gene Associated with Grain Protein Content in Chromosome 2B in Hard Red Winter Wheat (*Triticum aestivum* L.)

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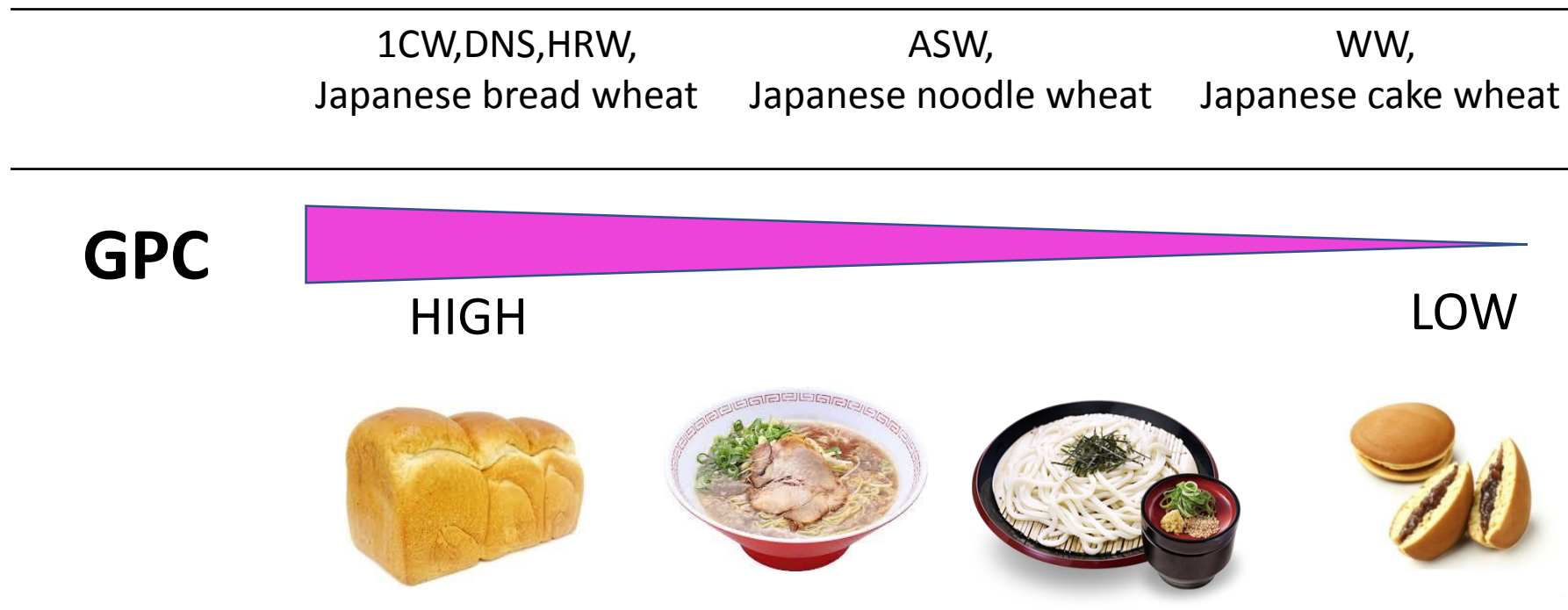
1) NARO, Hokkaido Agricultural Research Center, Memuro Research Station

2) NARO, National Institute of Crop Science

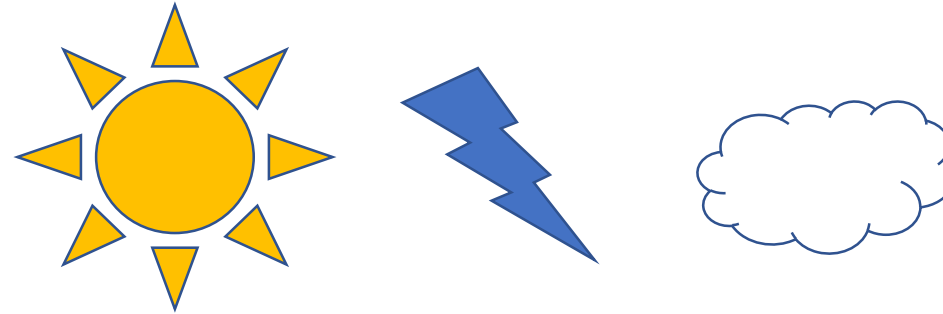
3) Tokyo University of Agriculture



Both **GPC** and protein quality are a major factor that determines the end-use quality of wheat.



Climate conditions



Soil conditions



High nitrogen

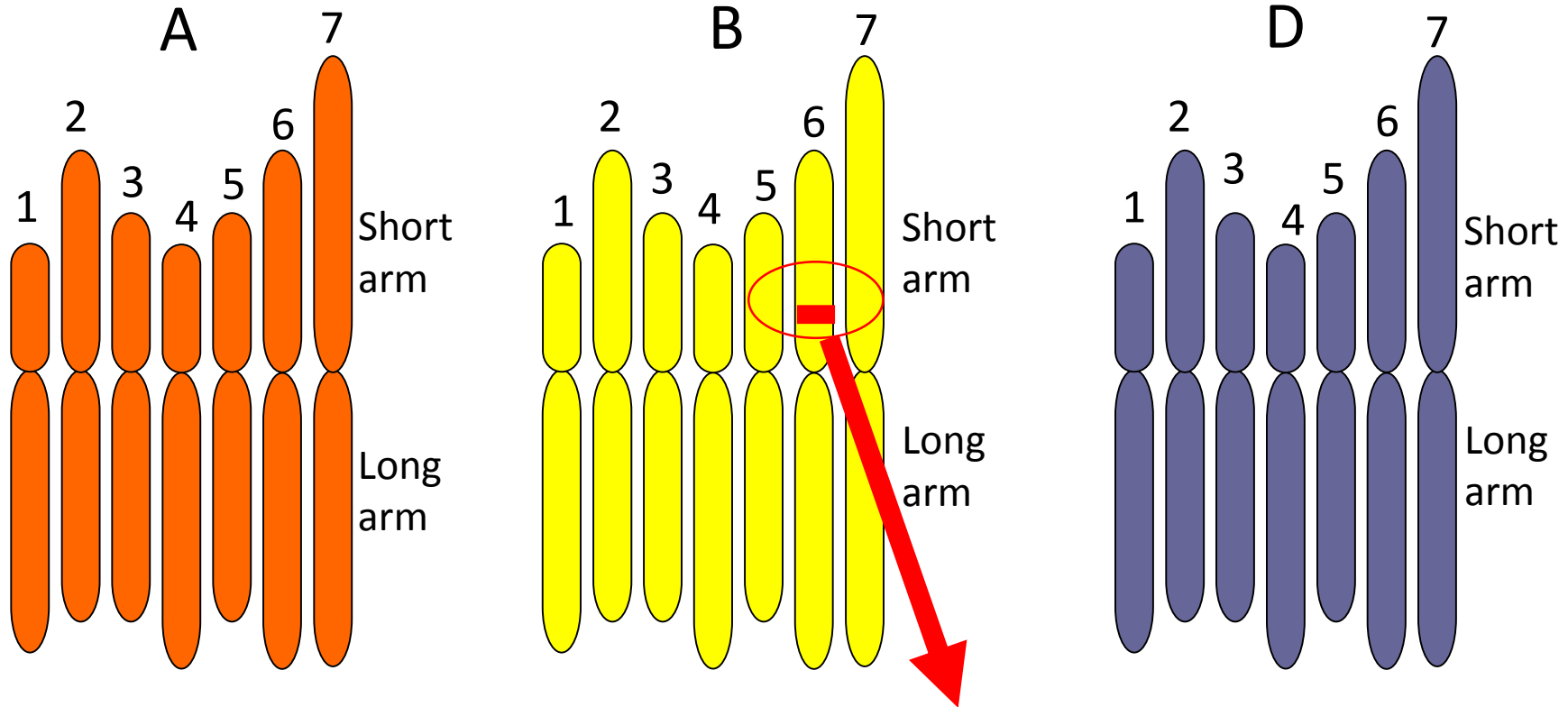


Low nitrogen

Cultivation conditions



➤ *Gpc-B1(NAM-B1)* has increased GPC (Uauy et al. Science 2006)

