

The New Zealand Institute for Plant & Food Research Limited

Plant & Food  
**RESEARCH**

RANGAHAU AHUMĀRA KAI



# Mapping quantitative loci conferring resistance to Psa in 'P1', a diploid male *A. chinensis* breeding parent

**Sue Gardiner, Heather Bassett, Jibrán Tahir, David Chagné, Luis Gea and friends**

# Strategy

## Why 'P1' ?

- Alive at Te Puke, central in breeding programme, 20K+ progeny in field
- Hort 16A x 'P1', 52 seedlings phenotyped in field, in replicate

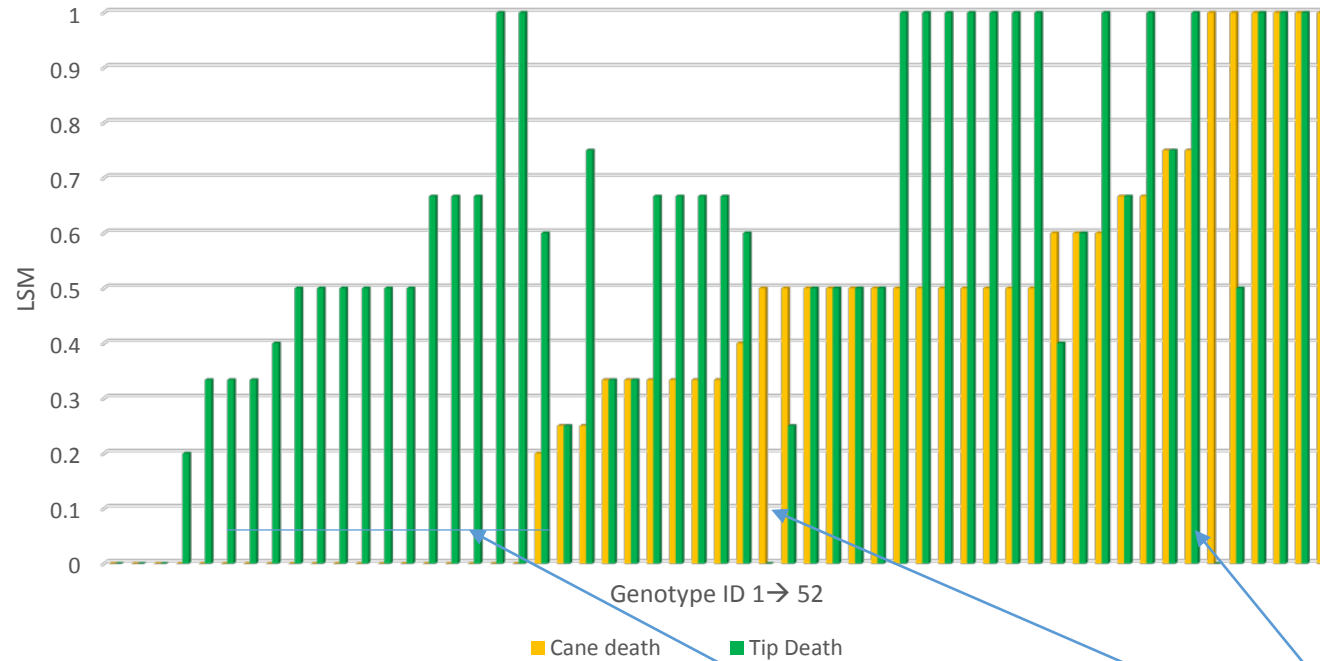
## Markers for mapping?

