

# Genome-wide association study to understand the genetics of manganese toxicity tolerance in rice

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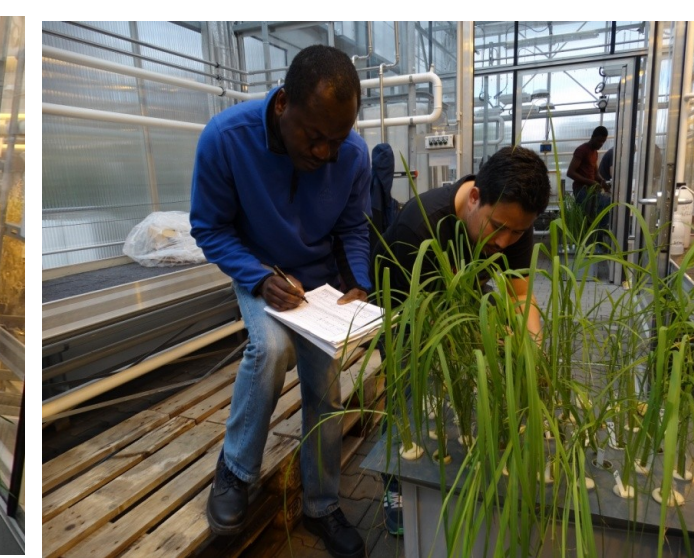
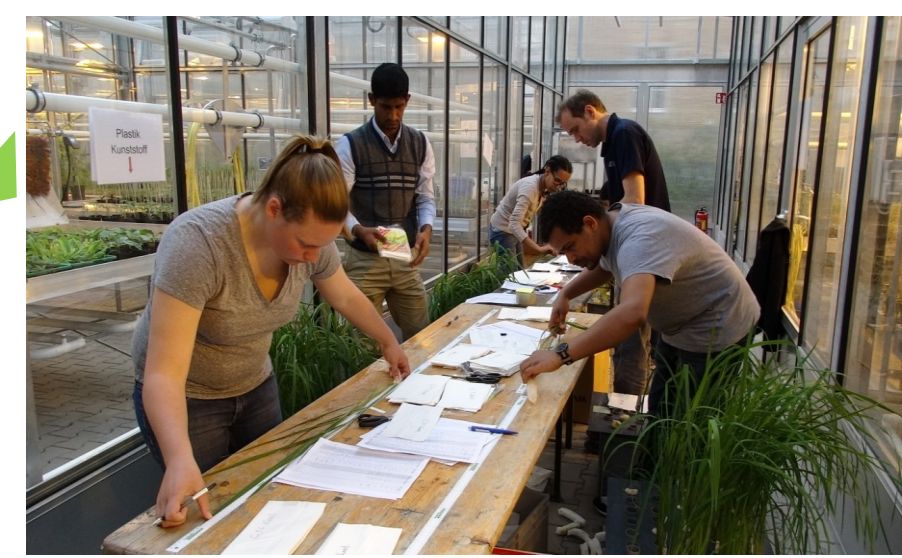


## Background

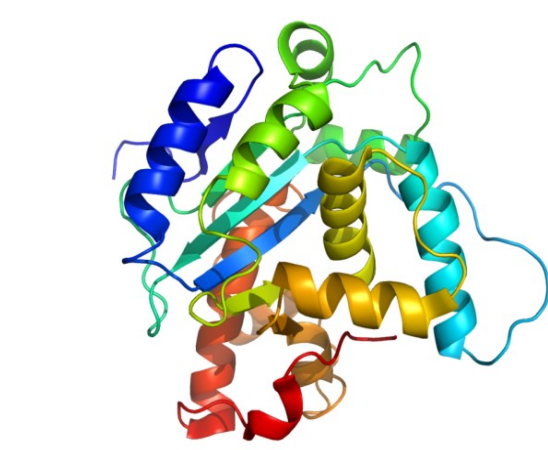
Manganese (Mn) is a plant essential micronutrient occurring in different oxidative states. Rice (*Oryza sativa* L.) is mostly cultivated in anaerobic (reducing) conditions where excess level of plant available  $Mn^{2+}$  ions are present in soil solution due to microbial energy metabolism. In cell apoplast,  $Mn^{2+}$  ions are oxidized to  $Mn^{3+}$  which is highly reactive (strong oxidizer of lipids and proteins). The typical symptom of Mn toxicity is the severe necrosis and rolling of older leaves.