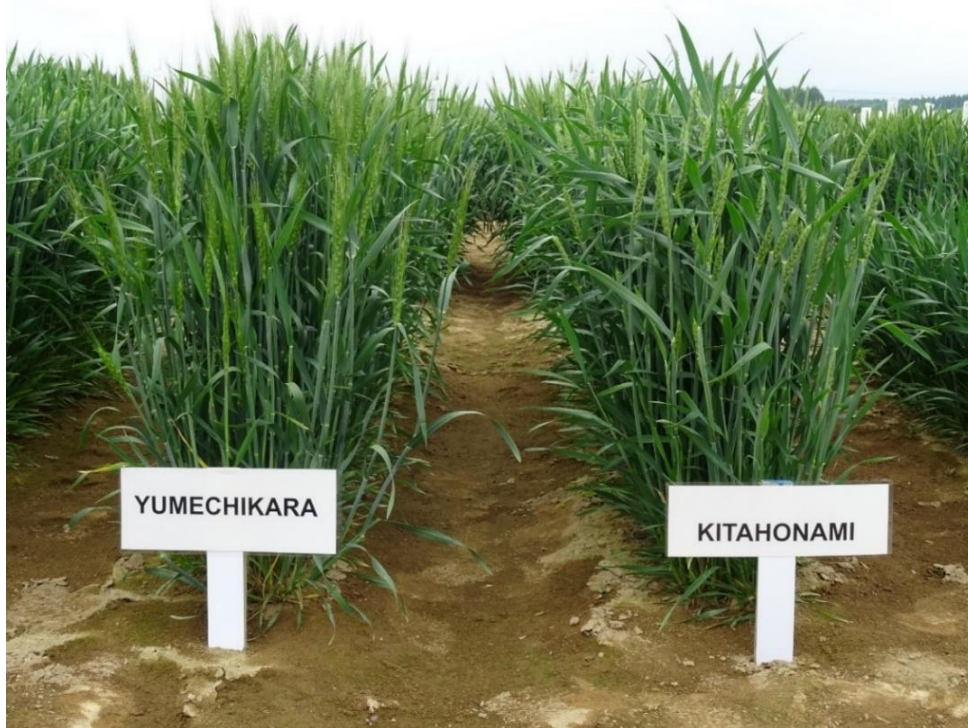


*Gpc-B1* (6BS)

*Gpc-B1* has negative effects on seed diameter.

Not only *Gpc-B1* but also another genes.

- Reveal the presence of the novel grain protein content gene.
- Produce the selection marker from the novel grain protein content gene.



**Yumechikara**

High protein content  
(about 14%)

**Kitahonami**

Low protein content  
(about 9%)

Cultivation area  
in Hokkaido

12,000ha

90,000ha

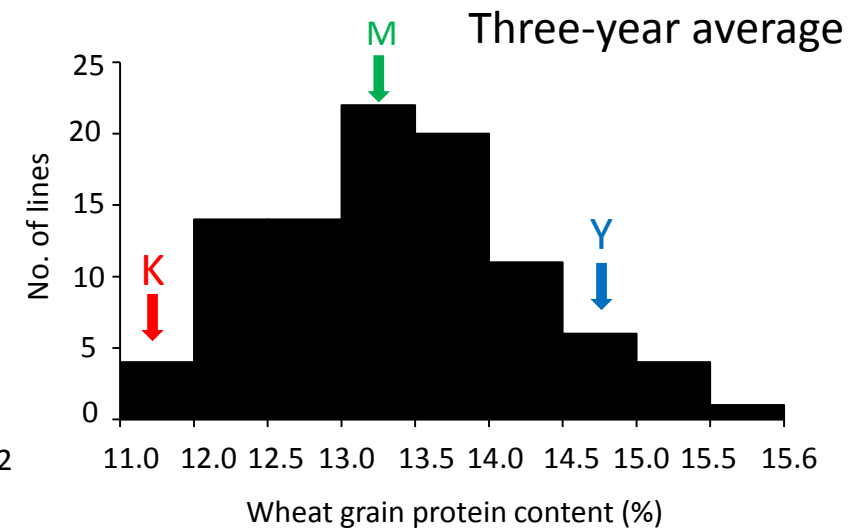
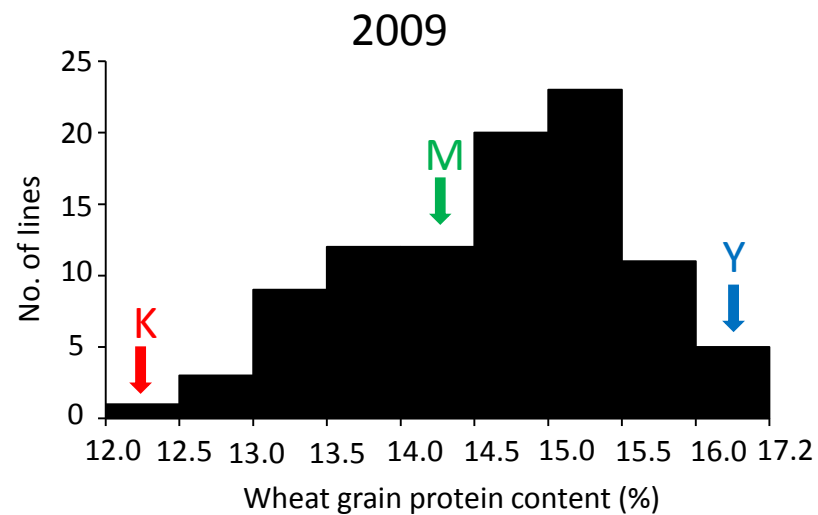
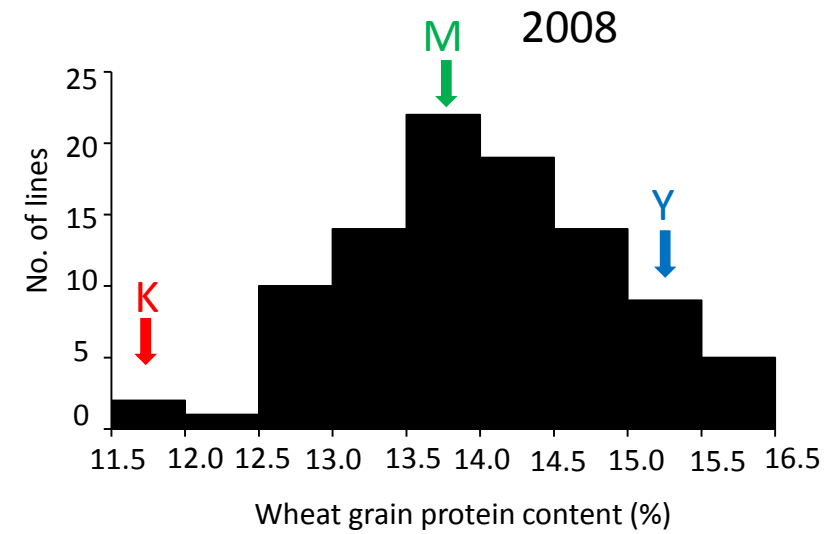
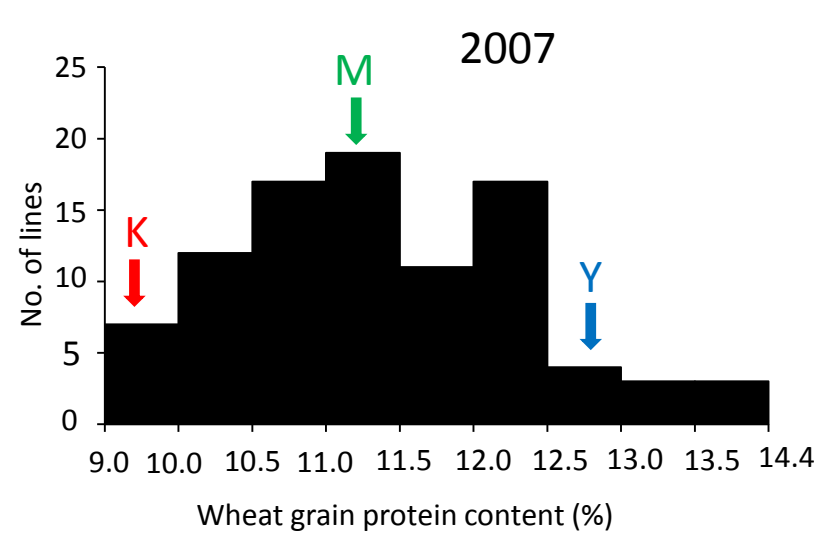
DH population