- Genotyping by sequencing produces thousands of markers for mapping
- Takes advantage of natural variation in genome (single nucleotide polymorphisms) → genetic markers

# Field phenotyping results for progeny are complex: (cane death and tip death)

Susceptible

Resistant



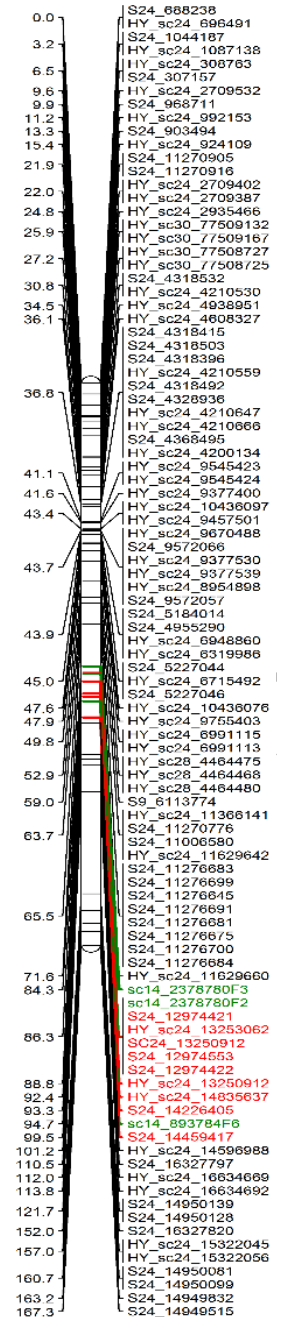
- Some plants susceptible to either tip death OR to cane death, some susceptible to both, some resistant to both
- **Different genetic controls?**

# GBS and mapping statistics – all great!

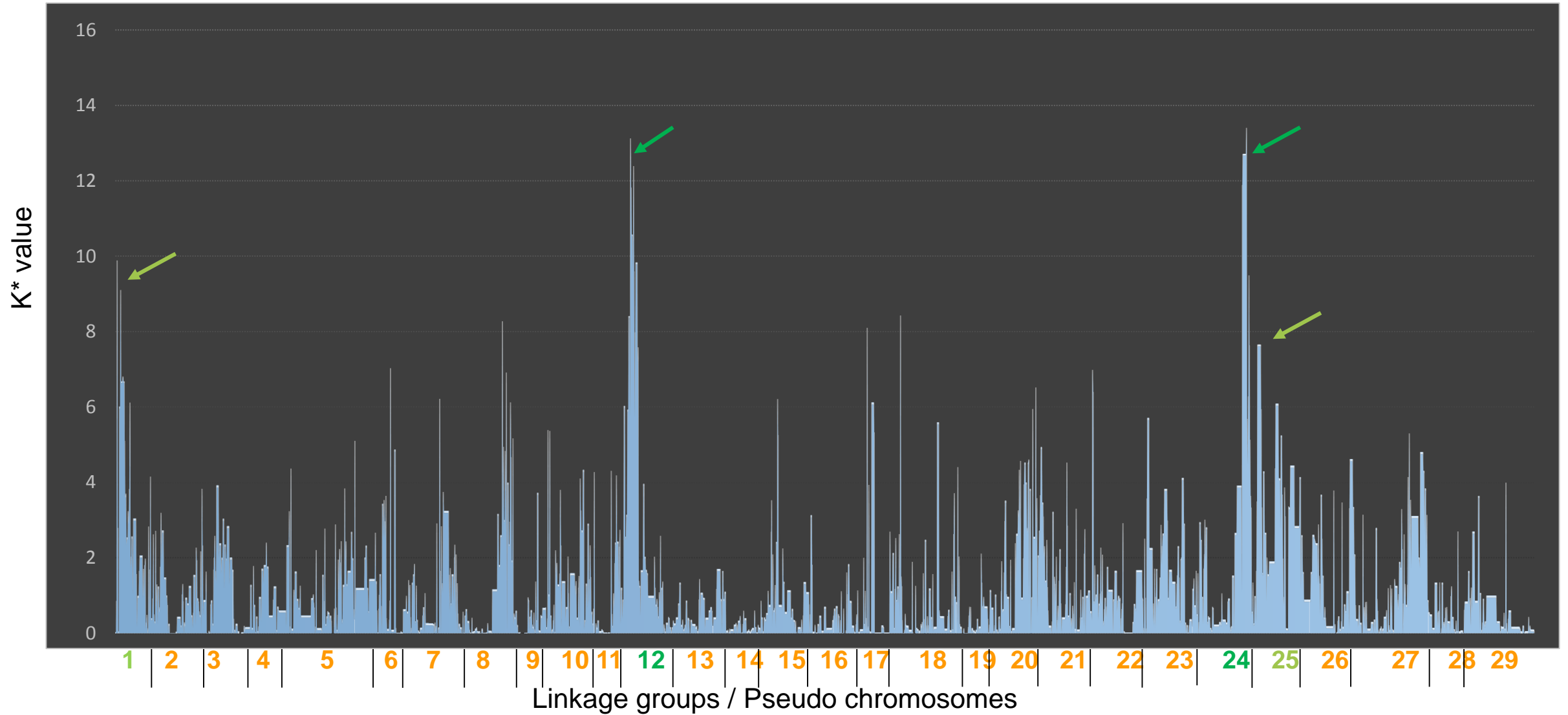
- *Bam*HI library sequencing → 321 million SE reads
- SNP sites in PS1 (improved) and ‘HongYang’ genomes → 29K each
- Filtered to 5K markers
- SNPs mapped in ‘P1’ = 3,000
- Mapped all 29 chromosomes of *A. chinensis*
- cM covered = 3,294