Cultivation and exposure to excess  $Mn^{2+}$

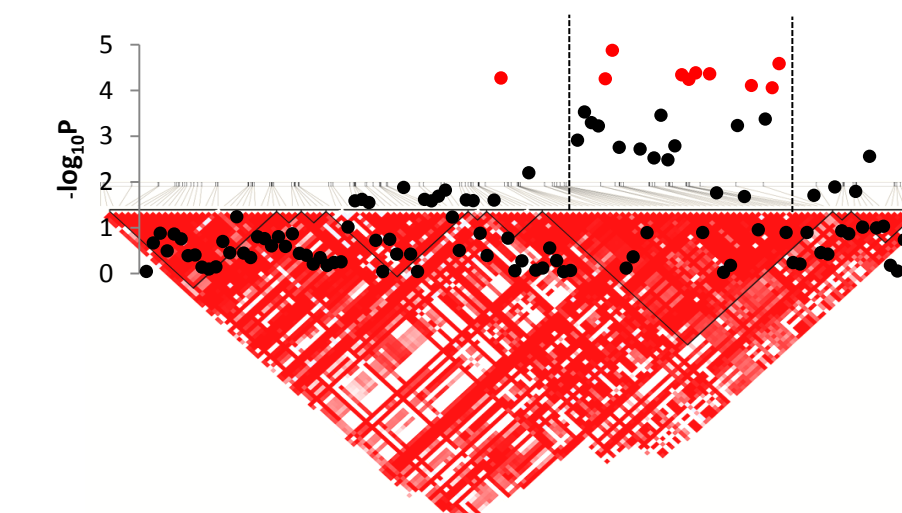


Phenotyping



Candidate genes

GWAS



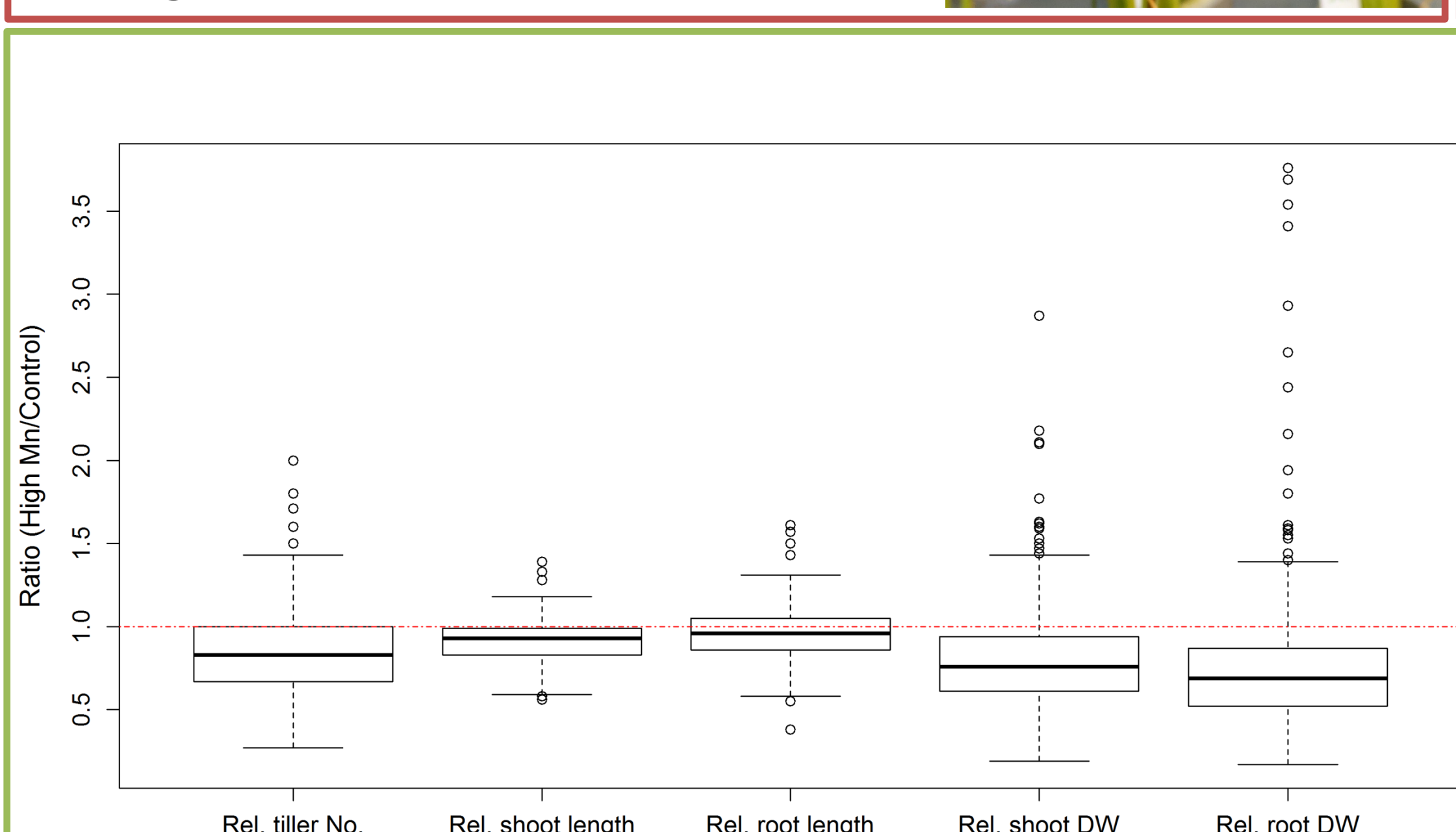
Mapping Data analysis

This study aims at using the genetic variation of rice to identify the candidate loci conferring Mn toxicity tolerance in rice.

GWAS: Genome-wide association study

## Materials & Methods

A diversity panel of 288 genotypes representing *indica* and *japonica* sub-groups of rice (*Oryza sativa* L.) were grown in hydroponics in control (0.5 ppm Mn) and Mn toxic conditions (5 ppm Mn). Phenotypic variation in root and shoot length, dry weight (DW), tiller numbers, leaf bronzing index and shoot Mn concentration were determined for mapping.