**Yumechikara × Kitahonami**

**94**lines 2007~2009

QTL analysis

**196**lines 2016~2017 Phenotyping for GPC

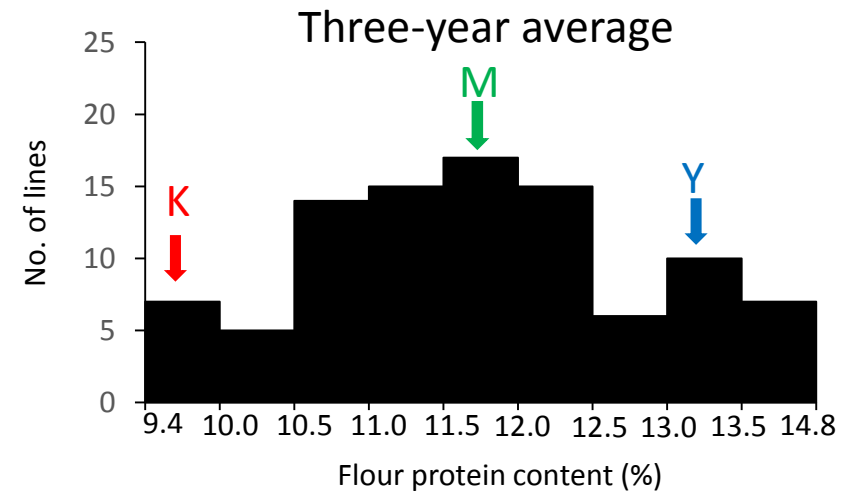
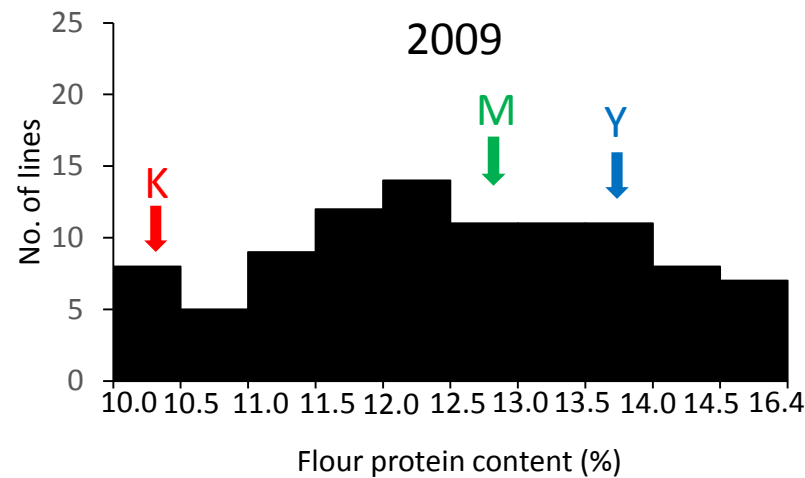
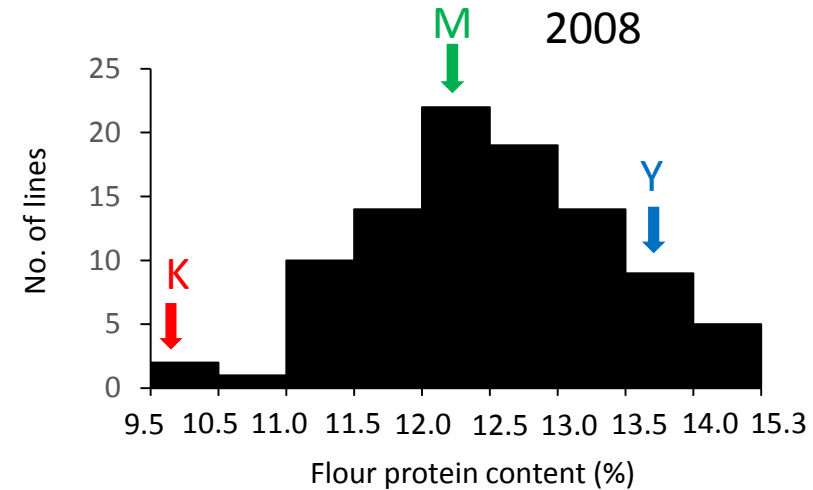
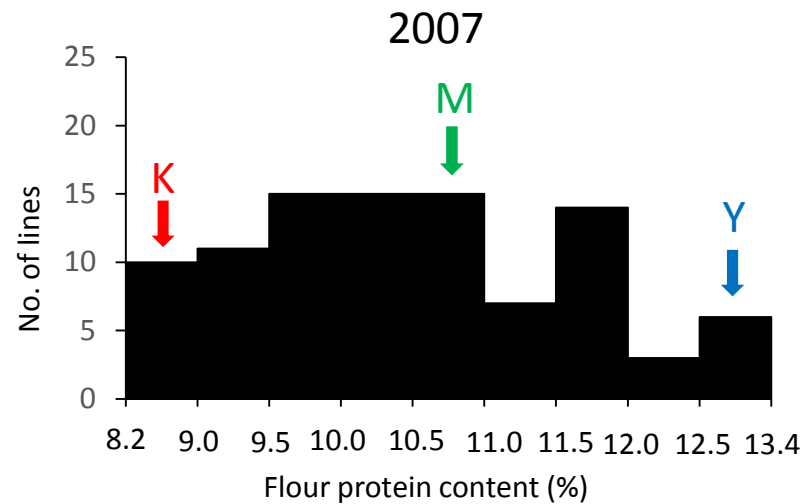


