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International Union for the Protection of New Varieties of Plants

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Technical Working Party on Testing Methods and Techniques **TWM/3/20**

Third Session
Beijing, China, April 28 to May 1, 2025

Original: English
Date: April 9, 2025

THE USE OF BIOMOLECULAR TECHNOLOGY IN DUS TESTING – A CASE STUDY ON BARLEY

Document prepared by an expert from the United Kingdom

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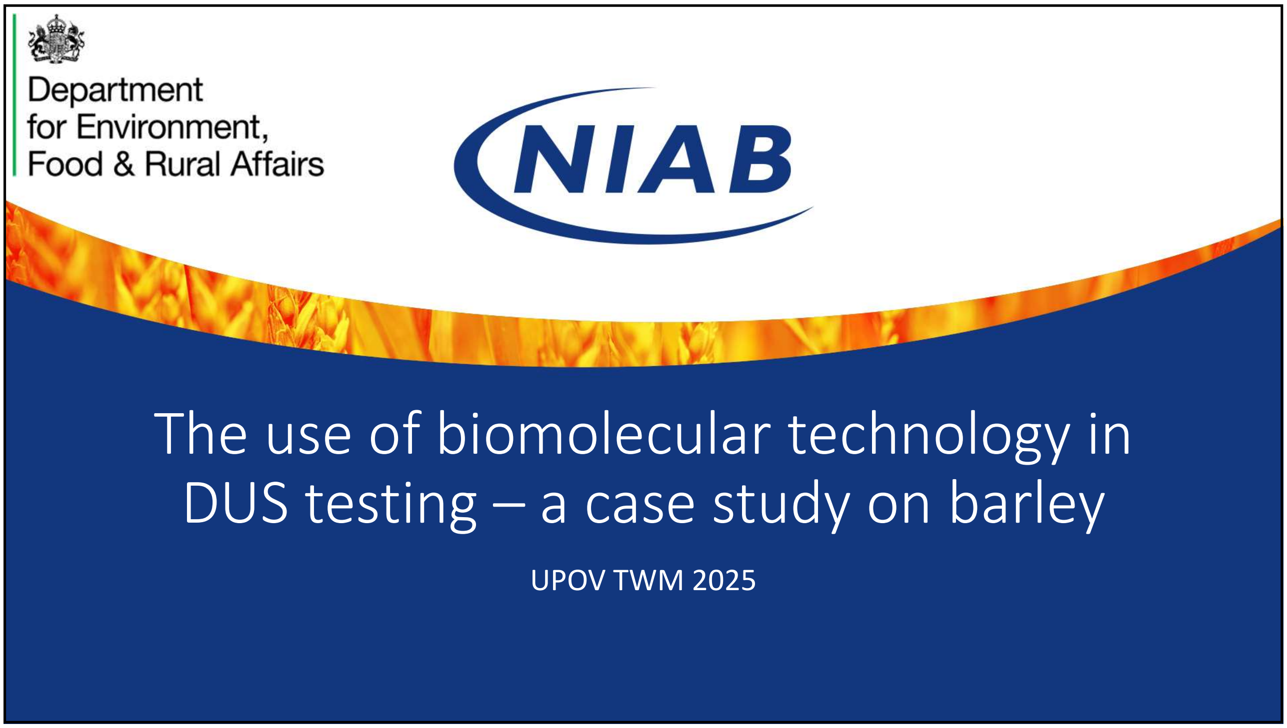
The annex to this document contains a copy of a presentation “The use of biomolecular technology in DUS testing – a case study on Barley”, to be made by an expert from the United Kingdom, at the third session of the TWM.