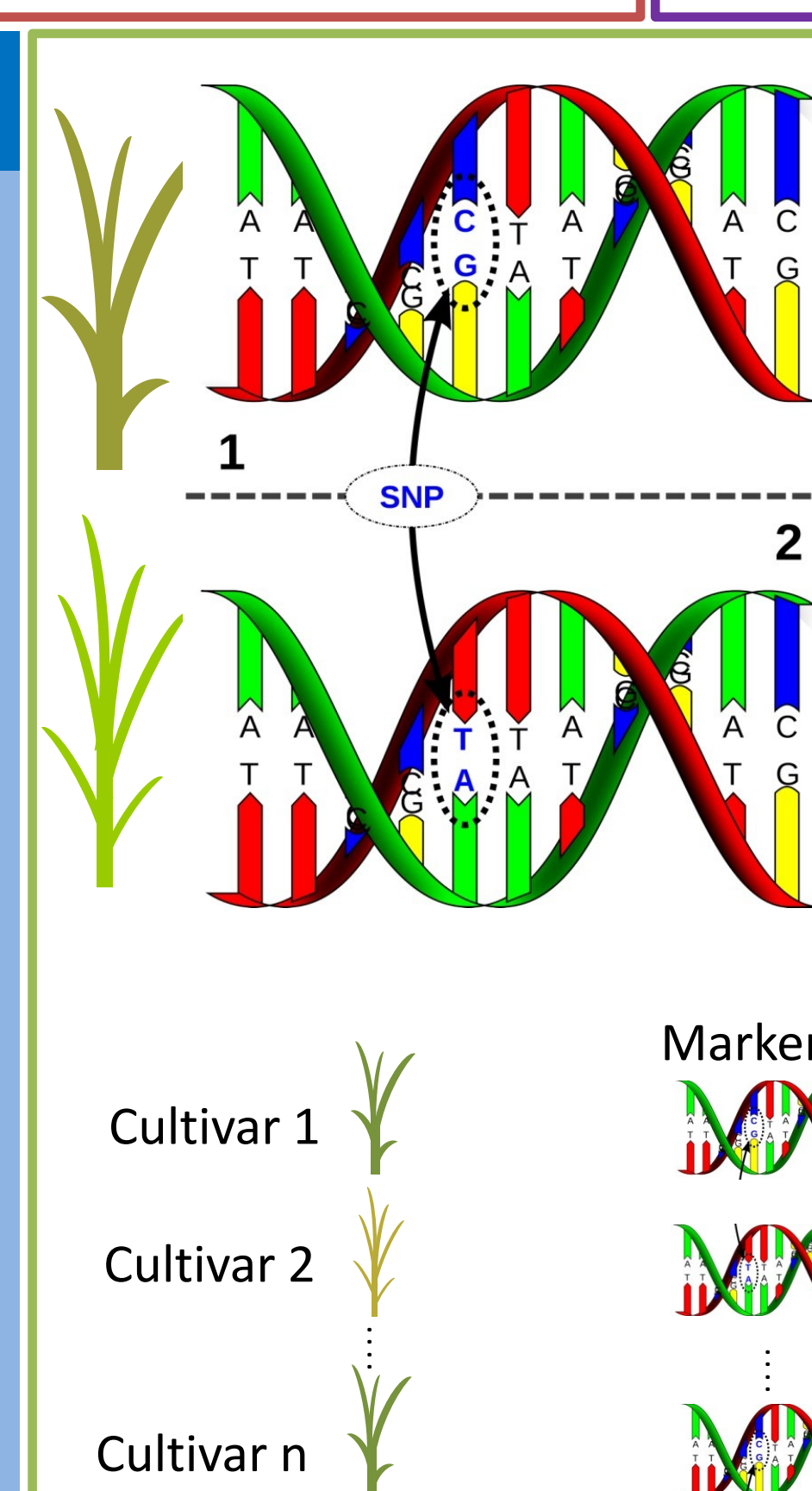


Box plots describing relative (Rel.) phenotypic values

Relative phenotypic value is the ratio of phenotypic value at toxic Mn condition / control condition.

