GENETIC CONTROL OF SUNFLOWER METABOLOME IN A DRY AGRONOMIC ENVIRONMENT

Marco Moroldo, Annick Moing, Stéphane Bernillon, Vincent Segura, Gabriela Bindea, Nicolas Blanchet, Nicolas Langlade and the SUNRISE consortium

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ASTR
Abiotic Stress Tolerance in sunfloweR

INRAE

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Outline of the presentation

1. Background and scientific aim
2. Experimental setup and analysis workflow
3. Results
4. Next steps

(1) Background and scientific aim

(1) Drought tolerance

- complex interplay among different mechanisms
- main strategies:

1) escape: phenological development matches periods of moisture availability
2) avoidance: water potential is maintained by increasing water uptake and reducing transpiration
3) plasticity: adaptation through osmotic adjustment, ROS scavenging and morphological mechanisms

(1) Molecular bases

Gene level

- heat shock proteins (HSP), late embryogenic abundant proteins (LEA), aquaporins

Pathway level

- response to abscisic acid (ABA)
- osmotic stress, cold stress, oxidative stress
- synthesis of osmolytes (e.g. sugars, aminoacids)
- synthesis of secondary metabolites (e.g. terpenes)

(1) Drought tolerance in sunflower

- drought tolerant (highly explorative root system)
- often grown in arid or semi-arid climates
- sensitivity to drought is nonetheless a concern
  from early flowering to achene filling
- genotypes show different levels of tolerance

need to better characterize
and improve the mechanisms
involved in drought tolerance

(1) Molecular bases in sunflower

- response to abscisic acid
- osmotic and oxidative stress
- cell wall modification
- glutathione synthesis
- sugar synthesis and starch degradation
- flavonoid and terpenes metabolism

List of pathways characterized using transcriptomics and metabolomics according to Rengel et al 2012, Moschen et al 2017, and Chunbo et al 2017

(1) Aim of the project

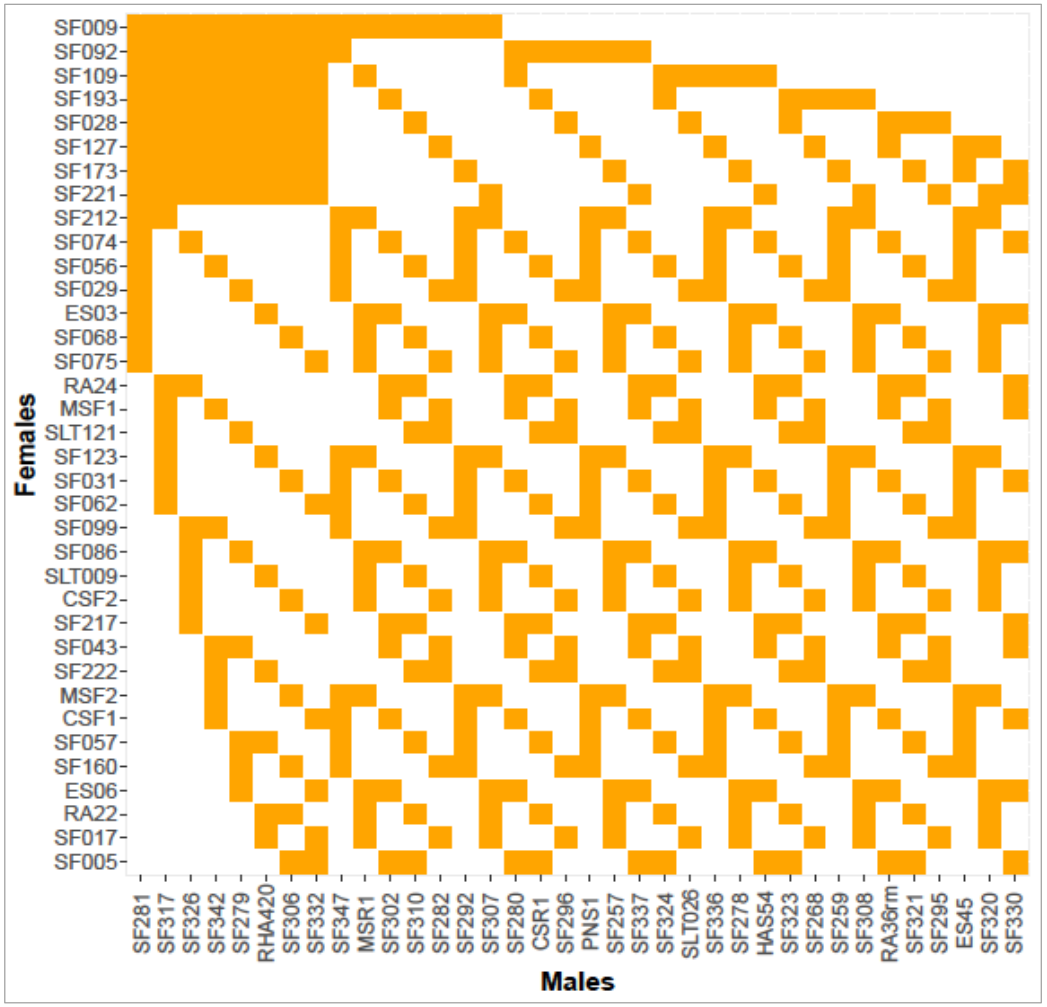
- to elucidate the basis of metabolic variation in response to drought in sunflower through a genetic association approach (metabolic QTLs or mQTLs)
- to complement the results already available obtained on transcriptome
- to functionally analyse the molecular pathways involved in the process

