

Improving phytosterol content and  
composition in *Brassica napus* oil

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BBSRC sLoLa Program – Renewable Industrial  
Products from Rapeseed (RIPR)

- Understand the genetic component which controls the quantitative variation observed for phytosterol content and composition in *B. napus* seed oil.

## Examples:

1. Steryl-esters/total sterol content (quantitative trait).
2. Brassicasterol levels (qualitative trait).
3. Is the campesterol/sitosterol ratio correlated to crop architecture (brassinosteroids biosynthesis)

# Methodology



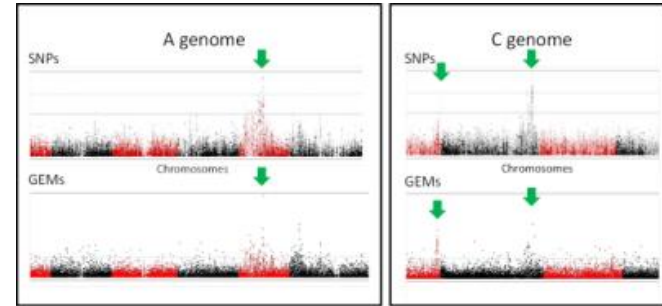
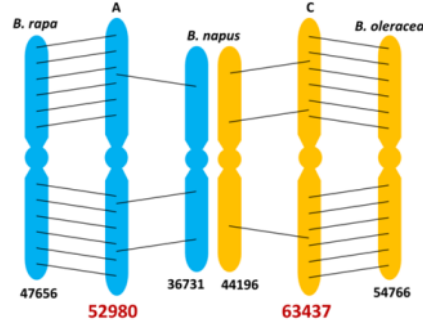
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ASSYST *B. napus* diversity panel

423 cultivars



Functional genotypes  
Illumina mRNAseq data



SNPs calling, transcript quantification (GEMs)

Associative  
Transcriptomic  
(AT) Analysis

SNP and GEM peaks  
detection. In some cases a  
good correlation is observed  
between SNPs and GEMs.

Trait analysis  
Biochemical Profiling



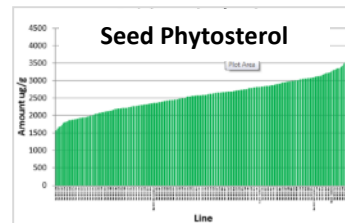
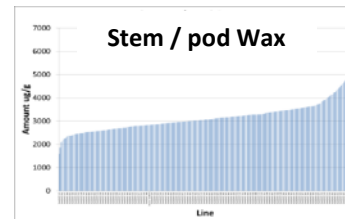
High Throughput  
Method development