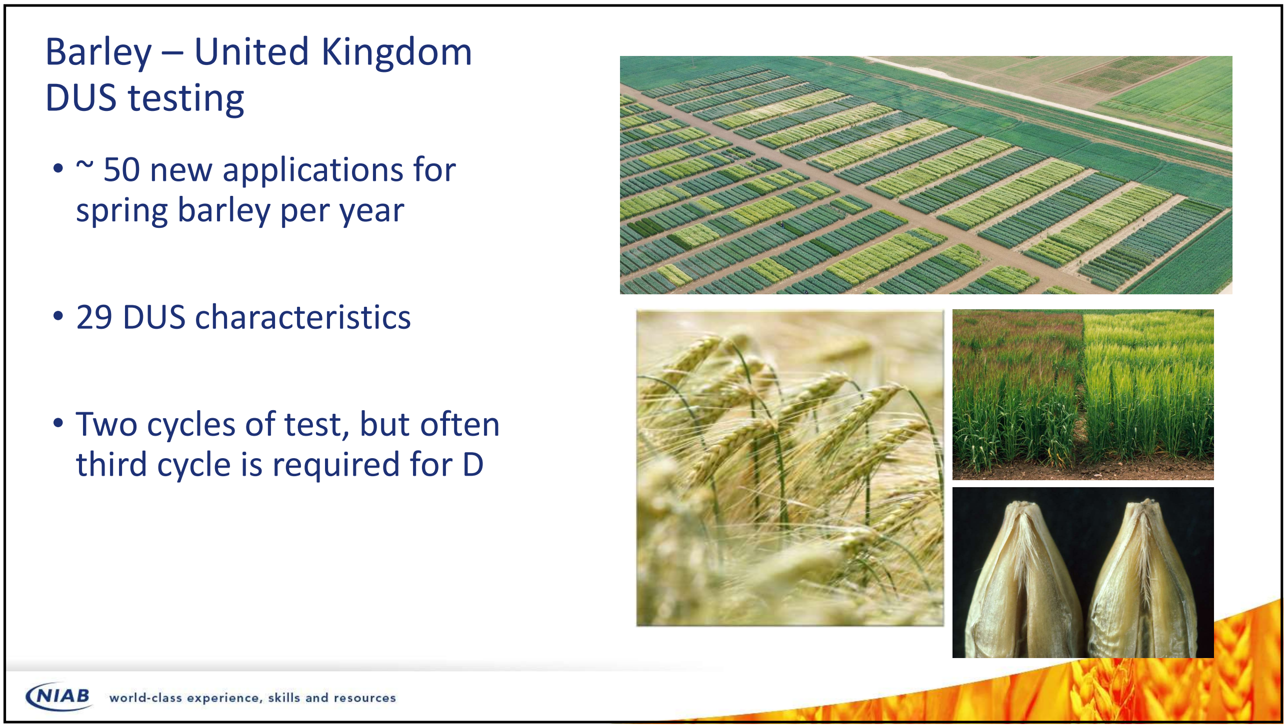
[Annex follows]



Department for Environment, Food & Rural Affairs

The use of biomolecular technology in DUS testing – a case study on barley

UPOV TWM 2025



Barley – United Kingdom DUS testing

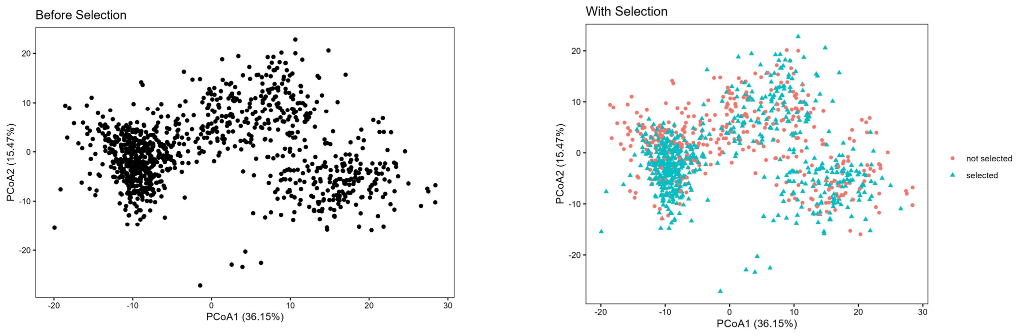
- ~ 50 new applications for spring barley per year
- 29 DUS characteristics
- Two cycles of test, but often third cycle is required for D