(2) Experimental setup

(2) Plant material

- 475 hybrids obtained by crossing 36 male genotypes and 36 female genotypes (incomplete factorial design)

(2) Plant material

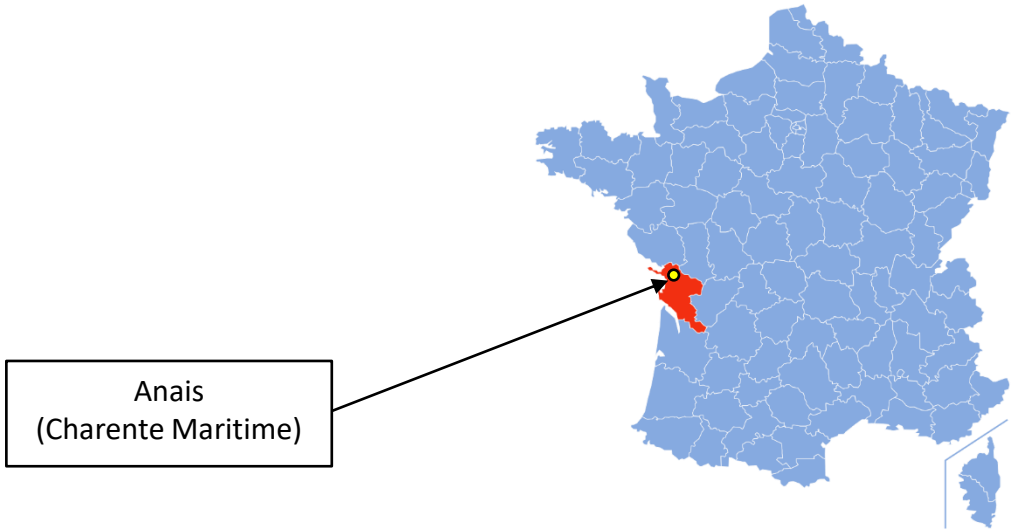


(2) Plant material

- 475 hybrids obtained by crossing 36 male genotypes and 36 female genotypes (incomplete factorial design)
- 1 plot per hybrid
- 4 hybrids used to correct for spatial biases
- good representation of the genetic diversity found in cultivated sunflower

(2) Experimental field

- trial conducted in Anais (Charente Maritime)
- type of soil: silty clay, 30 cm deep, 50% gravel