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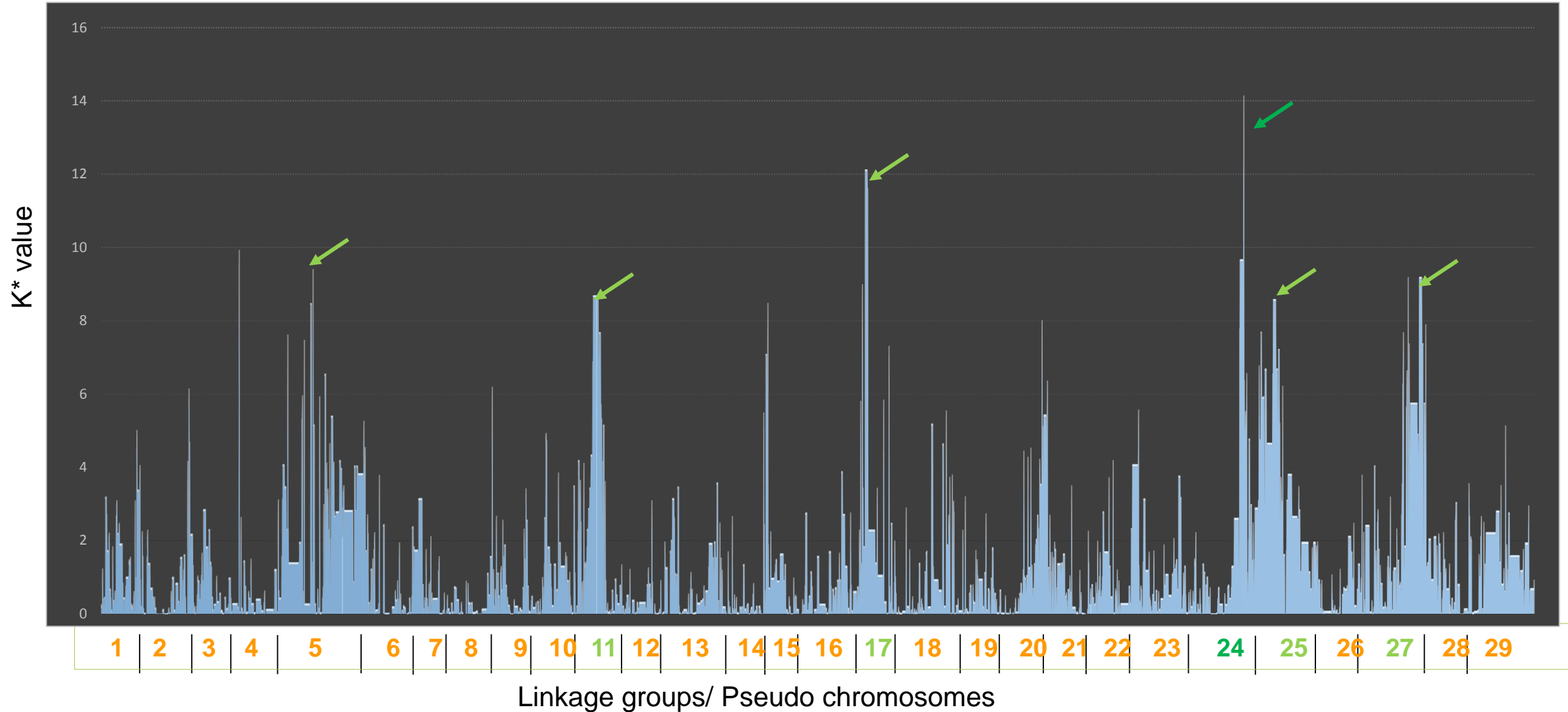
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# Cane death resistance QTLs - Kruskal-Wallis analysis