Extraction  
Purification  
Derivatisation



Wax / Phytosterol Analysis  
HPLC / GC / ESI-MS/MS

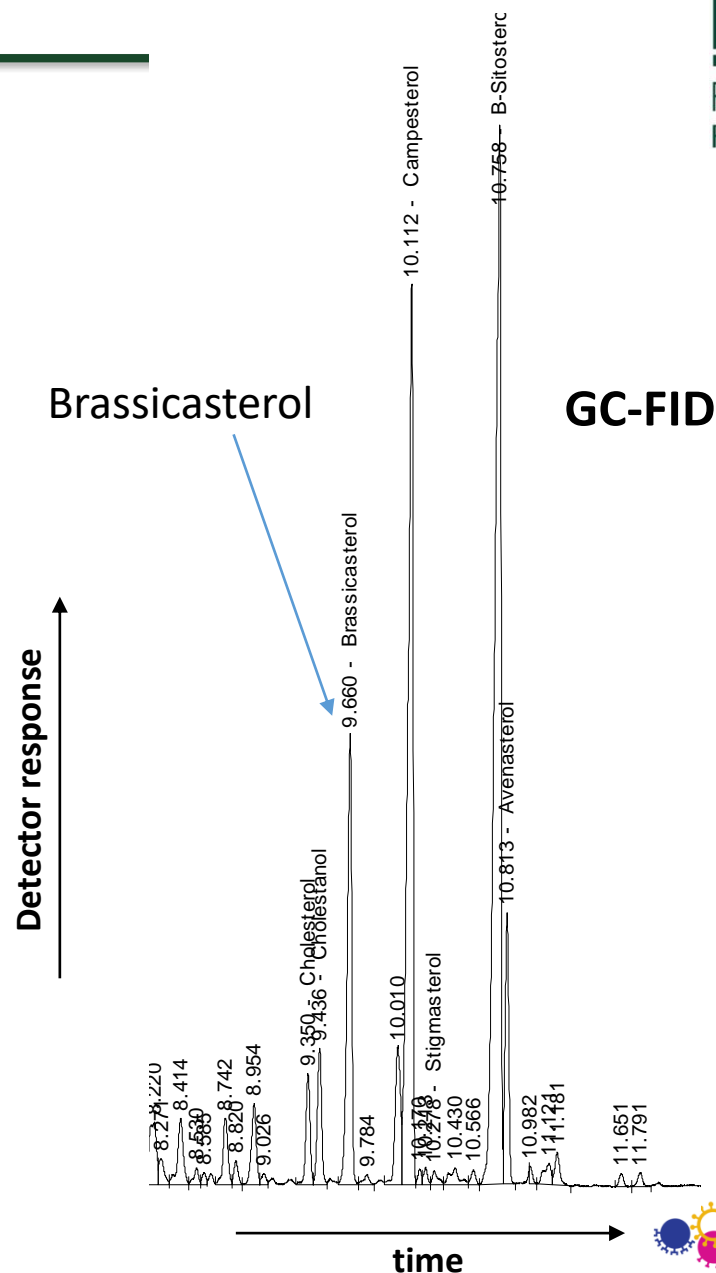


# Reducing Brassicasterol levels



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- Not necessarily harmful to health, however has not been tested and approved for use in food products.
- To comply with European regulation, and to compete with sunflower and soybean oil, brassicasterol level in OSR needs to be reduced.