(2) Experimental field

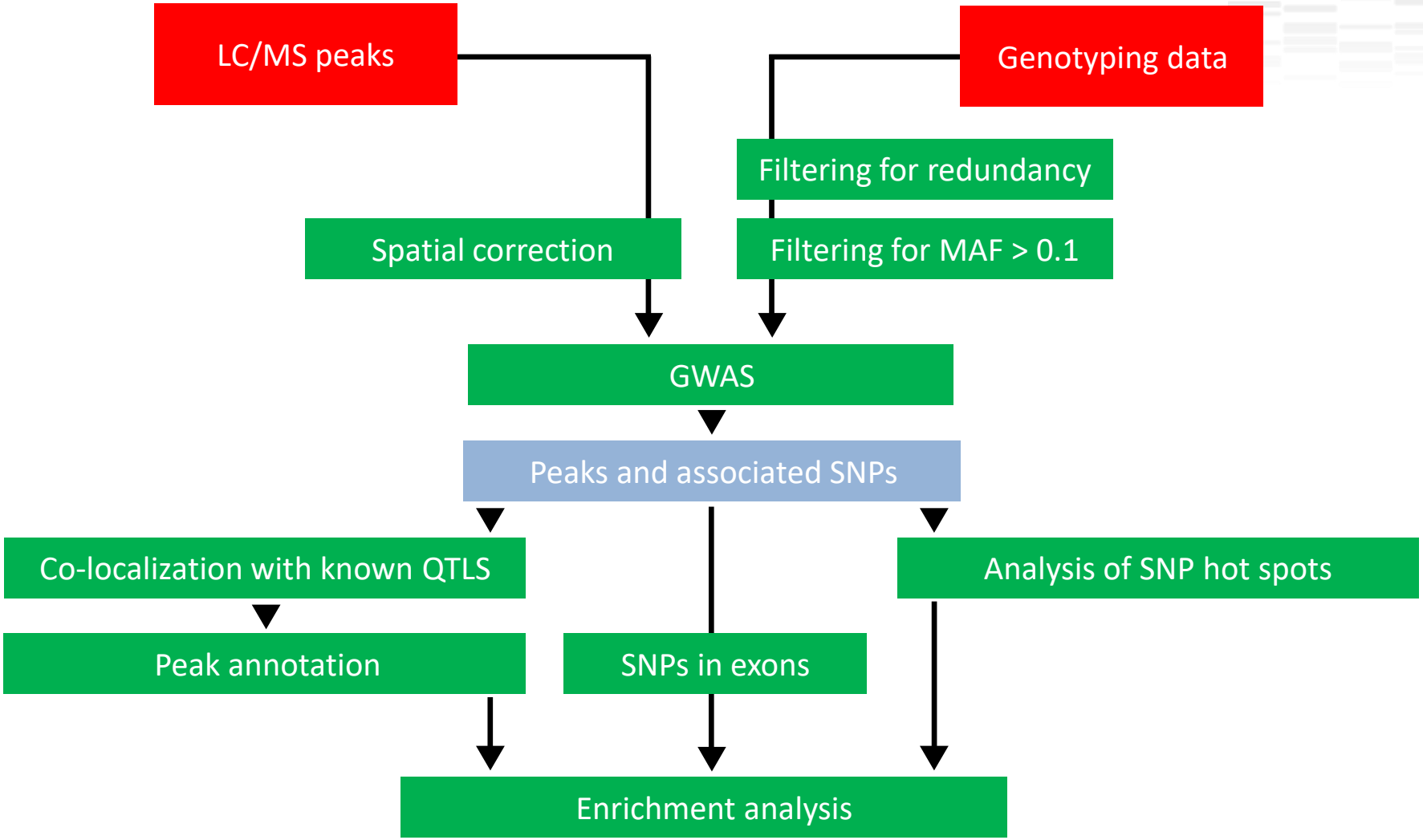
(2) Climatic conditions

- trial conducted from 02/05/2015 to 29/09/2015
- leaves at the *n*-4 topmost position were sampled on 22/07/2015, i.e. 7 days after blooming ± 3 days
- reduced rainfall in the 4 weeks before sampling (< 10 mm)
- average FTSW = 0.1 (simulation with ‘SUNFLO’)

(2) Metabolome and genotyping

- metabolome: untargeted analysis by LC/MS using an Orbitrap-MS (Thermo Fischer) after ethanol / water (80:20) extraction
- genotyping: Illumina resequencing of the parental lines