# Tip death resistance QTLs – Kruskal Wallis analysis



# QTLs for resistance to cane death (CD) and tip death (TD) are at different loci on 'P1' genome

P1:

- QTLs for resistance to CD LGs 12, 24 and maybe LGs 1, 25
- QTLs for resistance to TD LG 24 and maybe LGs 5, 11, 17, 25, 27



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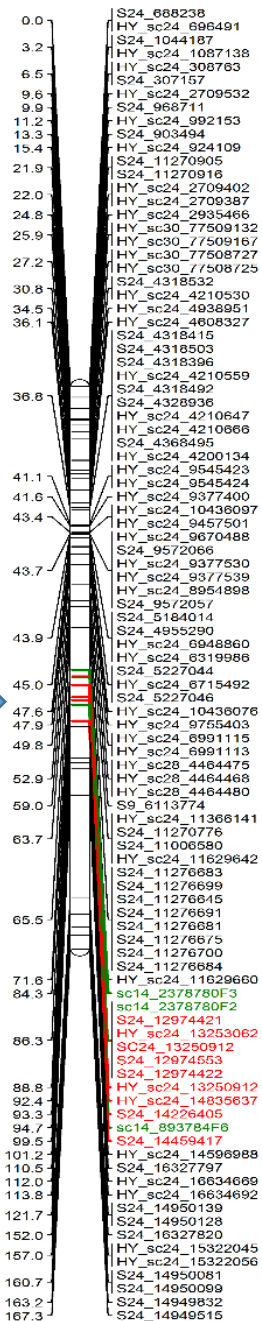
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- QTLs for resistance to TD LG 24 and maybe LGs 11, 17, 25, 27
- [ Ooze maps at LG 15, also LGs 10, 25, 28!!!]
- [QTL for final death in field maps to LG 28 (to be validated)]
- Complex situation...resistances may be related, but not genetically identical

# Validation of 'P1' QTLs

- **Heather** developed SSR markers at QTL peaks
- Mapped SSRs in Hort 16A x 'P1' population to determine resistance allelotype for each marker linked to resistance phenotype
- In order to track/validate QTLs in other breeding populations with 'P1' parent