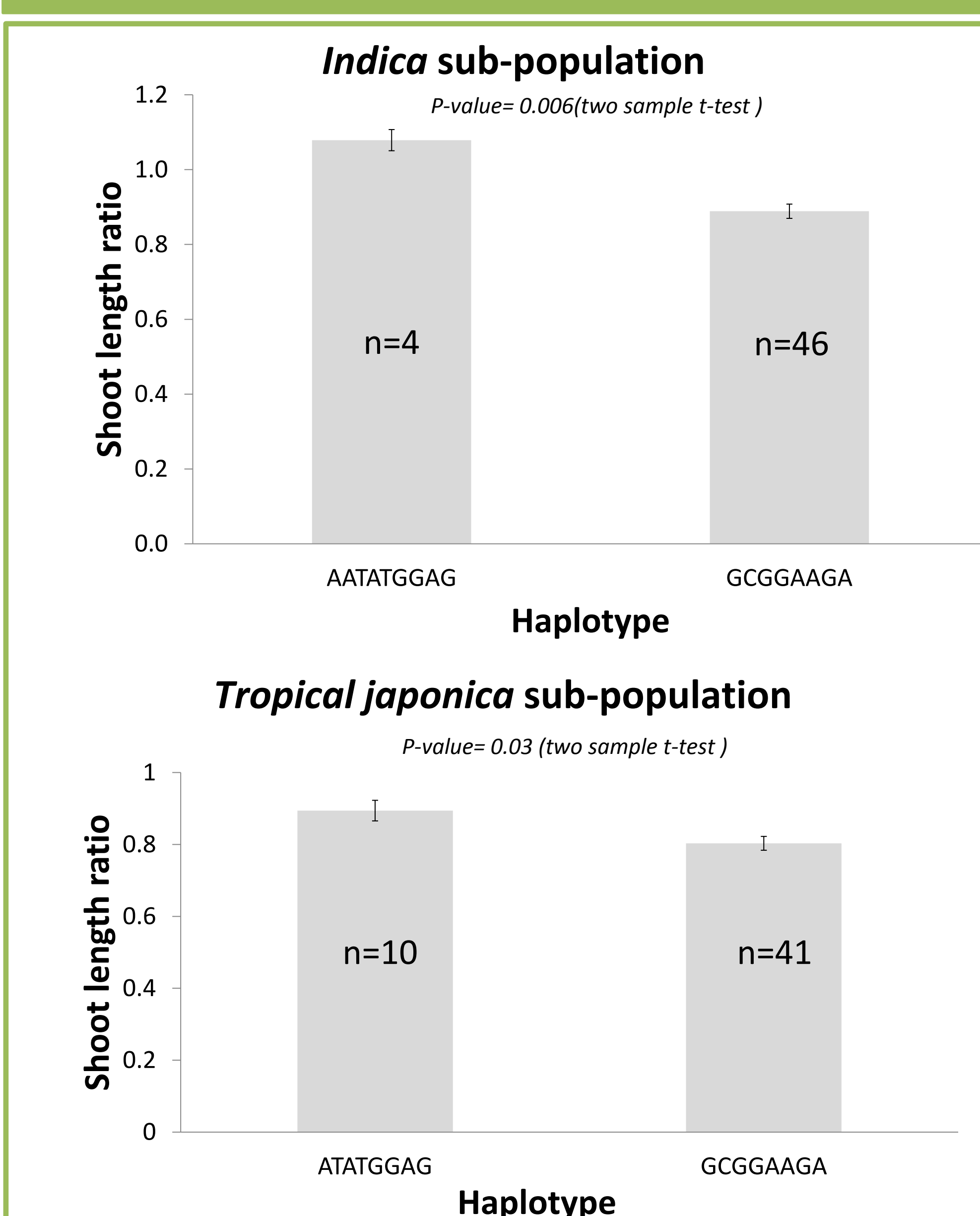
## Conclusion

- Significant natural variation in tolerance to Mn toxic conditions was observed in rice.
- 10 SNPs significantly associated with relative shoot length were detected on chromosome 6.
- Among 10 significant SNPs, 9 SNPs were located within a LD block.
- This LD block contained 58 candidate genes.



SNP is a single base-pair difference at a specific chromosomal position. They are abundant in the plant genome. Around 40,000 SNPs marker data were used for association mapping in this study.