(2) Analysis workflow

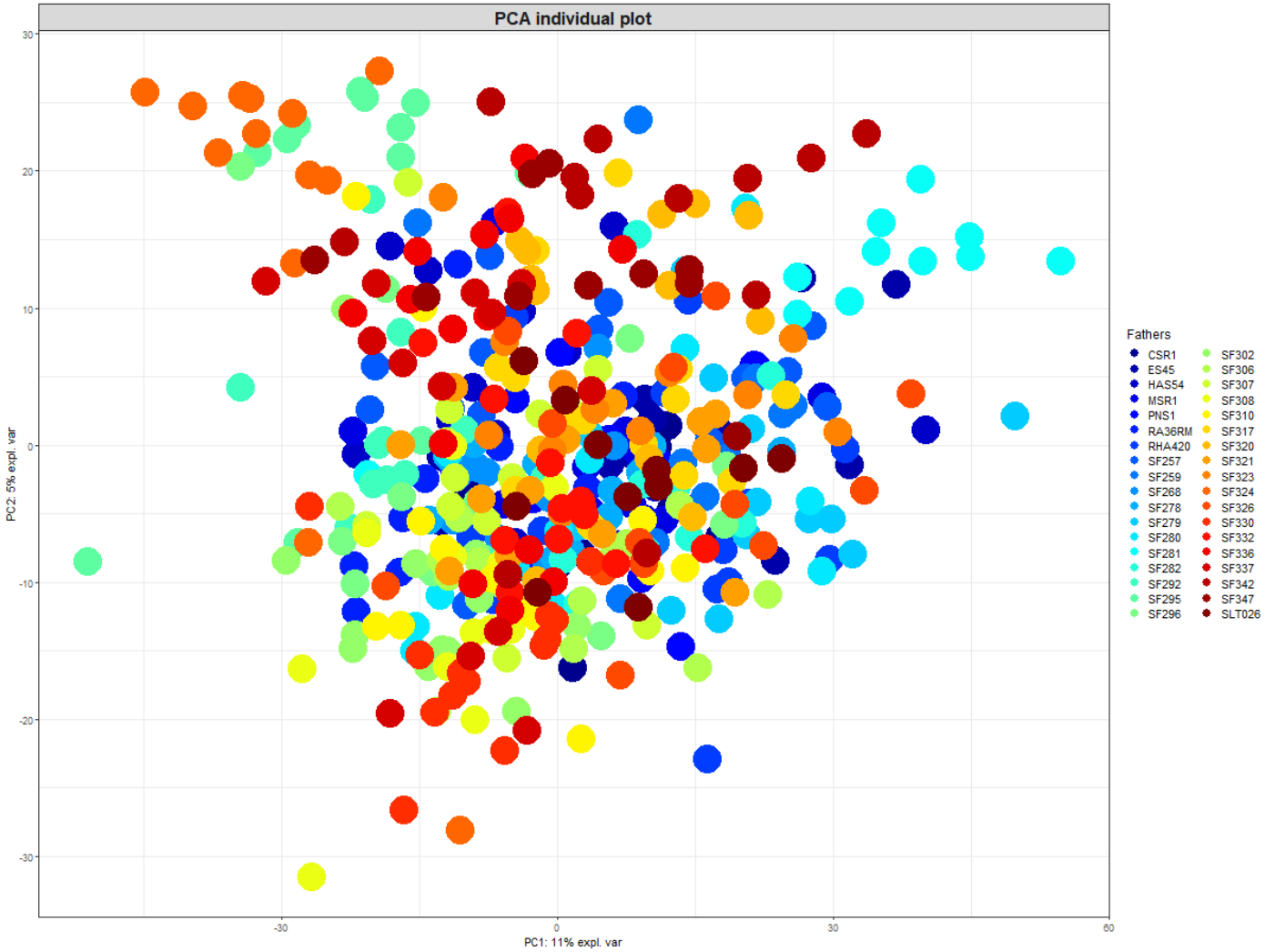


(3) Results

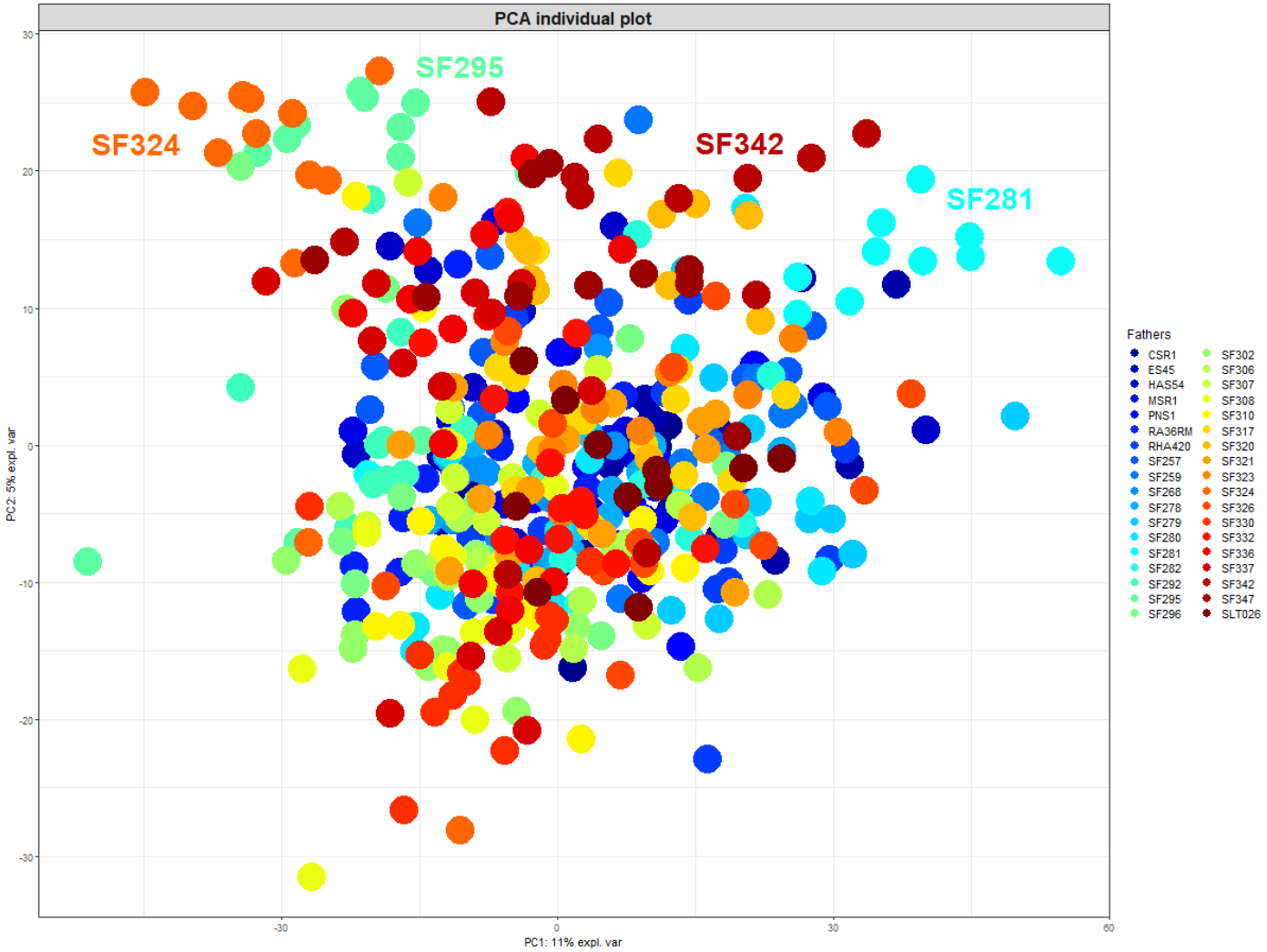
(3) Metabolome

- 450 samples retained
- 6,246 initially detected peaks
- 3,507 peaks retained after data filtering based on QC testing