K: Kitahonami

Y: Yumechikara

M: Means

# Flour protein content (FPC)



K:Kitahonami

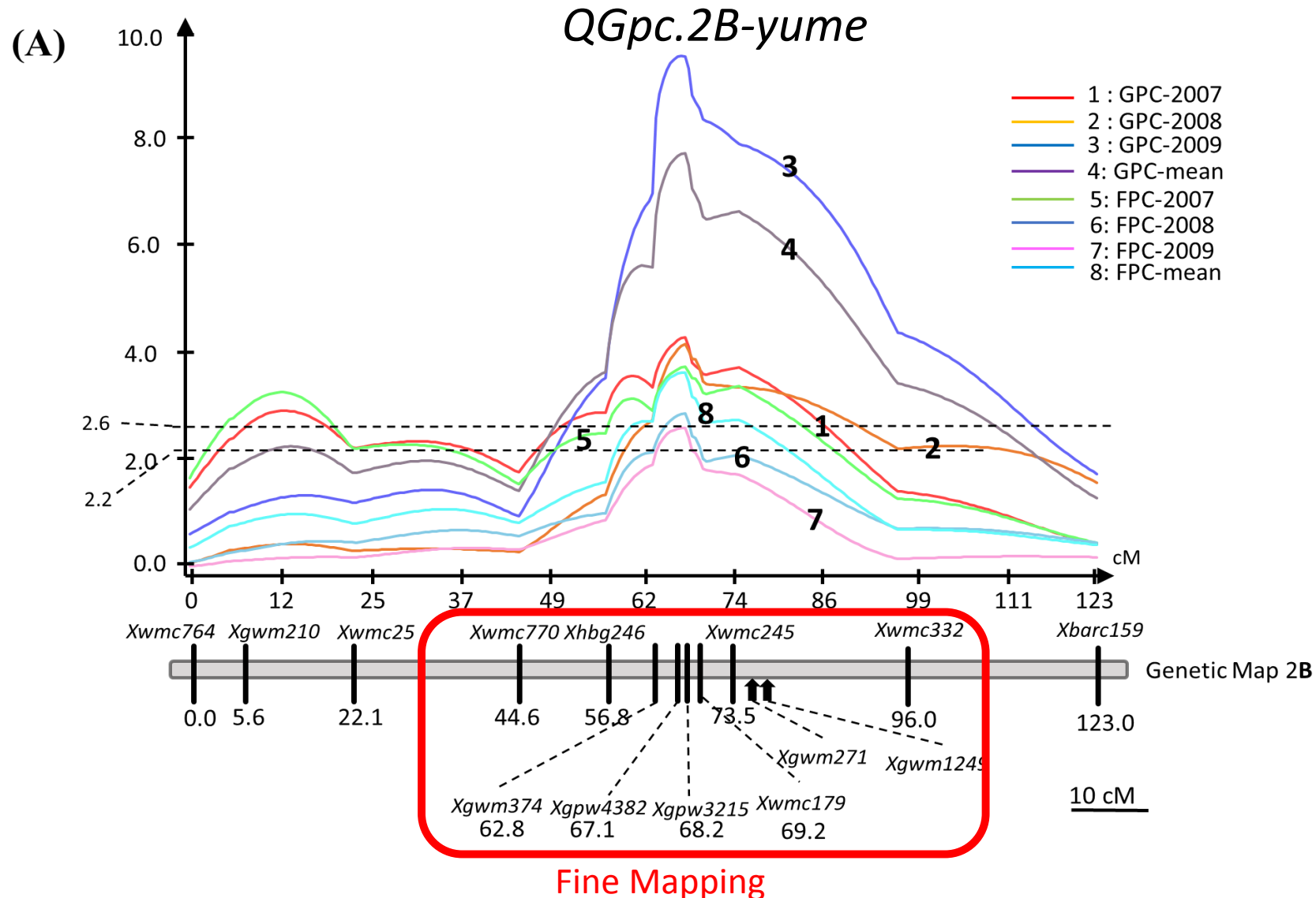
Y:Yumechikara

M: Means



# QTL analysis

QTL analysis using GPC, FPC and linkage map with 275 polymorphic SSR marker

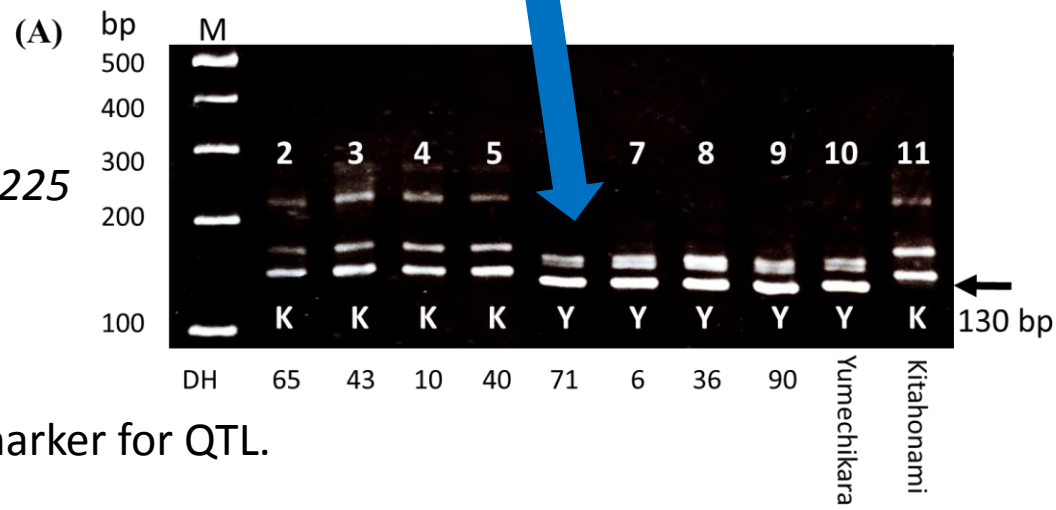


# Fine Mapping

DH	Marker						
	<i>Xhbg246</i>	<i>Xgwm374</i>	<i>Xgpw4382</i>	<i>Xgpw2225</i>	<i>Xgpw3215</i>	<i>Xwmc179</i>	<i>Xwmc245</i>
90	K	K	Y	Y	Y	Y	Y
36	K	K	Y	Y	Y	Y	Y
6	Y	Y	Y	Y	Y	K	K
71	Y	Y	Y	K	K	K	K
10	Y	K	K	K	K	K	K
40	Y	K	K	K	K	K	K
43	Y	K	K	K	K	K	K
65	K	K	K	K	K	K	K

PCR amplification product using *Xgpw4382* primers

Flanking crossovers in No.71  
between *Xgpw4382* and *Xgpw2225*

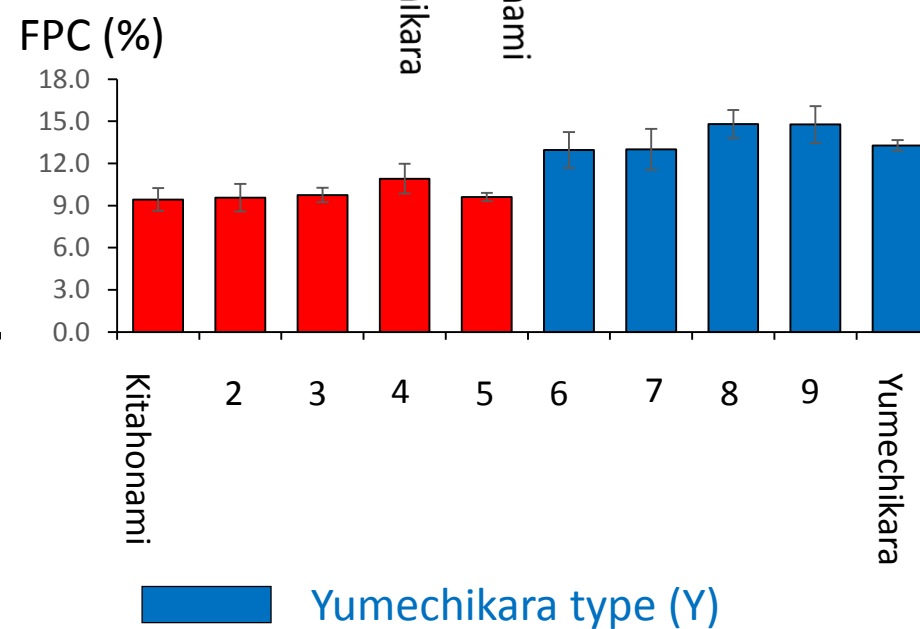
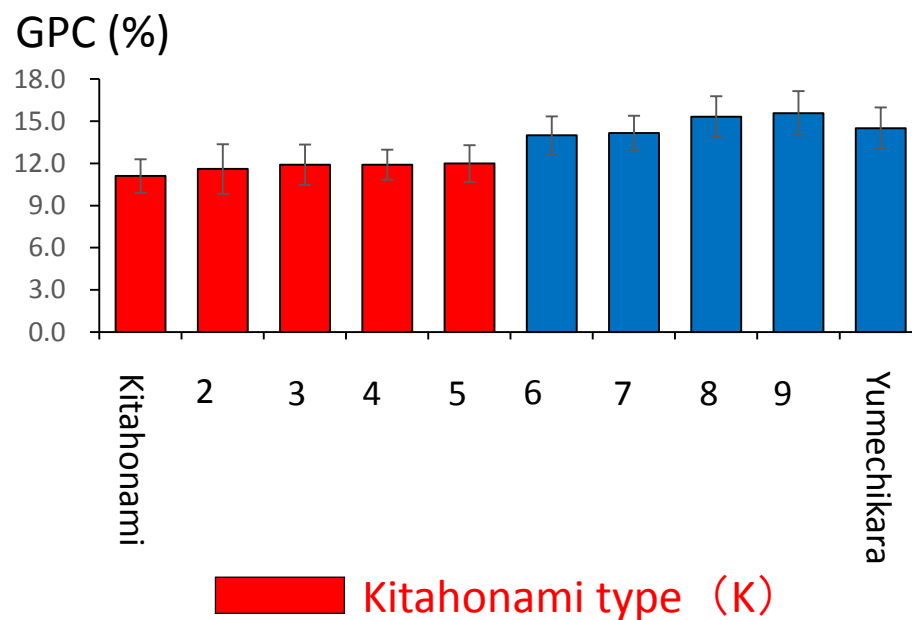
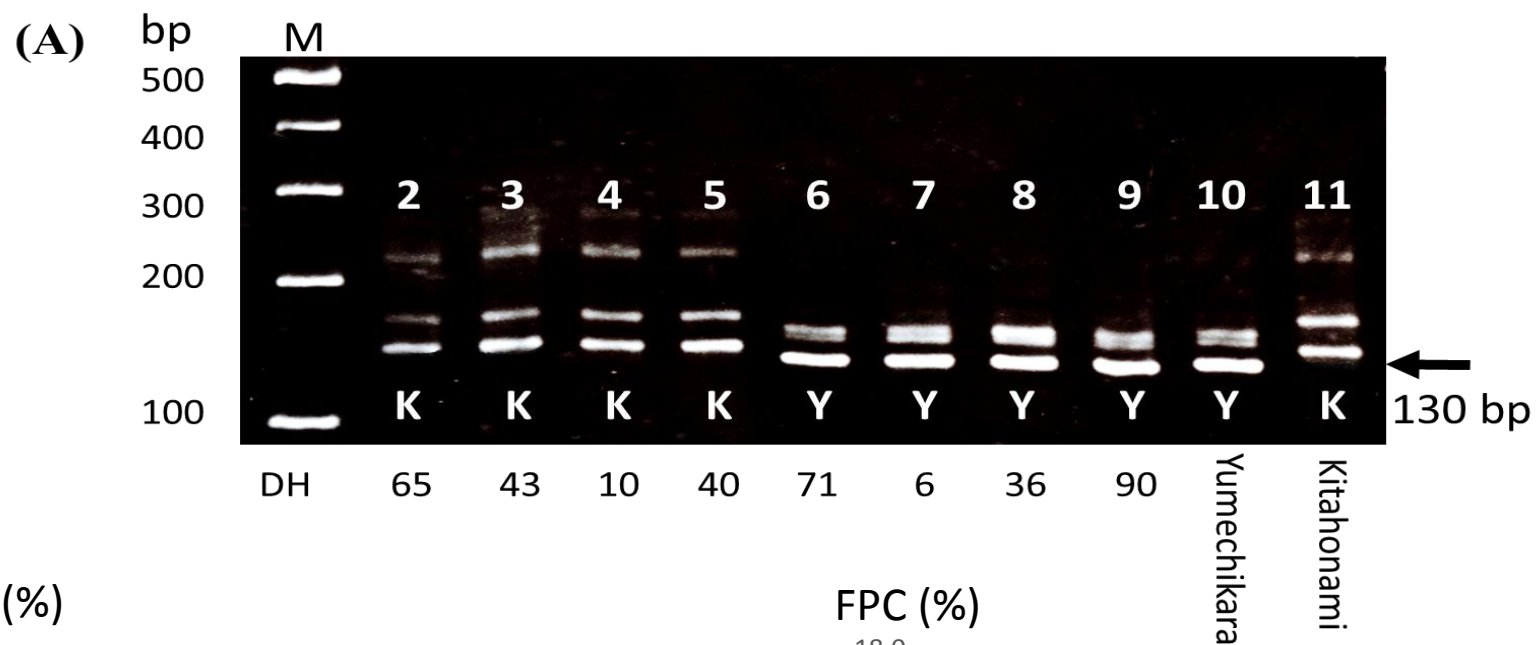


