

Technical Working Party on Testing Methods and Techniques**TWM/2/6 Add.****Second Session****Virtual meeting, April 8 to 11, 2024****Original:** English**Date:** April 10, 2024

**ADDENDUM TO:
MOLECULAR APPROACHES TO SUPPORT DUS TESTING***Document prepared by experts from the United Kingdom**Disclaimer: this document does not represent UPOV policies or guidance*

The annex to this document contains a copy of a presentation “Molecular approaches to support DUS testing”, made by an expert from the United Kingdom, at the second session of the Technical Working Party on Testing Methods and Techniques (TWM).

[Annex follows]



Molecular approaches to support DUS testing

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Concept

To improve the efficiency of DUS testing by:

- Using molecular markers to inform the selection of varieties of common knowledge to include in the growing trials
- Identifying characteristic-specific markers with the potential to replace phenotypic observations
- Developing molecular marker subsets for authentication of seed samples

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Molecular methods in DUS testing



Wheat



Barley



Raspberry

Molecular methods in DUS testing



Wheat



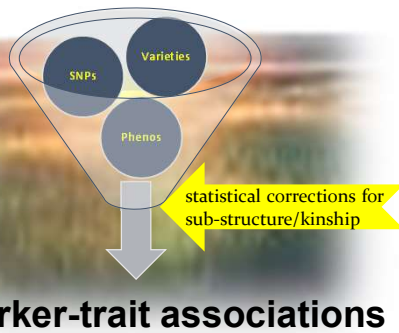
Barley



Raspberry

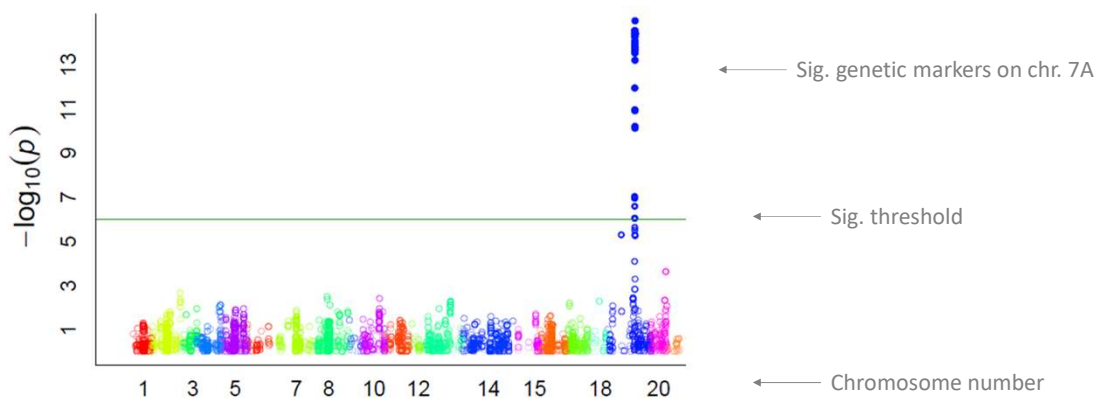
Historical work – marker trait analysis in wheat

- Wheat Association Genetics for Trait Advancement and Improvement in Lineages: WAGTAIL

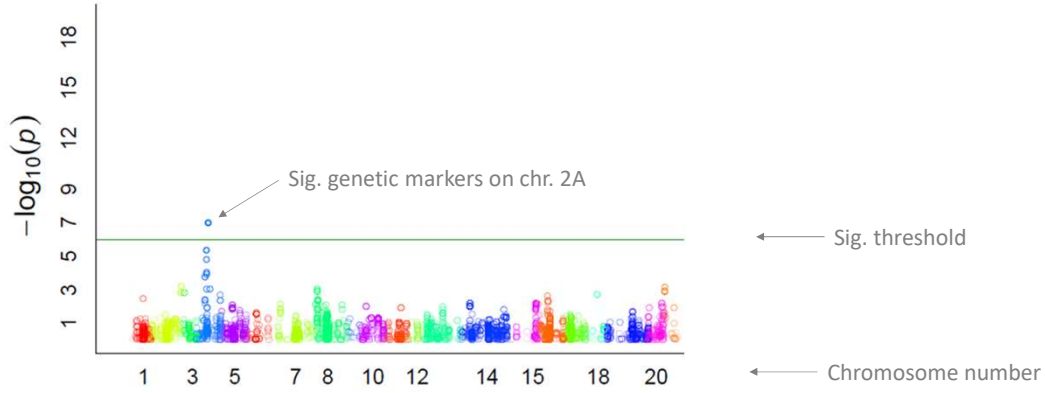


- BBSRC funded project
- Academic and industry partners, including many European wheat breeders
- 402 varieties (predominantly GB, FR, DE)
 - 365 with DUS descriptions
 - 216 with data for all 34 characteristics
- Illumina iSelect 90k chip
 - 60k worked
 - 30k polymorphic