- 2,557 peaks kept after the removal of artifacts (i.e. isotopes, adducts and losses)

(3) Metabolome



(3) Metabolome



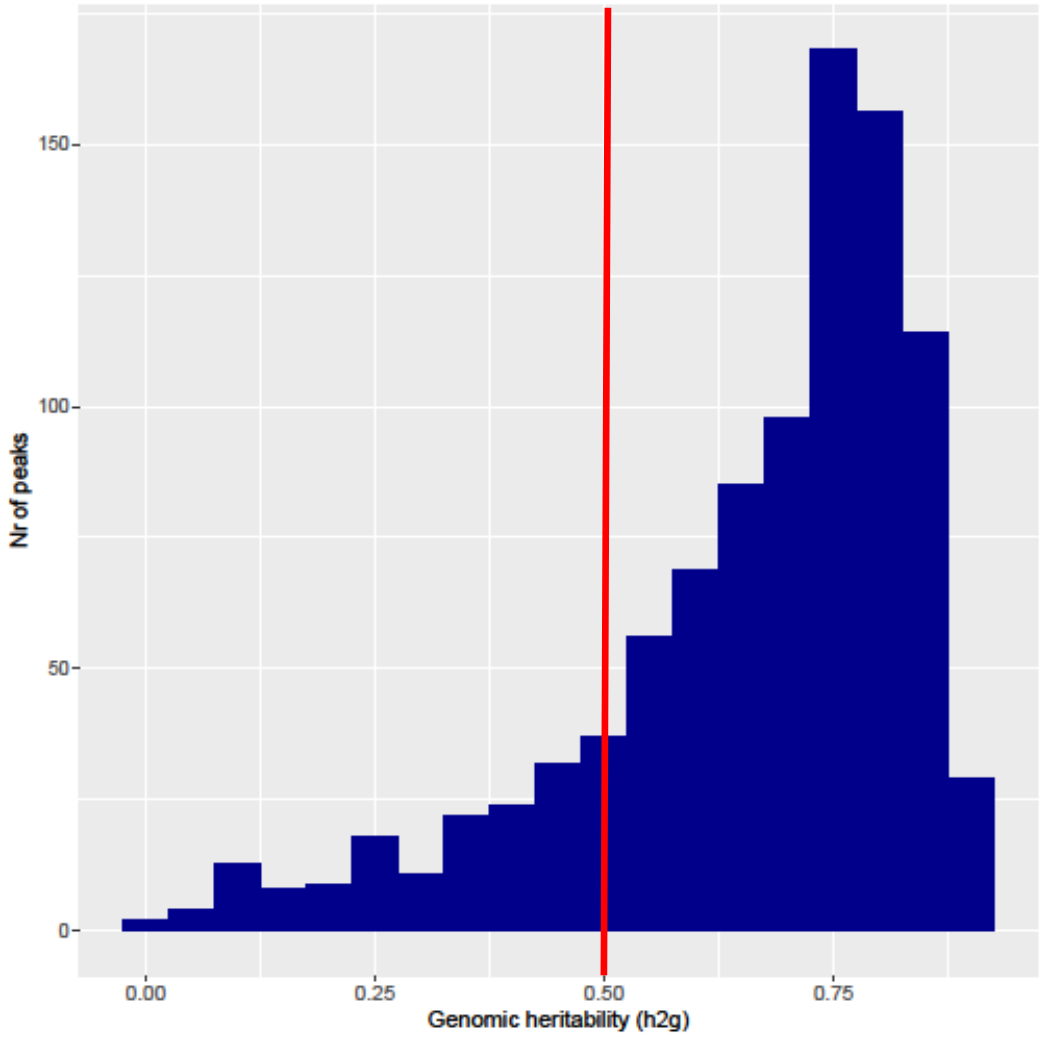
(3) Genotyping

- 450 samples retained
- filtering for redundance and MAF
- 350,052 SNPs used for GWAS