K:Kitahonami type

Y: Yumechikara type

*Xgpw4382* were the closest marker for QTL.

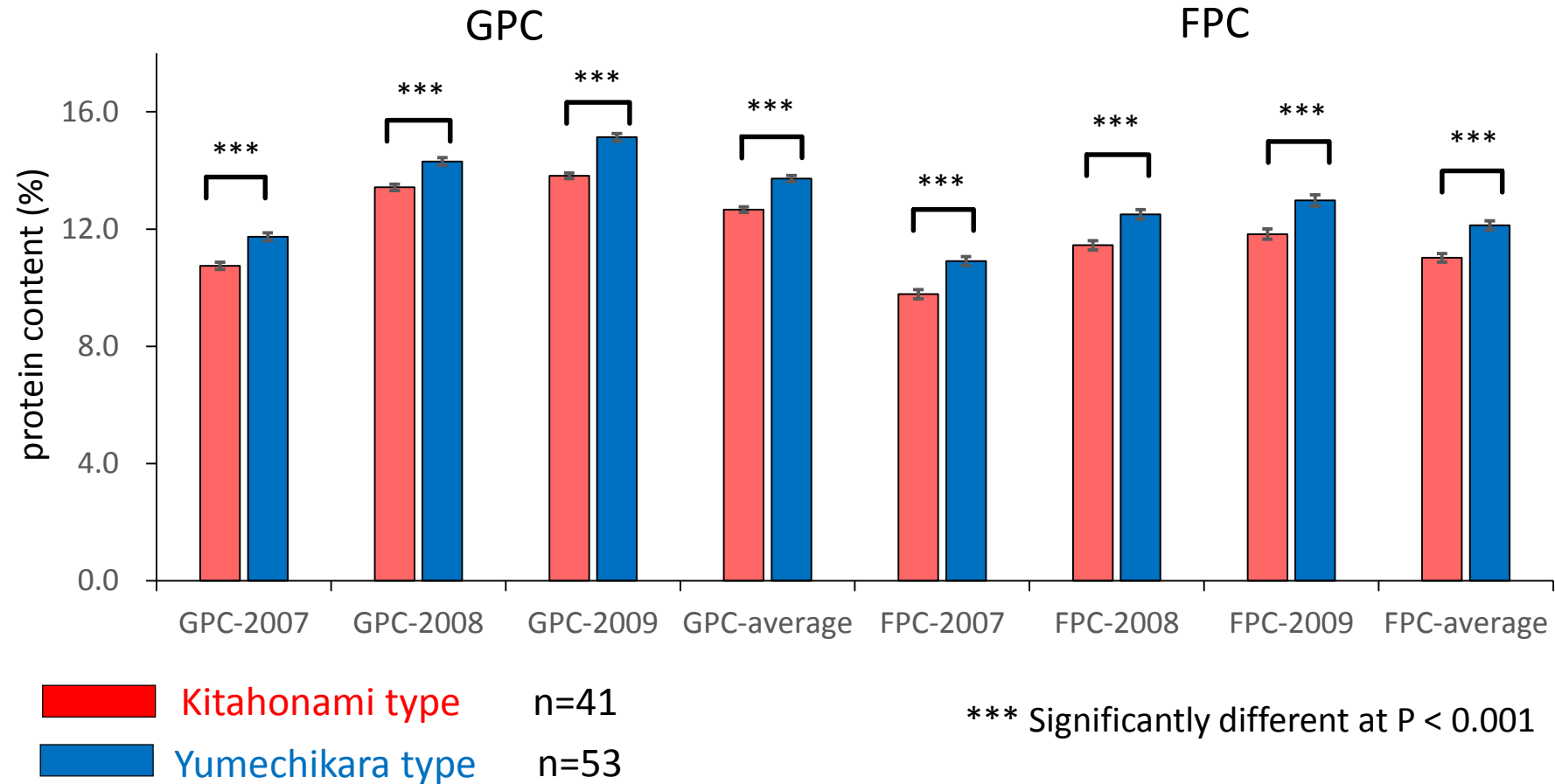
# The relationship between SSR marker and GPC,FPC





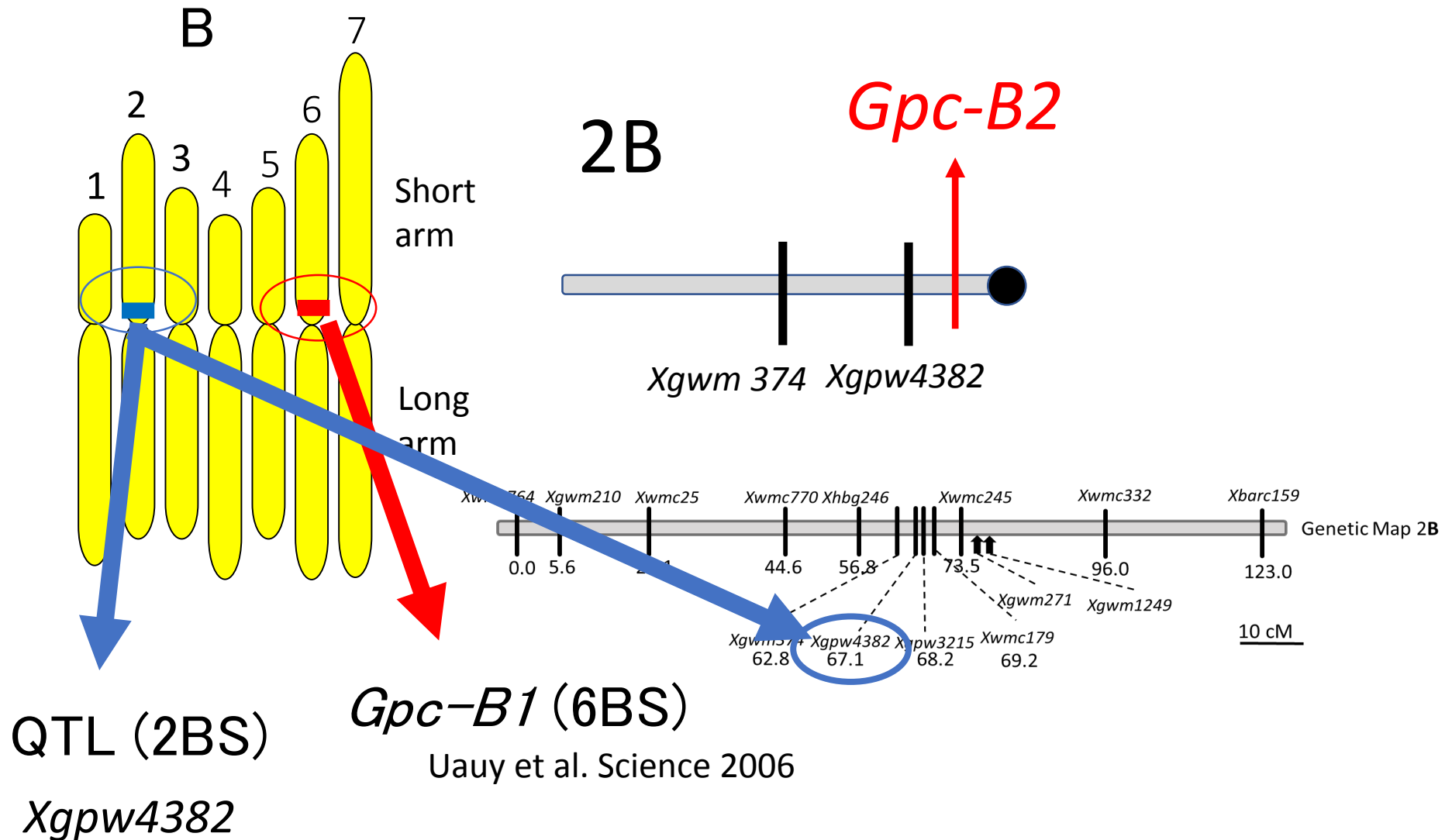
# Significance test

Mean values of the GPC and FPC in the 94 DH population grouped by Yumechikara and Kitahonami type for *Xgpw4382* on chromosome 2B.