# LG24 – Mapping of cane death, tip death resistance QTL in 'P1'



Markers supporting the QTLs using Kruskal Wallis  
Green= SSR  
Red = GBS

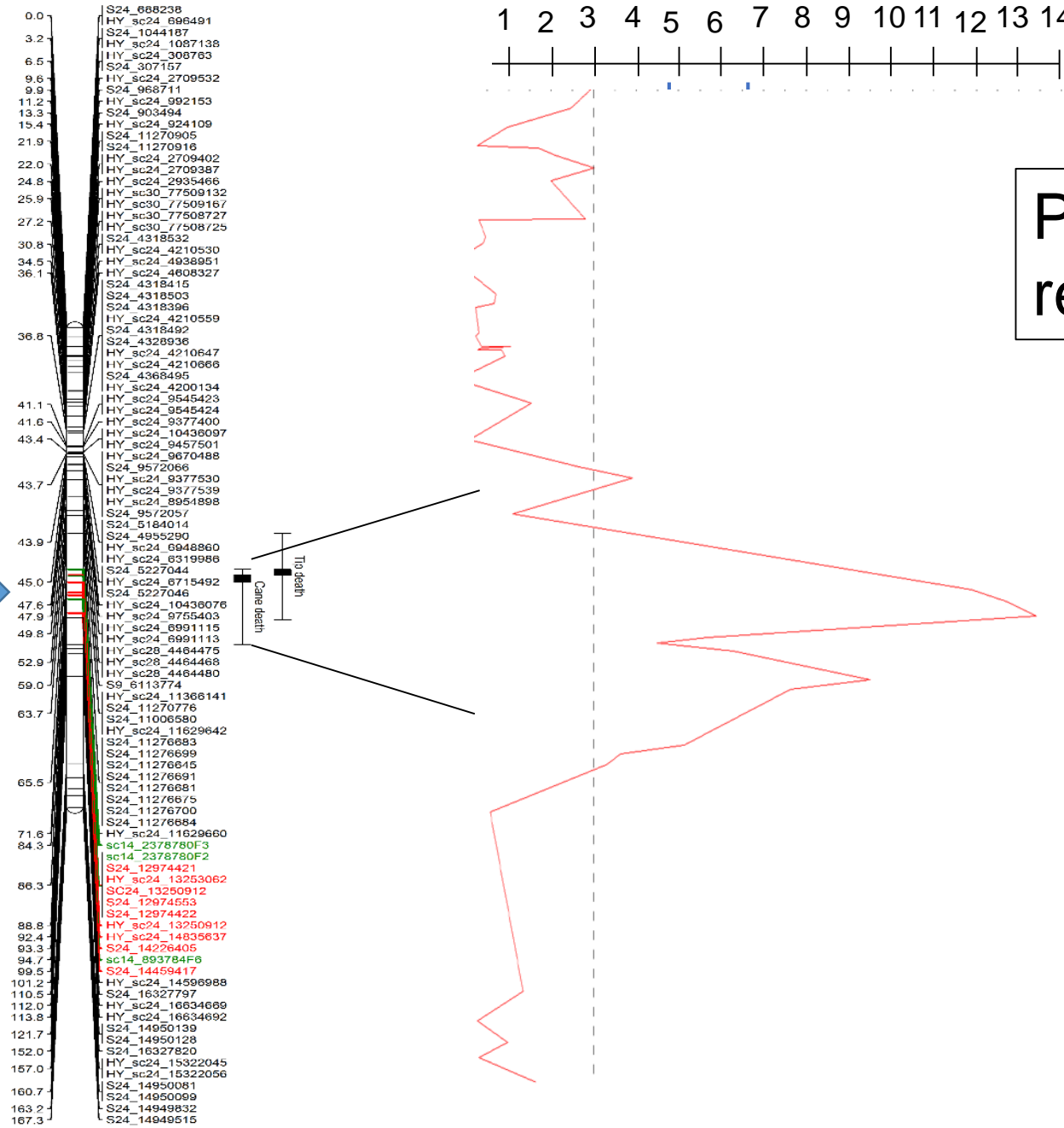
S24\_688238  
HY\_sc24\_696491  
S24\_1044187  
HY\_sc24\_1087138  
HY\_sc24\_308763  
S24\_307157  
HY\_sc24\_2709532  
S24\_968711  
HY\_sc24\_992153  
S24\_903494  
HY\_sc24\_924109  
S24\_11270905  
S24\_11270916  
HY\_sc24\_2709402  
HY\_sc24\_2709387  
HY\_sc24\_2935466  
HY\_sc30\_77509132  
HY\_sc30\_77509167  
HY\_sc30\_77508727  
HY\_sc30\_77508725  
S24\_4318532  
HY\_sc24\_4210530  
HY\_sc24\_4898951  
HY\_sc24\_4808327  
S24\_4318415  
S24\_4318503  
S24\_4318396  
HY\_sc24\_4210559  
S24\_4318492  
S24\_4328936  
HY\_sc24\_4210647  
HY\_sc24\_4210666  
S24\_4368495  
HY\_sc24\_4200134  
HY\_sc24\_9545423  
HY\_sc24\_9545424  
HY\_sc24\_9377400  
HY\_sc24\_10436097  
HY\_sc24\_9457501  
HY\_sc24\_9670488  
S24\_9572066  
HY\_sc24\_9377530  
HY\_sc24\_9377539  
HY\_sc24\_8954898  
S24\_9572057  
S24\_5184014  
S24\_4855290  
HY\_sc24\_6948860  
HY\_sc24\_6319986  
S24\_5227044  
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S24\_5227046  
HY\_sc24\_10436076  
HY\_sc24\_9755403  
HY\_sc24\_8991115  
HY\_sc24\_8991113  
HY\_sc28\_4464475  
HY\_sc28\_4464468  
HY\_sc28\_4464480  
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HY\_sc24\_11366141  
S24\_11270776  
S24\_11006580  
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S24\_11276681  
S24\_11276675  
S24\_11276700  
S24\_11276684  
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sc14\_2378780F2  
S24\_12974421  
HY\_sc24\_13253062  
SC24\_13260912  
S24\_12974553  
S24\_12974422  
HY\_sc24\_13250912  
HY\_sc24\_14835637  
S24\_14226405  
sc14\_893784F6  
S24\_14459417  
HY\_sc24\_14598988  
S24\_16327797  
HY\_sc24\_16634669  
HY\_sc24\_16634692  
S24\_14950139  
S24\_14950128  
S24\_16327829  
HY\_sc24\_15322045  
HY\_sc24\_15322056  
S24\_14950081  
S24\_14950099  
S24\_14949832  
S24\_14949515