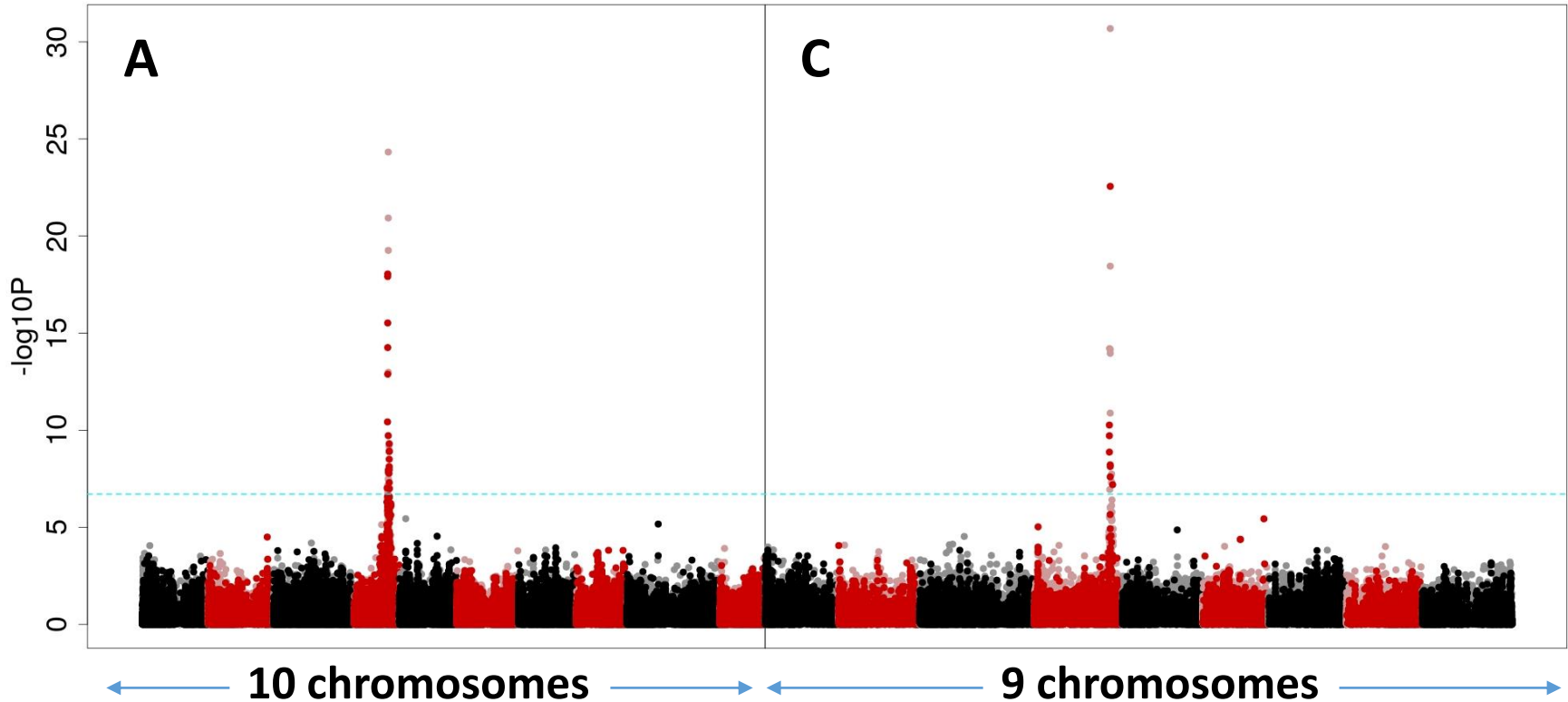


# Brassicasterol – SNP ( $\mu\text{g/g}$ seed, GC-FID)

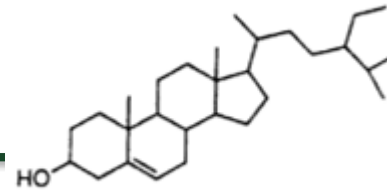


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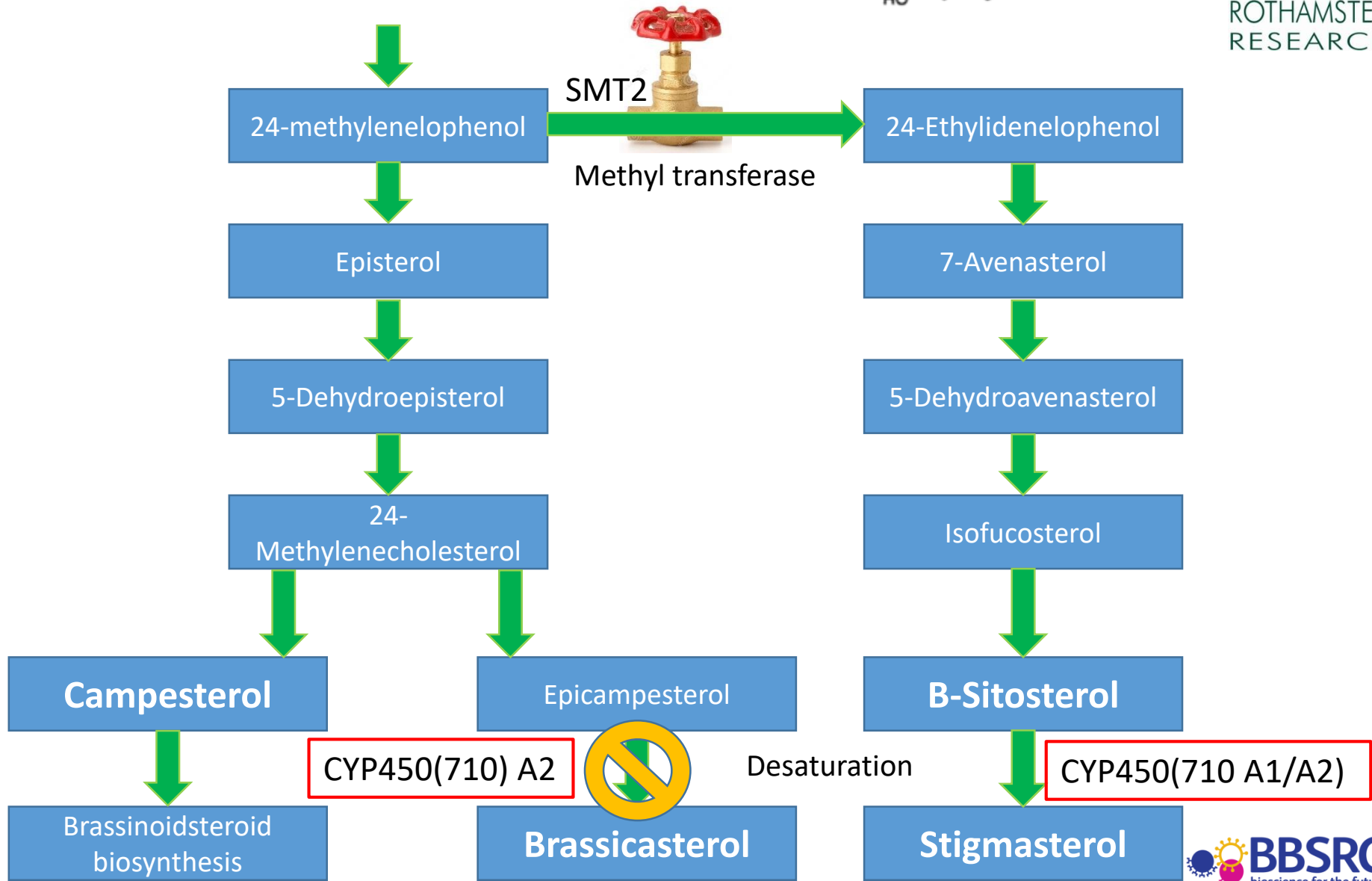
Trait control genes with variation in the panel