The use of biomolecular technology in DUS testing

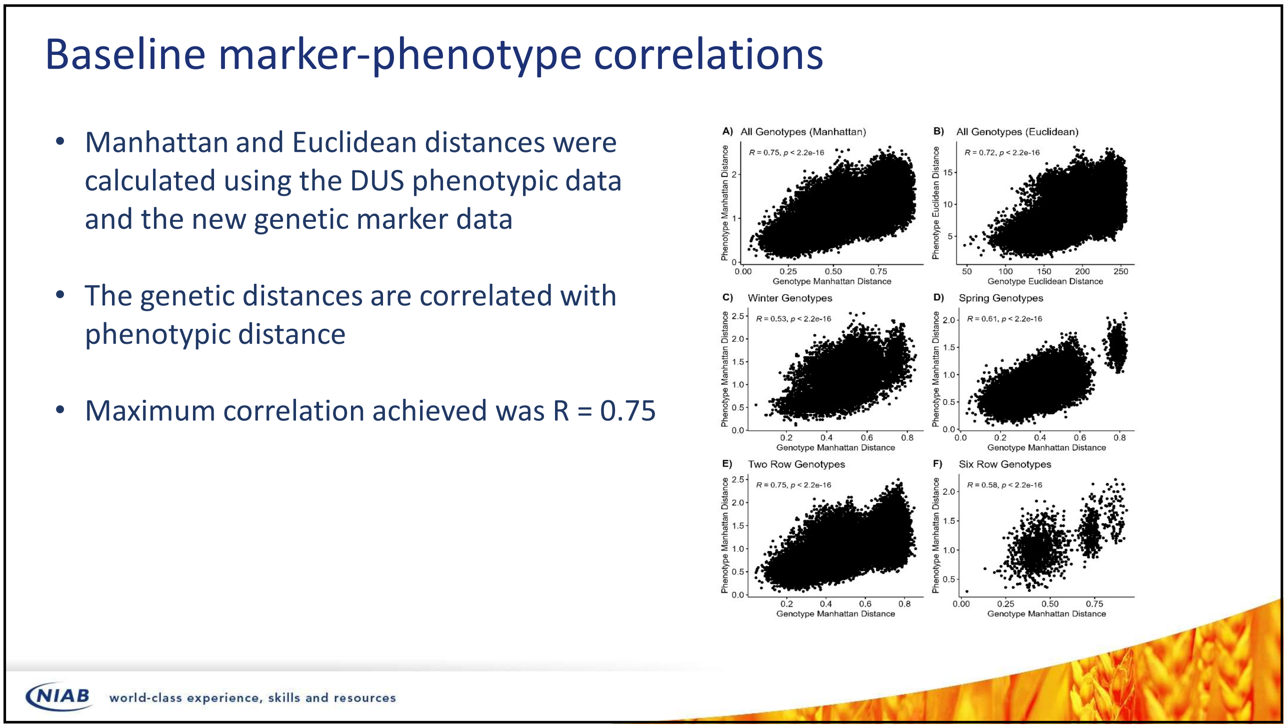
Defra funded project (24 months ending Spring 2025). The aims are to:

- Explore the use of molecular marker data to inform the selection of similar varieties for the growing trials
  - Earlier identification could remove the need for the third test year and improve trial design
- Identify and develop a smaller set of markers for varietal identity and seed stock authentications
- Develop our existing DUS database structure to enable inclusion of genotypic data and associated metadata