(3) GWAS

- 450 samples retained
- analysis carried out with the R package ‘mlmm-gwas’ (Segura et al 2012): multi-locus mixed model
- no multiple testing correction and filtering on eBIC
- 955 LC/MS peaks (37%) associated to 27,246 unique redundant SNPs
- high levels of genomic heritability (h2g)

(3) GWAS



H2g > 0.5 in 84% of the cases

(3) GWAS

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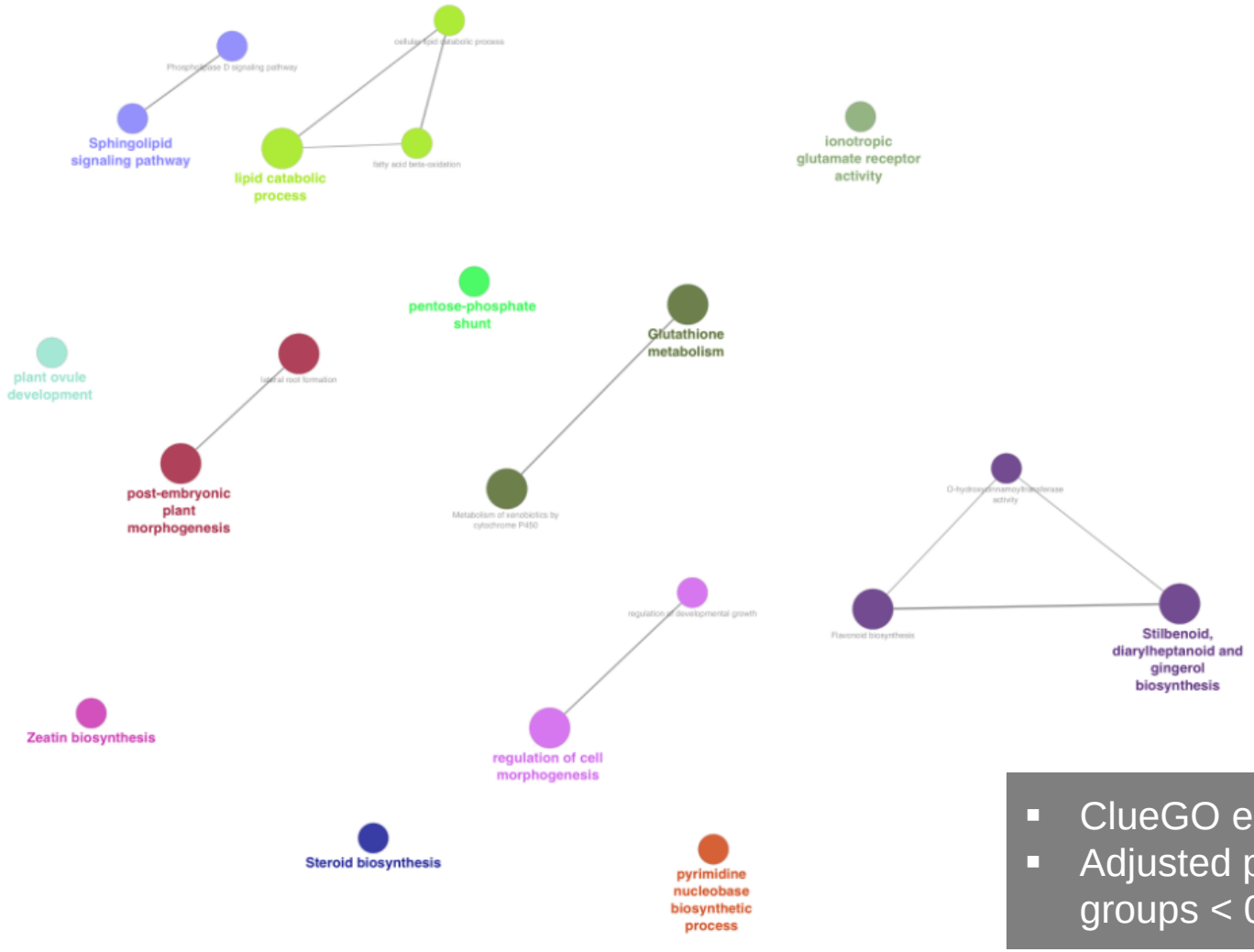
- 955 LC/MS peaks (37%) associated to 27,246 unique redundant SNPs
- high levels of genomic heritability (h2g)
- 1,768 SNPs located in exons, i.e. 555 genes, 385 LC/MS peaks