# Sterol Biosynthesis



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# Cytochrome P450 710 TILLING lines



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# Cytochrome P450 710 TILLING lines



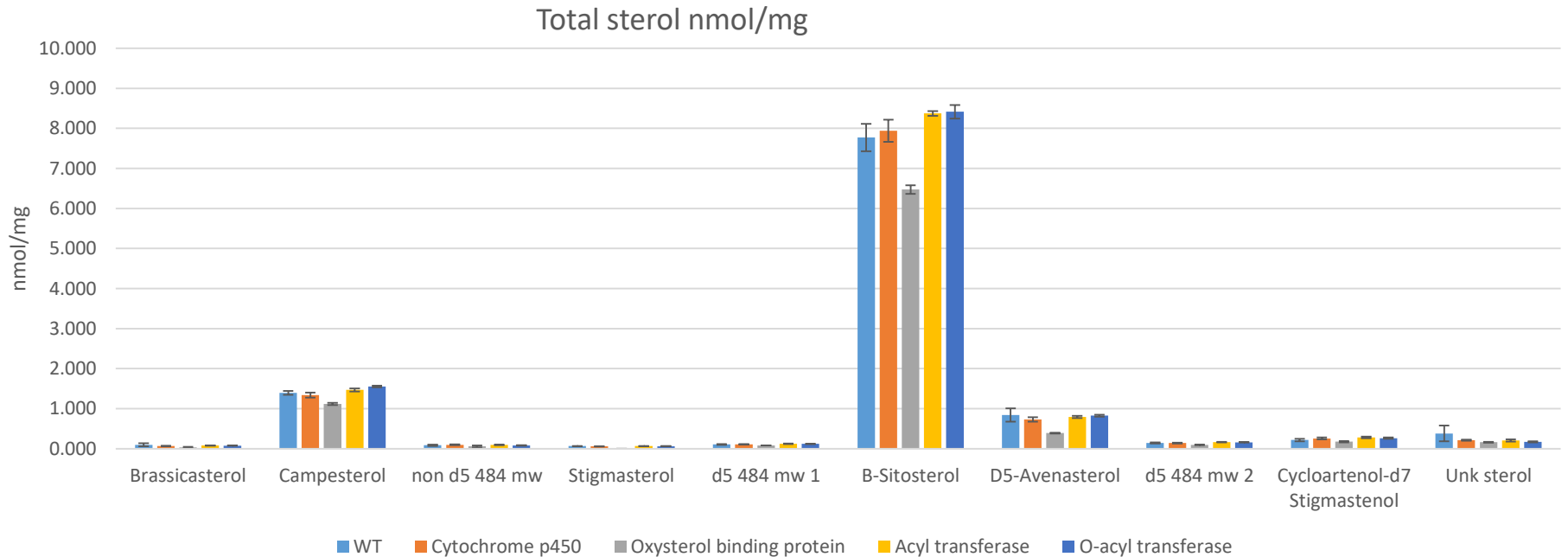
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# TDNA Arabidopsis



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# Further Work

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- Profile Cyp450 710 A2 form TILLING mutants sterol profiles.
  - Subsequent back crossings
- Crispr K.O. of Cyp450 (J.I.C)- Varying combination of A1/A2
- Gamma ray TILLING population – Genes relating to total sterol and steryl-ester selected to be characterised.