Genotyping

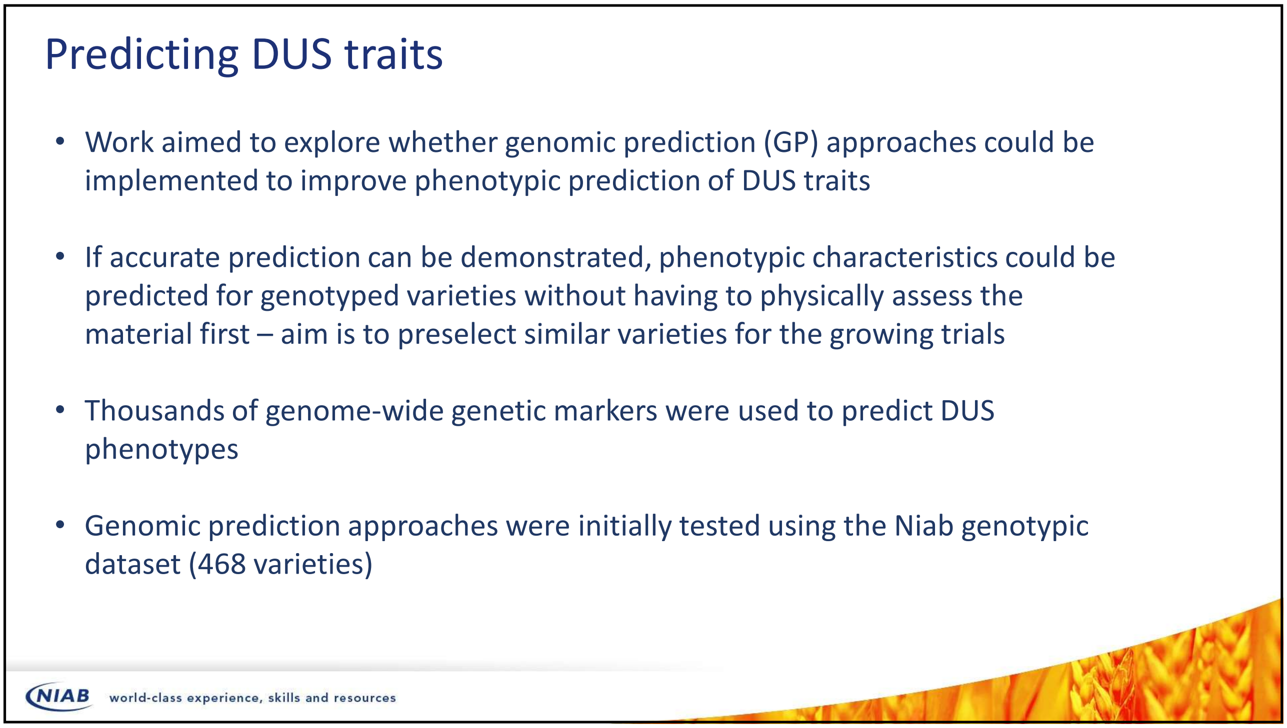


- Varieties for genotyping selected, where sufficient seed was available, to cover both spring and winter barley varieties that had undergone United Kingdom DUS testing up to 2022, alongside European reference varieties
- > 500 varieties genotyped using 50k SNP feature genotyping array
- Working with the James Hutton Institute this data was combined with previously genotyped varieties from the IMPROMALT project to obtain a final dataset of >1000 varieties