(3) Gene-level enrichment



- ClueGO enrichment analysis
- Adjusted p-value for pathway groups < 0.05

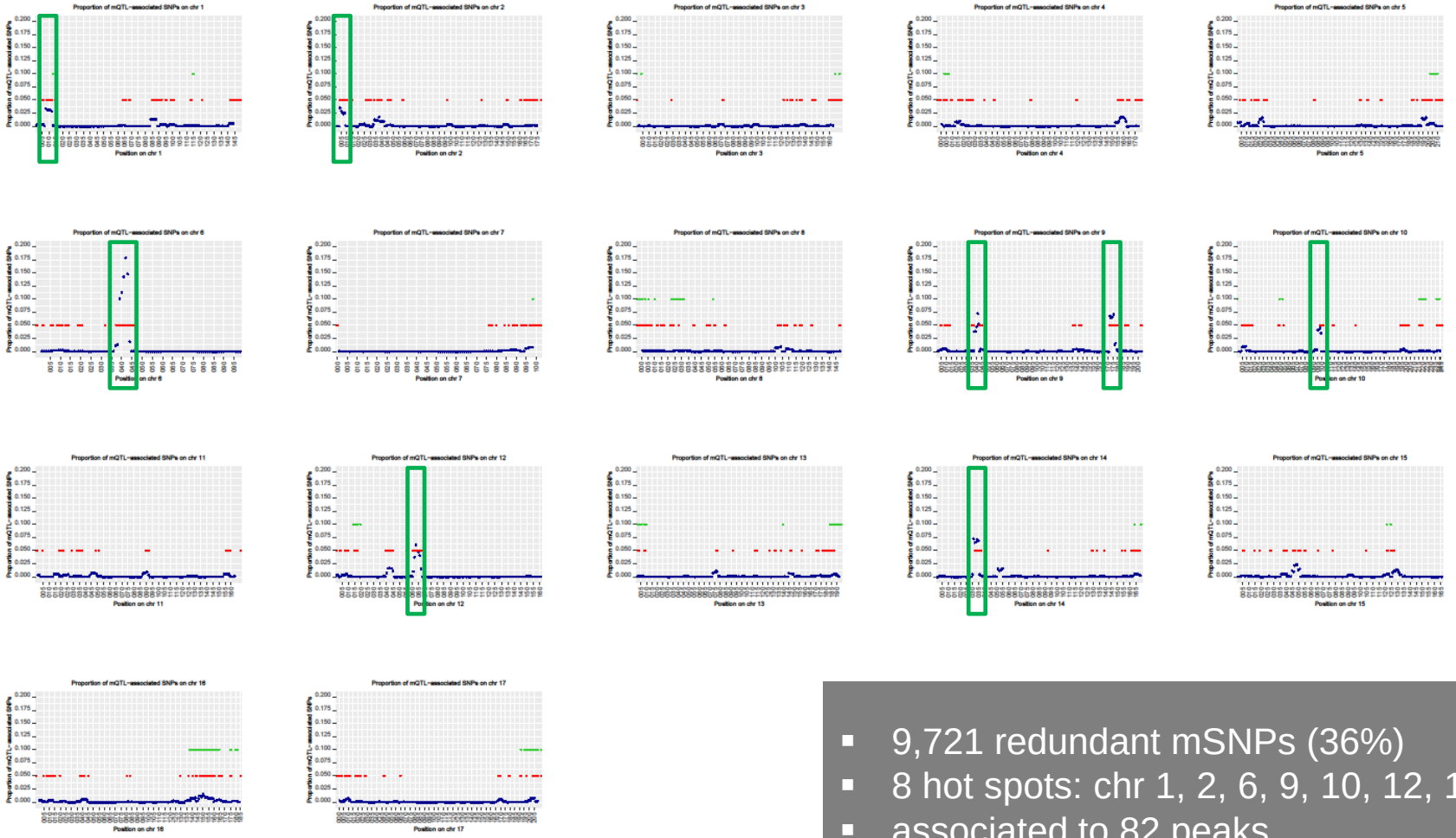
(3) Gene-level enrichment

Term	ontology source	BH adjusted p-value
post-embryonic plant morphogenesis	GO	0.0042
glutathione metabolism	KEGG	0.0014
sphingolipid signaling pathway	KEGG	0.0242
flavonoid biosynthesis	KEGG	0.0016
stilbenoid, diarylheptanoid and gingerol biosynthesis	KEGG	0.0016
lipid catabolic process	GO	0.0012
cellular lipid catabolic process	GO	0.0012