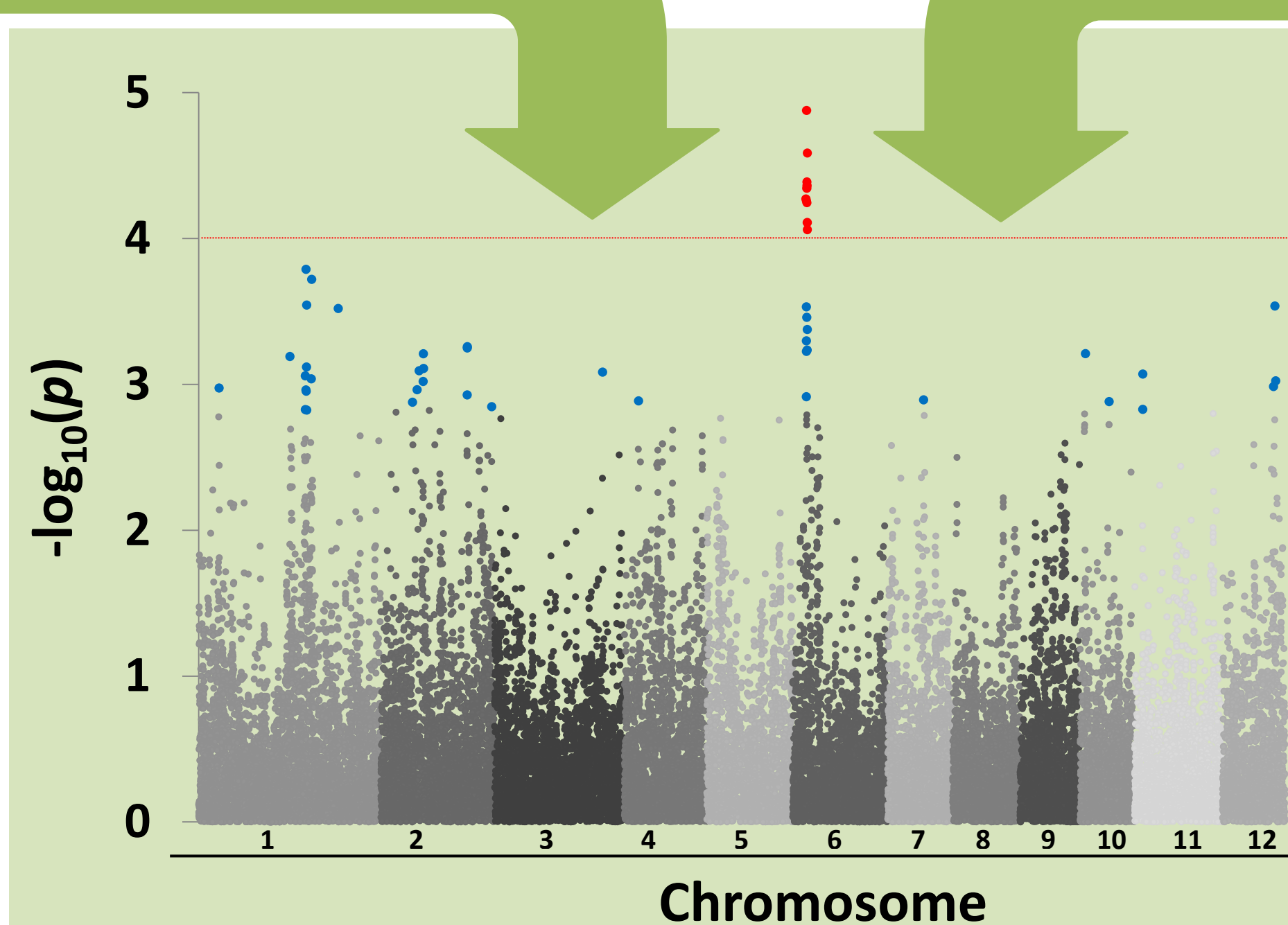
## Phenotype data



Haplotype analysis for *indica* and *tropical japonica* sub-population for relative shoot length