To death  
Cane death

# LG24 – Mapping of cane death resistance QTL in ‘P1’

KW score K\*

1 2 3 4 5 6 7 8 9 10 11 12 13 14



Plot for cane death resistance QTL

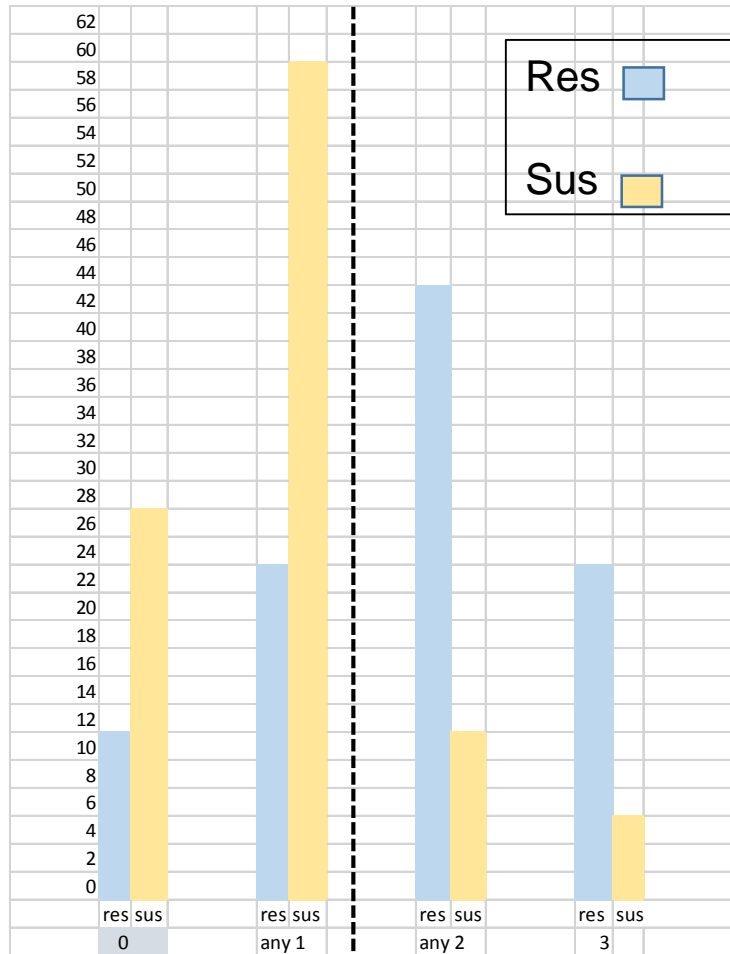
Markers supporting the QTLs using Kruskal Wallis  
 Green= SSR  
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No death  
 Cane death