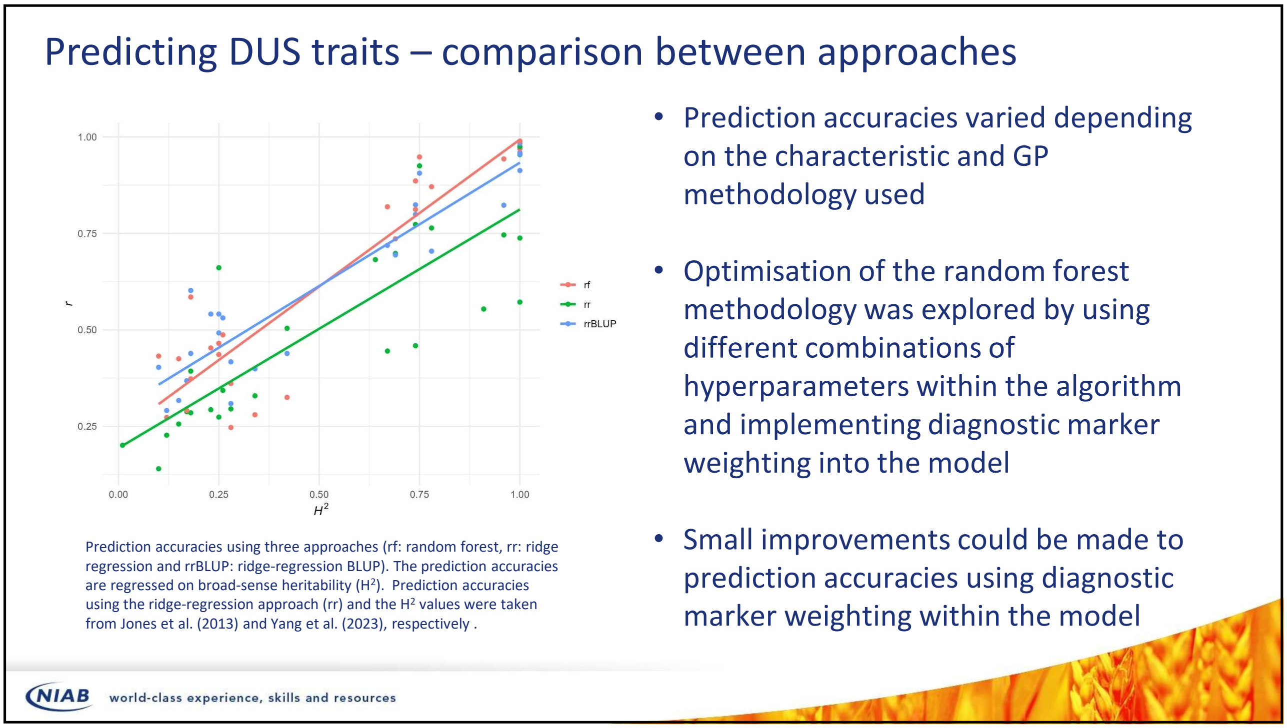
Baseline marker-phenotype correlations

- Manhattan and Euclidean distances were calculated using the DUS phenotypic data and the new genetic marker data
- The genetic distances are correlated with phenotypic distance
- Maximum correlation achieved was R = 0.75



Predicting DUS traits

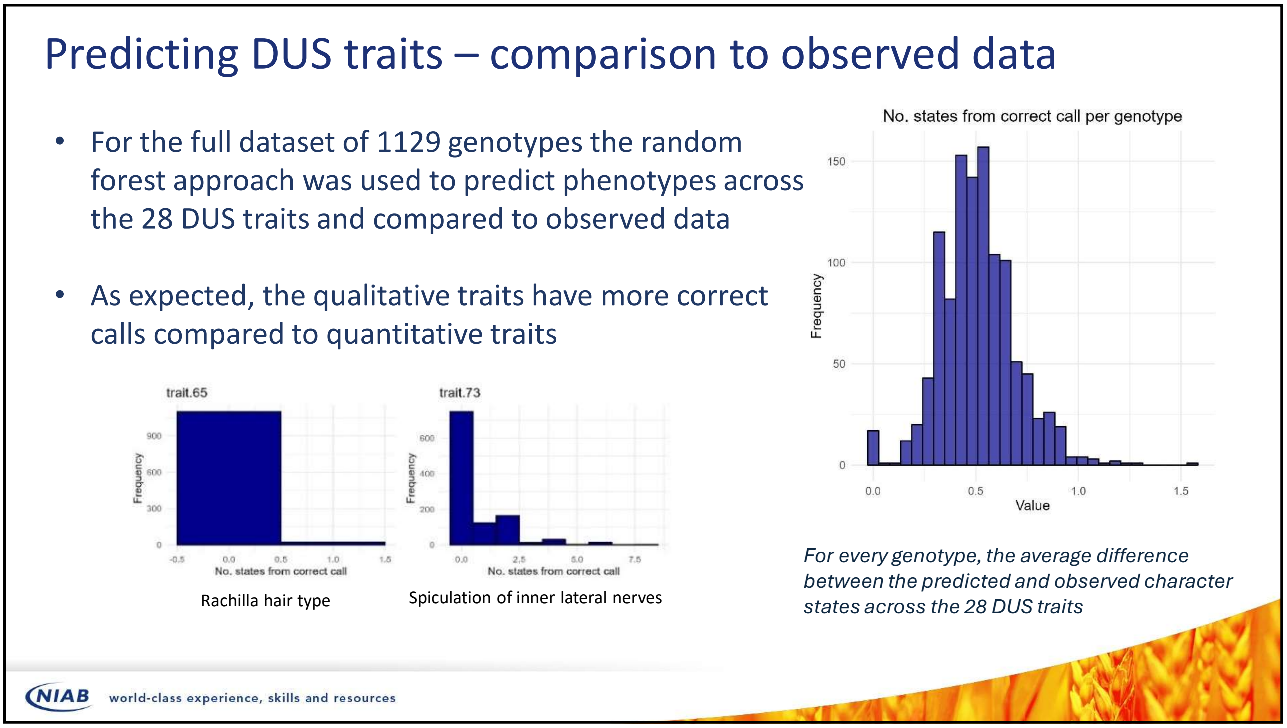
- Work aimed to explore whether genomic prediction (GP) approaches could be implemented to improve phenotypic prediction of DUS traits
- If accurate prediction can be demonstrated, phenotypic characteristics could be predicted for genotyped varieties without having to physically assess the material first – aim is to preselect similar varieties for the growing trials
- Thousands of genome-wide genetic markers were used to predict DUS phenotypes
- Genomic prediction approaches were initially tested using the Niab genotypic dataset (468 varieties)