# AT analysis: Comparison between total sterol and steryl-ester content



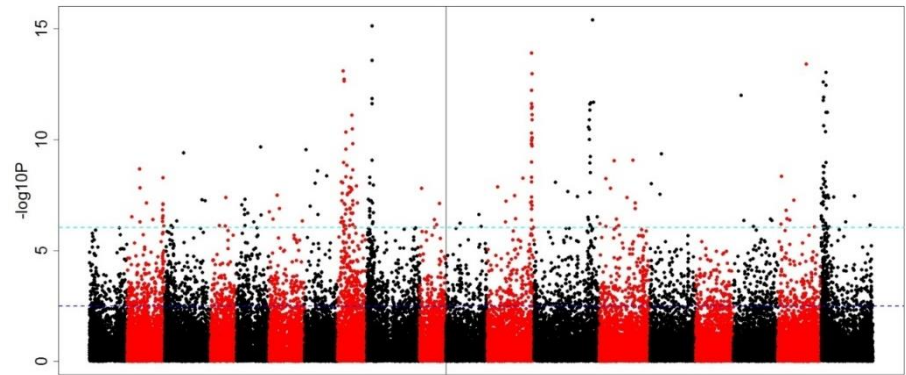
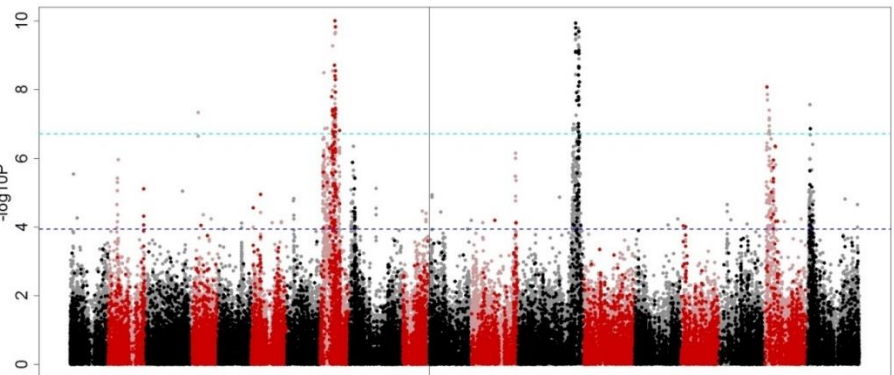
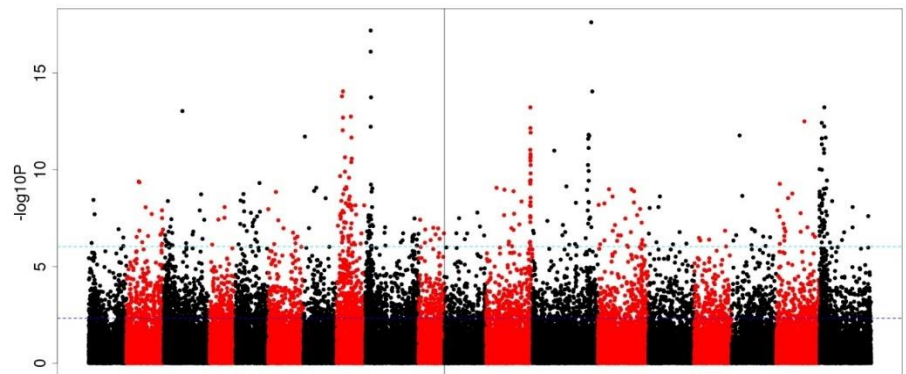
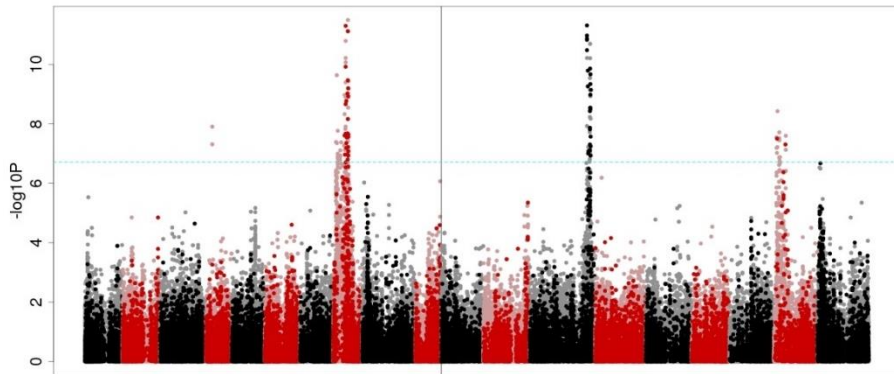
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**SNPs**

**GEMs**

Total sterol (GC-FID)

Total sterol (GC-FID)



Steryl ester ESI-MS/MS

Steryl ester (ESI-MS/MS)

Several gene targets have been identified and will be characterised  
By *B. napus* gamma ray TILLING panel

## Collaborators and Thanks:

- Ian Bancroft
- Lenka Havlickova
- Zhesi Hi
- Andrea Harper
- Frédéric Beaudoin
- JIC – TILLING germplasm