# Validation of SSR markers in population ('A'); 87 phenotyped plants, screened with SSRs for 'cane death' resistance

% plants with QTL combination in res or sus class, respectively

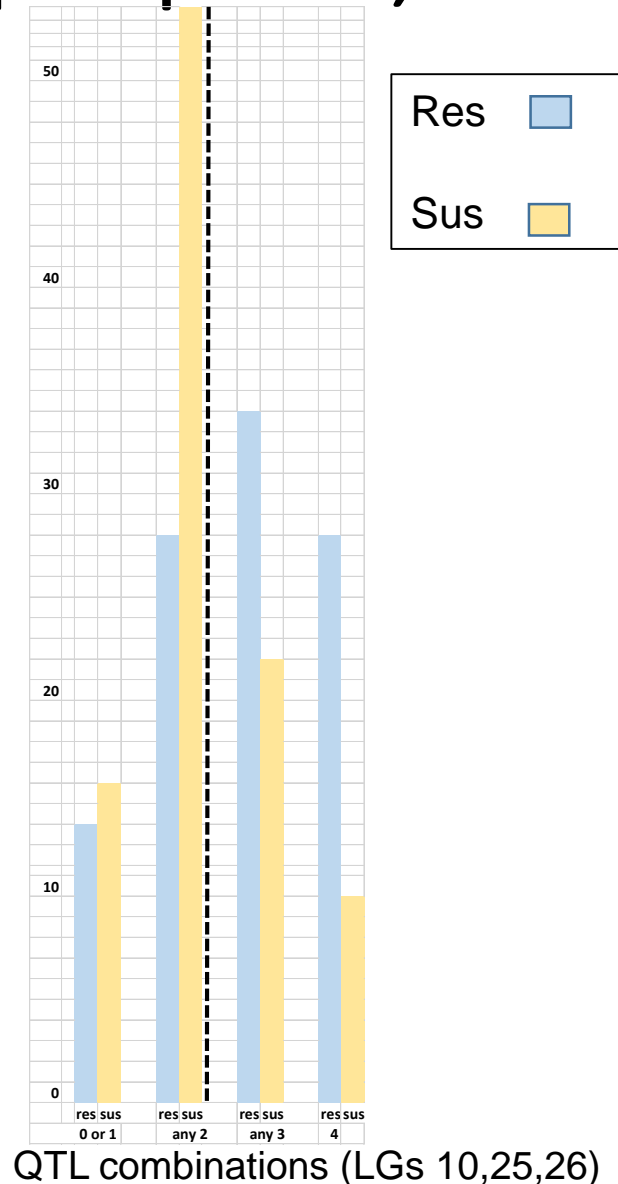


QTL combinations (LGs 12, 24, 25)

- SSR markers identify cane death resistance QTLs in validation population 'A'
- QTLs more effective in combination