This QTL might be influential in GPC

# The search for the novel GPC gene

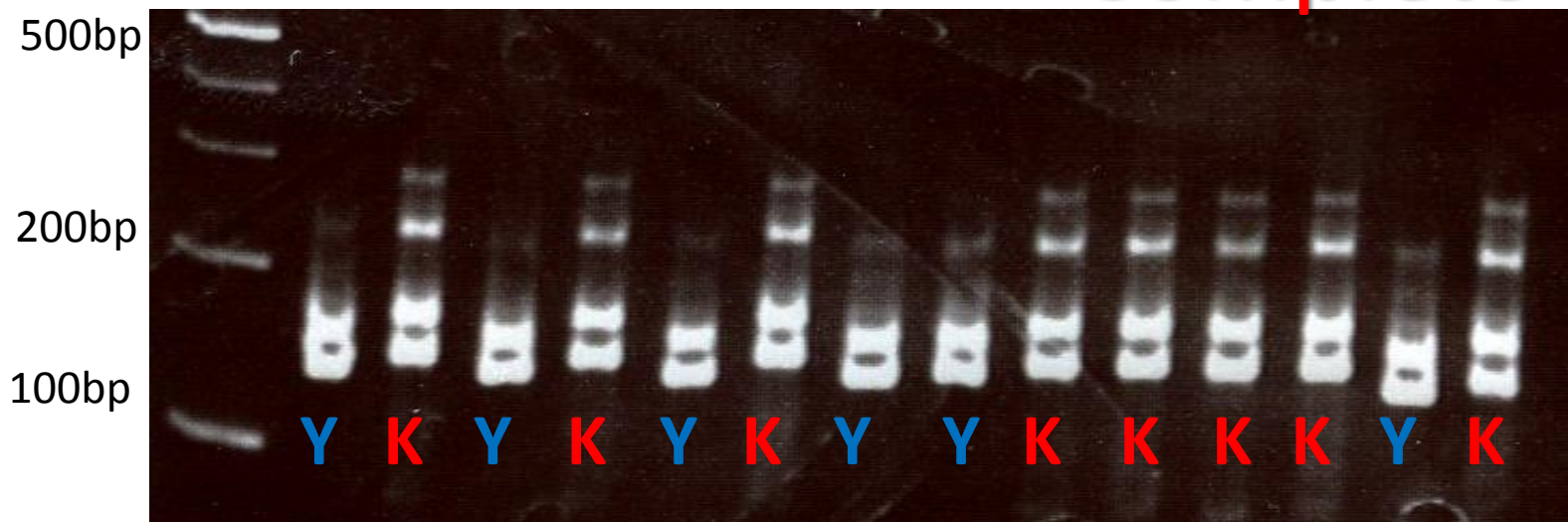


Developed *Gpc-B2* specific PCR primers from database information

## Complete Same Result

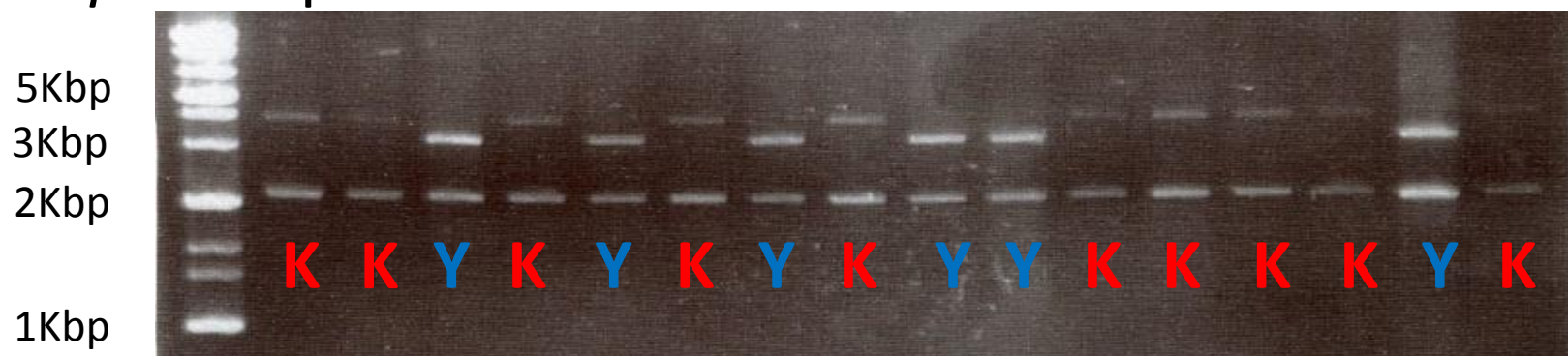
same  
genotype

*Xgpw4382*



83 84 85 86 87 88 89 90 91 92 93 94 S\* S\*

*Gpc-B2* specific marker

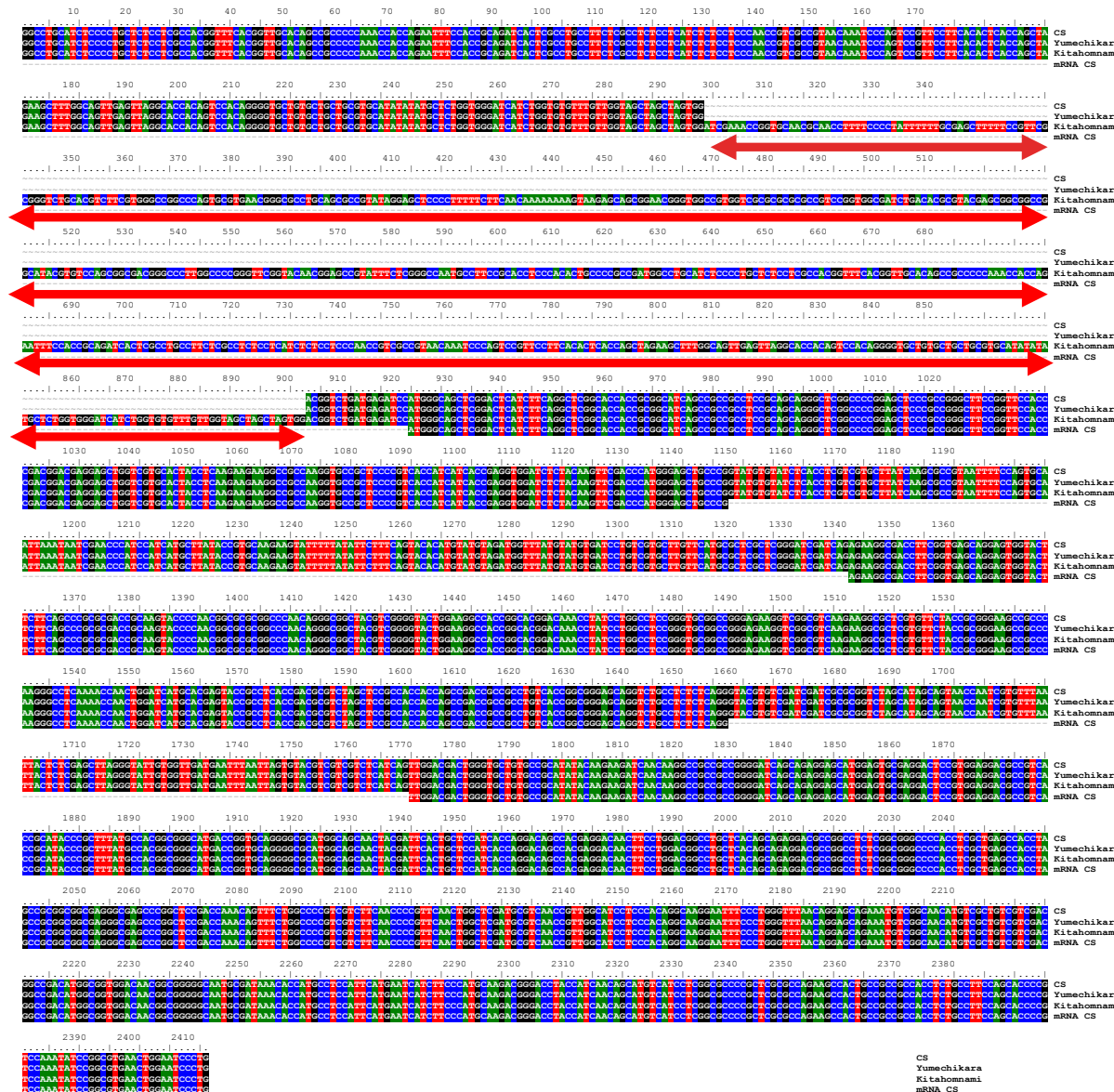


81 82 83 84 85 86 87 88 89 90 91 92 93 94 S\* S\*