GWAS example 1: Coleoptile colour

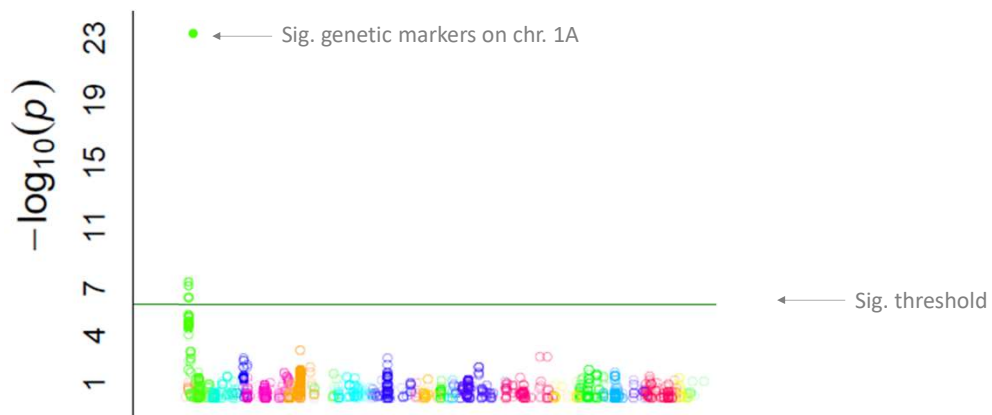


GWAS example 2: Grain colouration with phenol



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GWAS example 3: Glume hairiness



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Molecular methods in DUS testing



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Barley – United Kingdom DUS testing

- ~ 50 new applications for spring barley per year
- 29 DUS characteristics
- Two cycles of test, but often third cycle is required for D

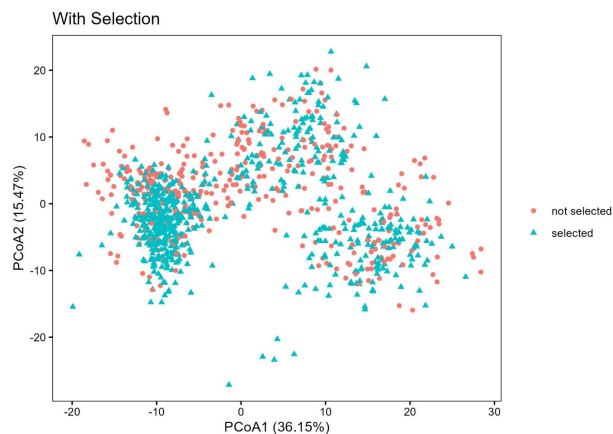
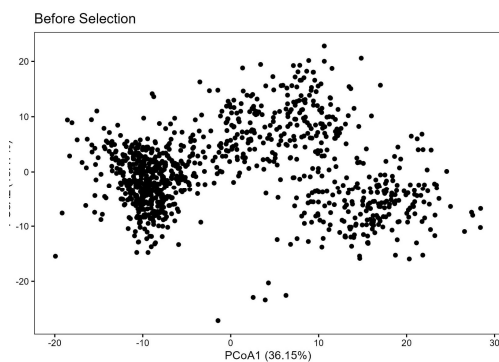


Barley – current project

Defra funded project (24 months starting January 2023). The aims are to:

- Use molecular marker data to inform the selection of similar varieties for the growing trial
 - Earlier identification could remove the need for the additional test year and improve trial design
- Identify and validate a smaller set of markers for seed sample authentications and varietal identity checks
- Modify our existing DUS database structure to enable inclusion of genotypic data

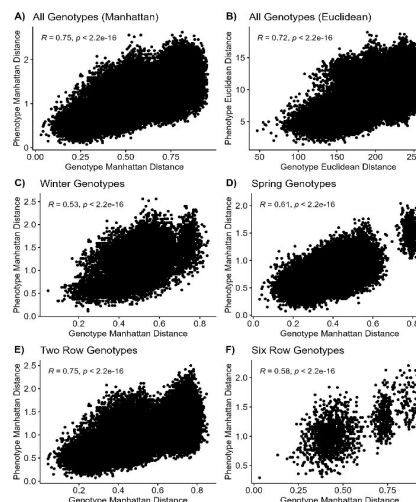
Barley – Genotyping



- Varieties selected to cover the full trait diversity space
- > 500 varieties genotyped using 50k marker array

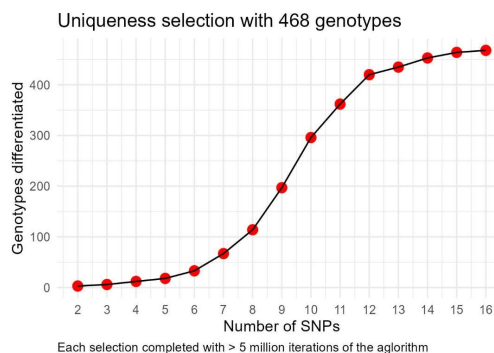
Barley – Baseline marker-trait correlations

- Maximum correlation achieved is 75%
- Molecular marker number is not the limiting factor
- Next steps:
 - Expanding the genotypic and phenotypic datasets
 - Exploring methodological improvements to optimize the phenotypic predictions



Barley – Marker selection for varietal ID and seed sample authentications

- Open source software “Uniqueness” used to identify a subset of markers based on their discrimination ability
- 16 SNPs can differentiate our test collection of 468 genotypes
- Next steps:
 - Expanding the varietal collection analysed
 - Design and experimental validation of KASP markers



Molecular methods in DUS testing



Wheat



Barley



Raspberry

Raspberry

- Defra funded project
- Aim: Develop an Artificial Intelligence program to identify genomic markers with the potential to distinguish between varieties of *Rubus idaeus* L.
- Phenotype data
- Exome capture and sequencing, Whole genome sequencing, Artificial Intelligence and deep learning



Current work – obtaining representative raspberry material for genotyping