(3) Co-localization with QTLs

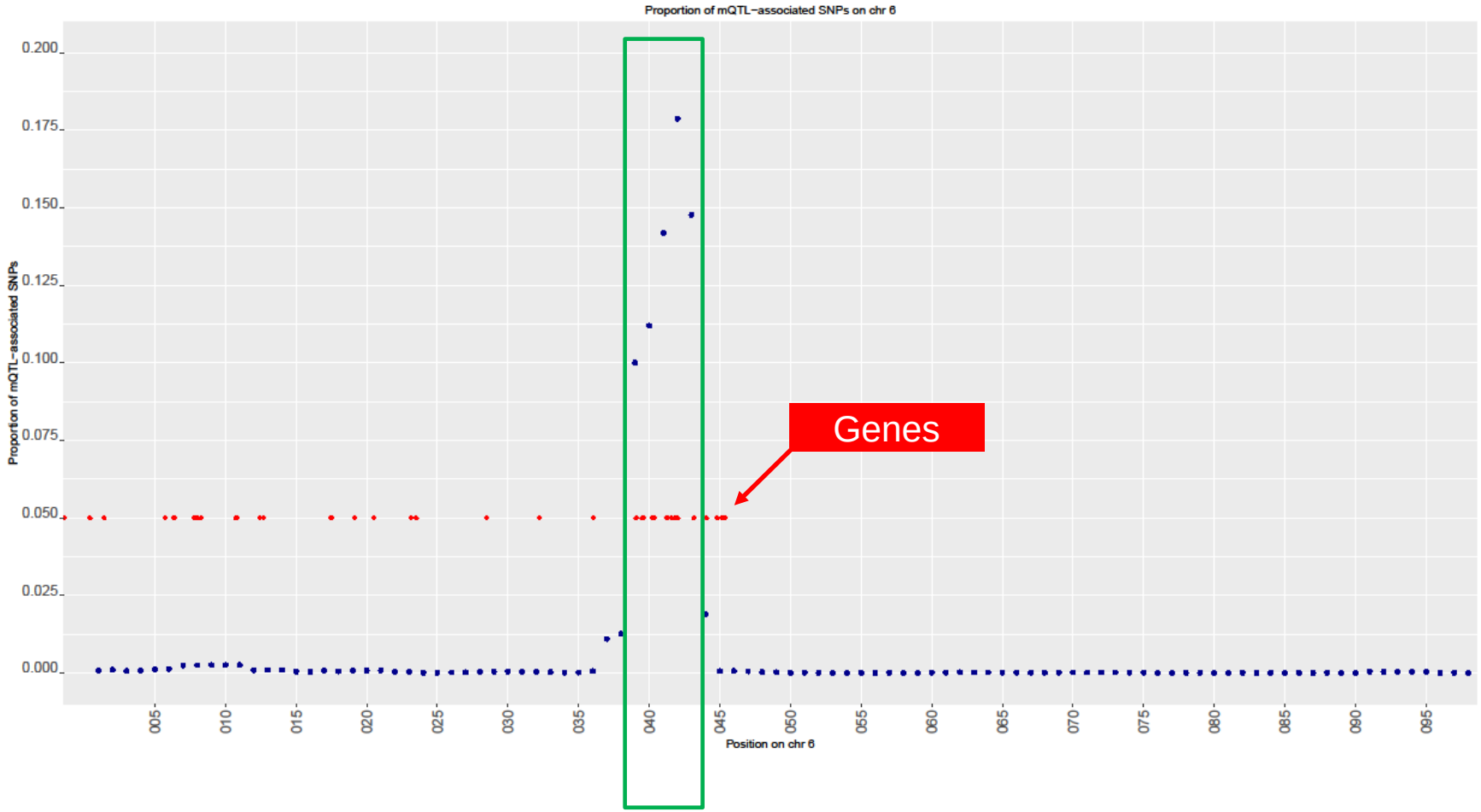
- interval QTL – mSNPs = 50 kb
- 638 total SNPs
- 120 peaks co-localizing with QTLs controlling either drought, cold or nutritive tolerance
- 19 peaks co-localizing with productivity traits (i.e. total yield and oil yield)
- 137 total peaks co-localizing with QTLs
- annotation of peaks ongoing

(3) Results: SNP hot spots



- 9,721 redundant mSNPs (36%)
- 8 hot spots: chr 1, 2, 6, 9, 10, 12, 14
- associated to 82 peaks

(3) Results: SNP hot spot on chr 6



(4) Conclusions and next steps

- mSNP tend to cluster in hot spots
- genes associated to mSNP seem to follow similar patterns of localization
- genes associated to mSNP seem to belong to drought-related pathways

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- annotation of the peaks corresponding to QTLs
- other strategies of annotation of the peaks
- other enrichment analyses (e.g. on hot spots)

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