S\*:Parental lines K:Kitahonami type Y:Yumechikara type



# Sequence analysis

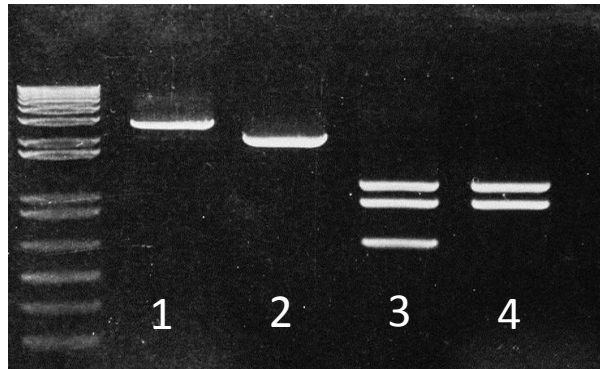


the 5' UTR and promoter region

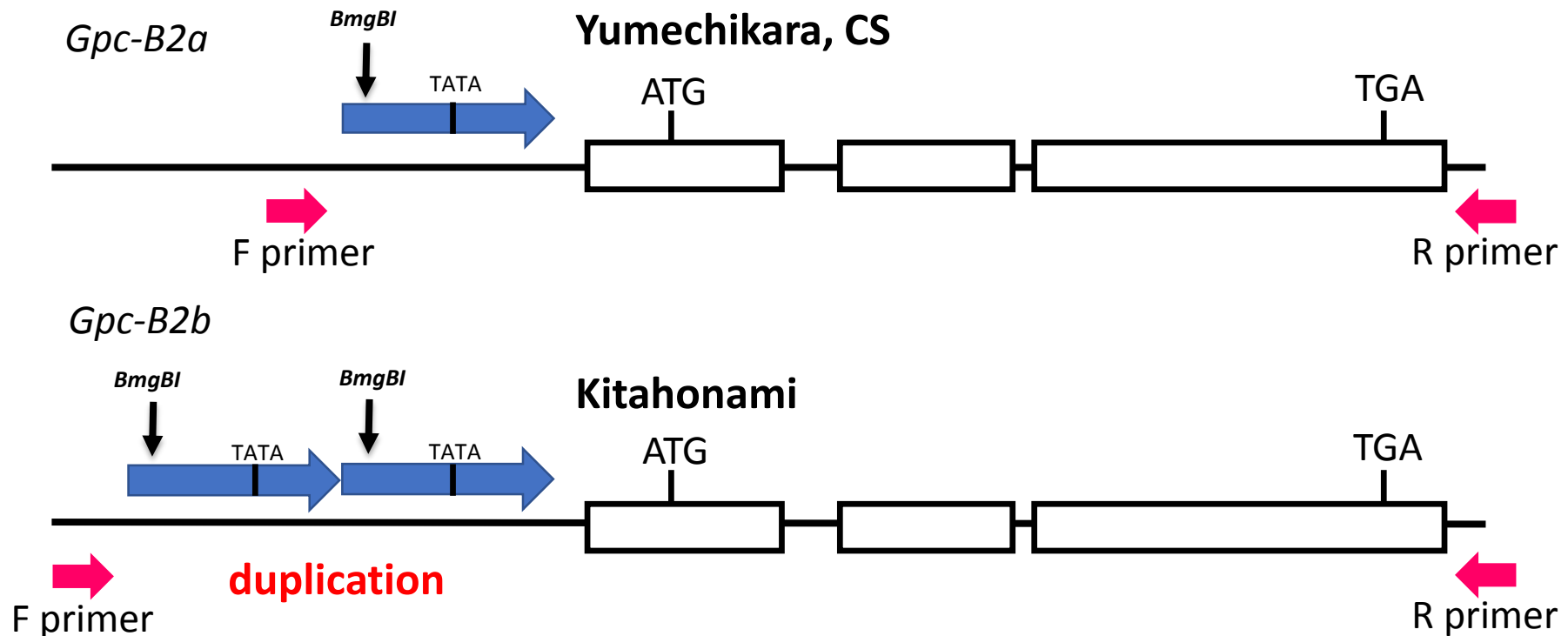
615bp

1. Chinese spring (CS)
2. Yumechikara
3. Kitahonami
4. mRNA CS

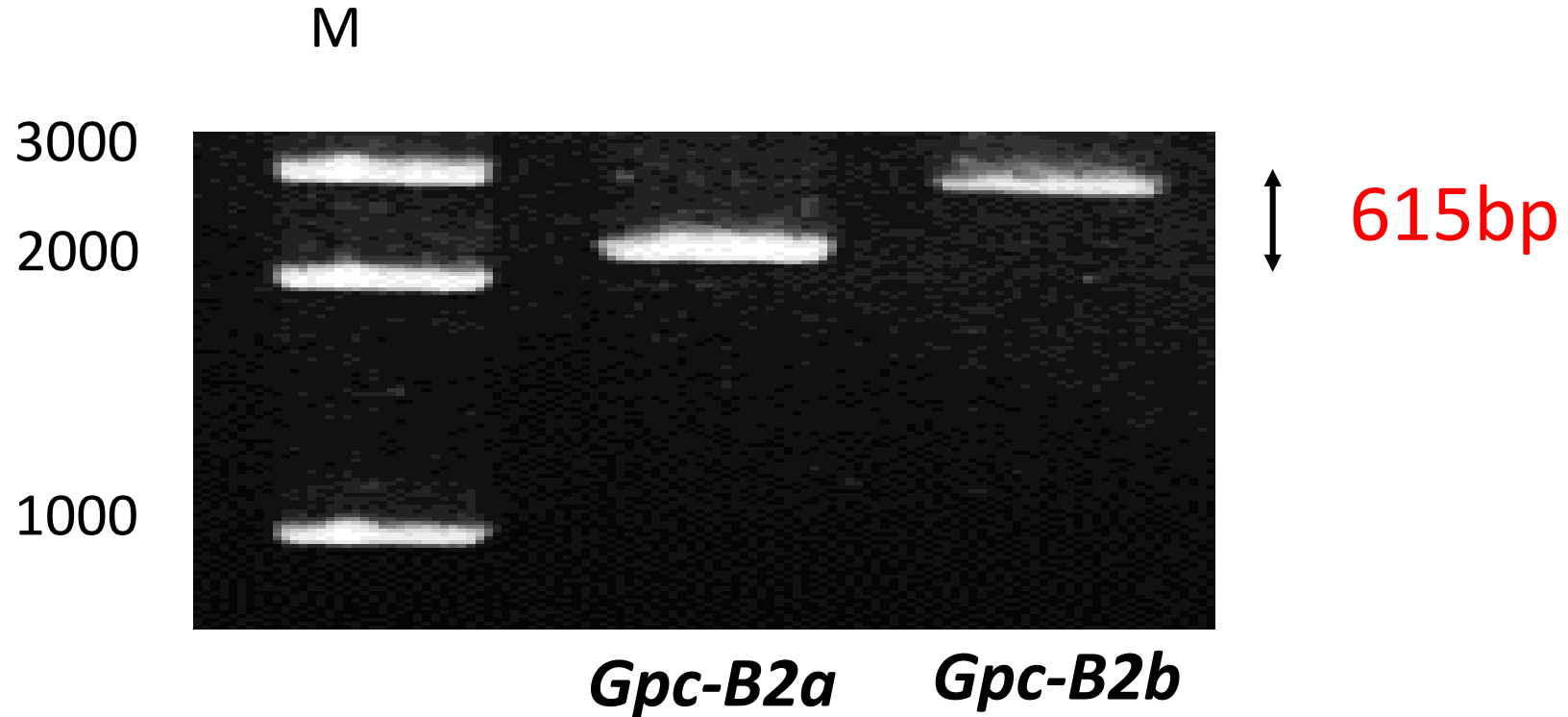
# Confirmed by gene digestion



- 1: Nested PCR Kitahonami
- 2: Nested PCR Yumechikara
- 3: Digested by *BmgBI*
- 4: Digested by *BmgBI*