# Validation of 'tip death' resistance markers in population ('G'); 93 phenotyped plants, screened with SSRs

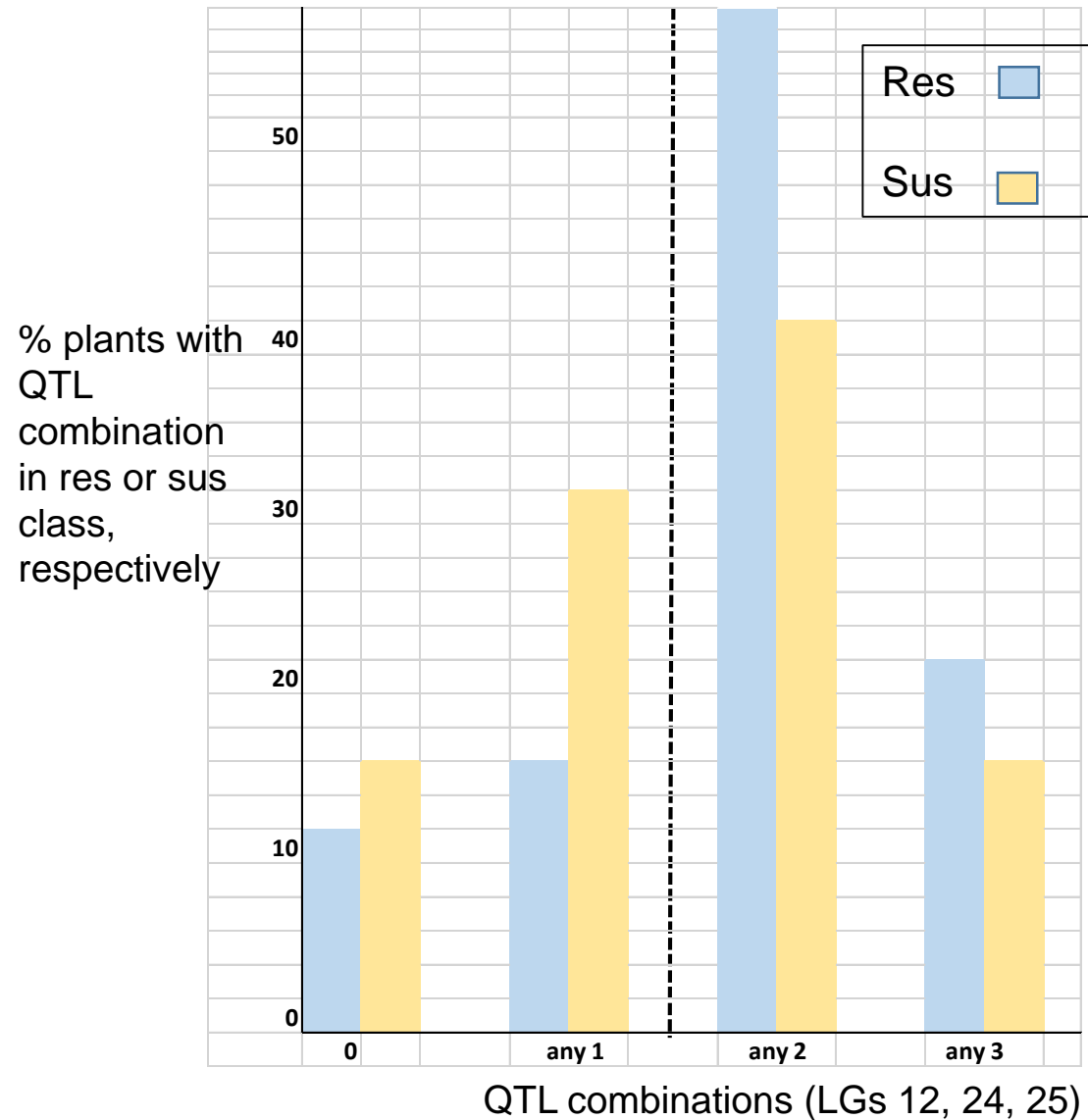
% plants with  
QTL  
combination  
in res or sus  
class,  
respectively



- SSR markers identify tip death resistance QTLs in validation population 'G'
- QTLs more effective in combination



# Validation of 'ooze' resistance markers in population ('A'); 87 phenotyped plants, screened with SSRs



- SSR markers identify 'ooze' resistance QTLs in validation population 'A'
- QTLs more effective in combination

## Now:

1. **Heather:** QTL validation in a larger (372 plants) diploid population with 'P1'
  - look at interactions of QTLs more precisely
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3. **New PFR resource for c.g identification:** whole genome sequence of 'P1'

- Sequencing complete

# Marker assisted selection – for what; and when?

- QTL based resistance is complex....
  - Phenotypes are independent, which trait breed for?
  - Interaction among QTLs for single resistance phenotype, screen for which QTLs?
- Will construct robust markers from candidate genes
- → high throughput automated screens (Taqman, array based?) of DNA of known females (sex tested)

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