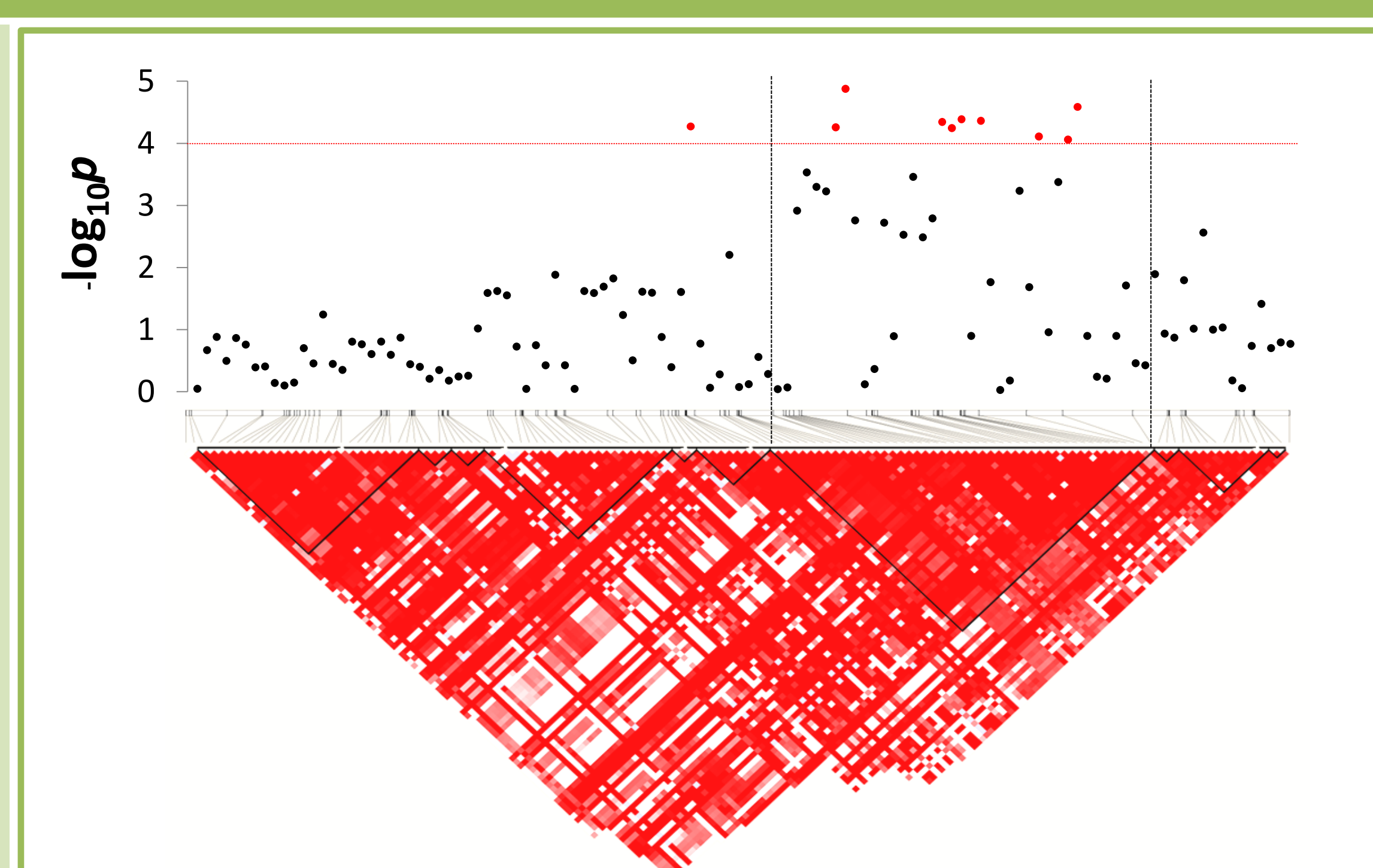
Haplotypes represent certain allele combinations at significant SNPs. Two major haplotypes were observed for highly significant SNPs associated with RSL.

## SNP genetic marker data



Manhattan plot from association mapping between relative shoot length (RSL) and SNP based data. Each point represents a SNP.

The graph shows the association between each SNP marker and relative shoot length. The SNPs in blue colour indicate top 50 SNPs (based on  $-\log_{10}p$  value) while those in red colour are significant SNPs (significance threshold of  $p < 0.0001$ ). 10 significant SNPs were detected on chromosome 6.



Pair-wise linkage disequilibrium (LD),  $D'$  between SNP markers on chromosome 6 (regions including significant markers). Dark red indicates  $D'$  value 1 and white indicates 0.

LD is the non random association of alleles. LD blocks containing highly significant SNPs are considered as candidate loci. A sharp peak with nine significant SNPs was observed in one LD block on chromosome 6. The LD block comprises 58 genes coding for proteins like receptor kinase, RING-H2-finger, leucine repeat region domain, resistance LR10 and caffeoyl-Coenzyme.

Analysis of sequence variation is ongoing to test the validity of candidate genes.