# Developed specific Marker



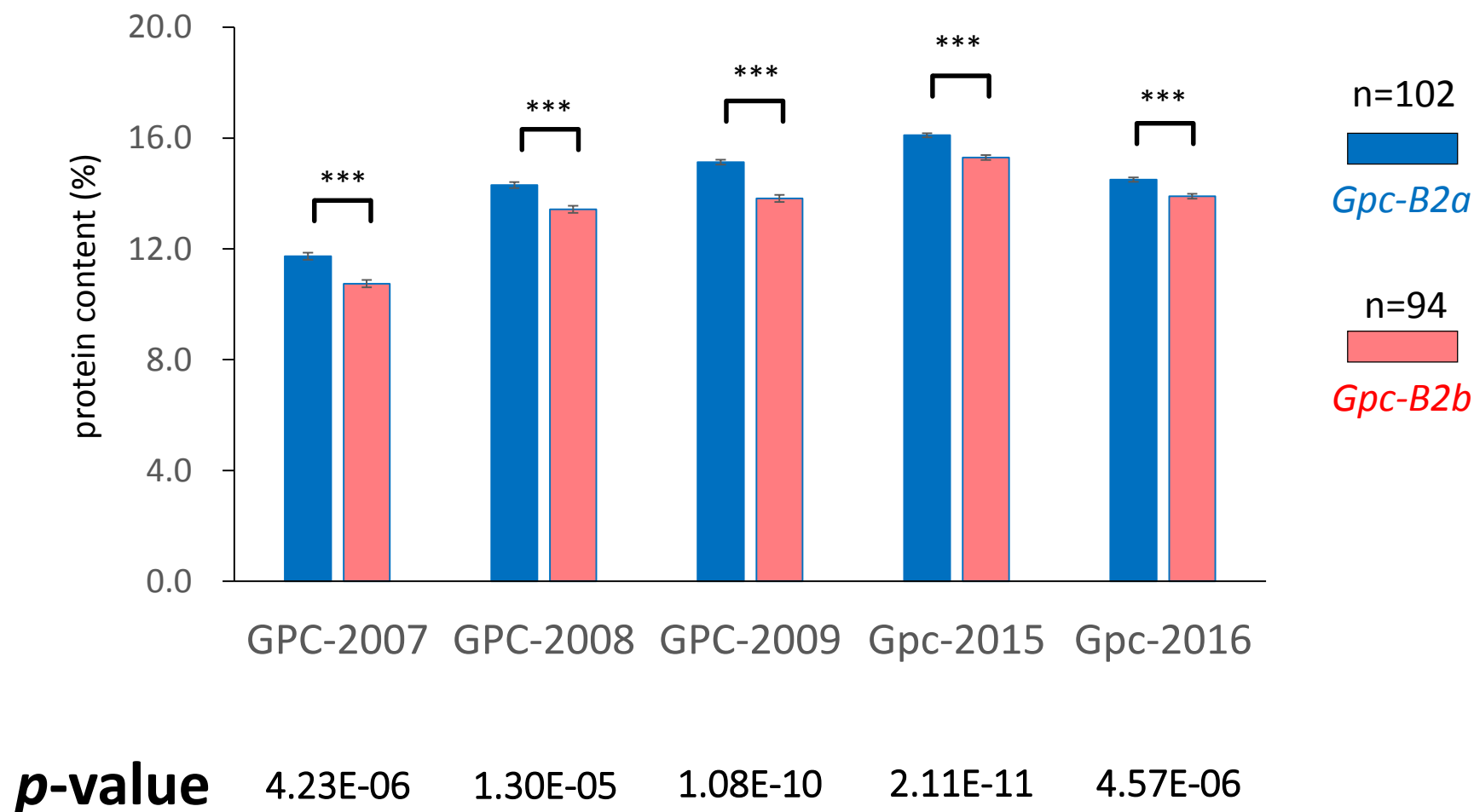
This specific marker is able to identify the *Gpc-B2* polymorphism.



# Significance test

Mean values of the GPC in the 196 DH population grouped by *Gpc-B2a* and *Gpc-B2b* for *Gpc-B2* specific marker.

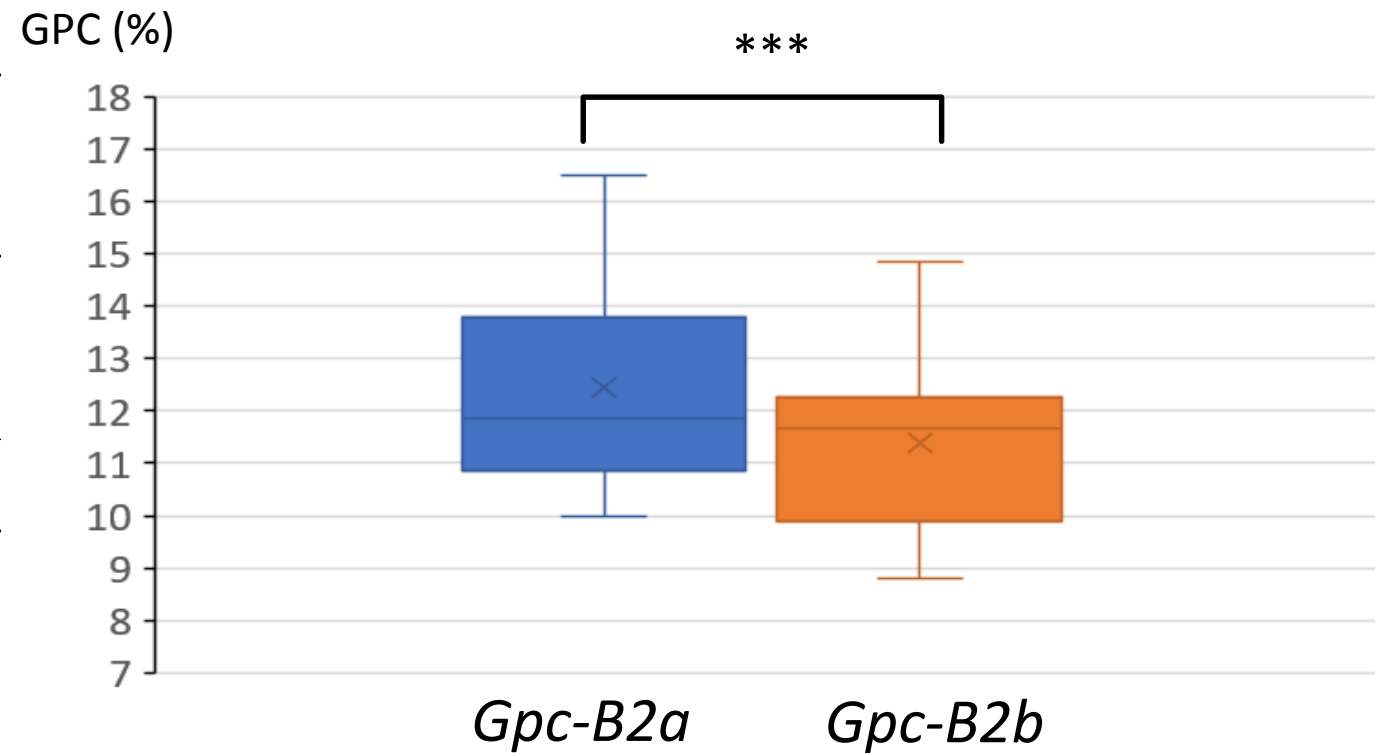
These groups showed differences of 1.0% in the mean GPC over 5 years of testing.



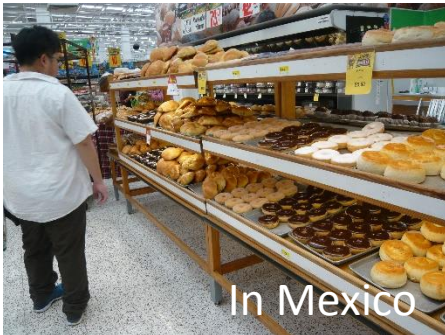
The *Gpc-B2b* might have a decreasing effect on GPC.

Mean values of the GPC in the 254 cultivated varieties grouped by *Gpc-B2a* and *Gpc-B2b* for *Gpc-B2* specific marker.

Allele	No. of varieties
<i>Gpc-B2 a</i>	239
<i>Gpc-B2 b</i>	15
Total	254



- *Gpc-B2* is most probably involved in GPC.
- The tandem duplication in *Gpc-B2* contribute to the decrease in GPC.
- *Gpc-B2b* is available for the improvement of low protein content wheat varieties.





Thank you for your attention!

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