Predicting DUS traits – comparison between approaches

Prediction accuracies using three approaches (rf: random forest, rr: ridge regression and rrBLUP: ridge-regression BLUP). The prediction accuracies are regressed on broad-sense heritability (H^2). Prediction accuracies using the ridge-regression approach (rr) and the H^2 values were taken from Jones et al. (2013) and Yang et al. (2023), respectively .

- Prediction accuracies varied depending on the characteristic and GP methodology used
- Optimisation of the random forest methodology was explored by using different combinations of hyperparameters within the algorithm and implementing diagnostic marker weighting into the model
- Small improvements could be made to prediction accuracies using diagnostic marker weighting within the model



Predicting DUS traits – comparison to observed data

- For the full dataset of 1129 genotypes the random forest approach was used to predict phenotypes across the 28 DUS traits and compared to observed data
- As expected, the qualitative traits have more correct calls compared to quantitative traits

Rachilla hair type

Spiculation of inner lateral nerves

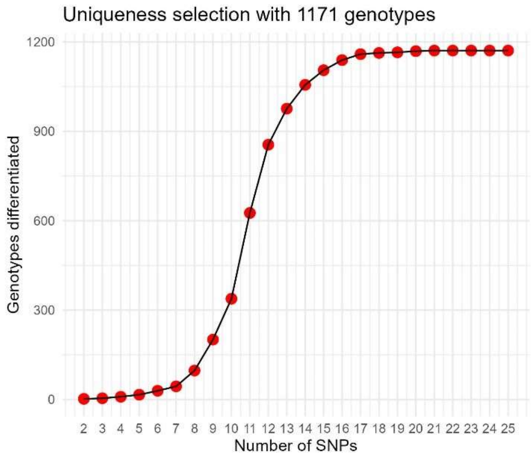
For every genotype, the average difference between the predicted and observed character states across the 28 DUS traits

Predicting DUS traits – current and future work

- Current work - optimisation of similar variety selection using predicted phenotypes and comparison to historic DUS decisions.
- Recommendation for future research to focus on implementation. For example, possible genotyping of remaining barley variety collection and assessment in parallel to current methods to explore real-world working scenario.

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
- 20 SNPs can completely differentiate between the 1171 genotypes in our collection

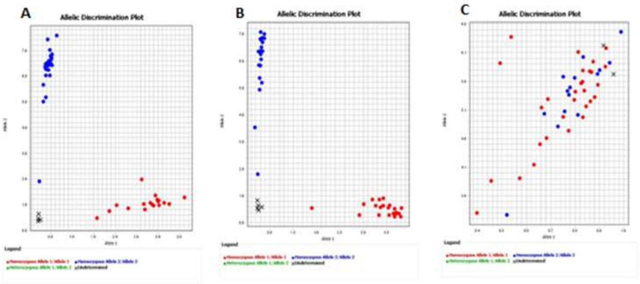


Uniqueness selection with 1171 genotypes

Each selection completed with ~ 2 million iterations of the aglorithm

Design and experimental validation of KASP markers

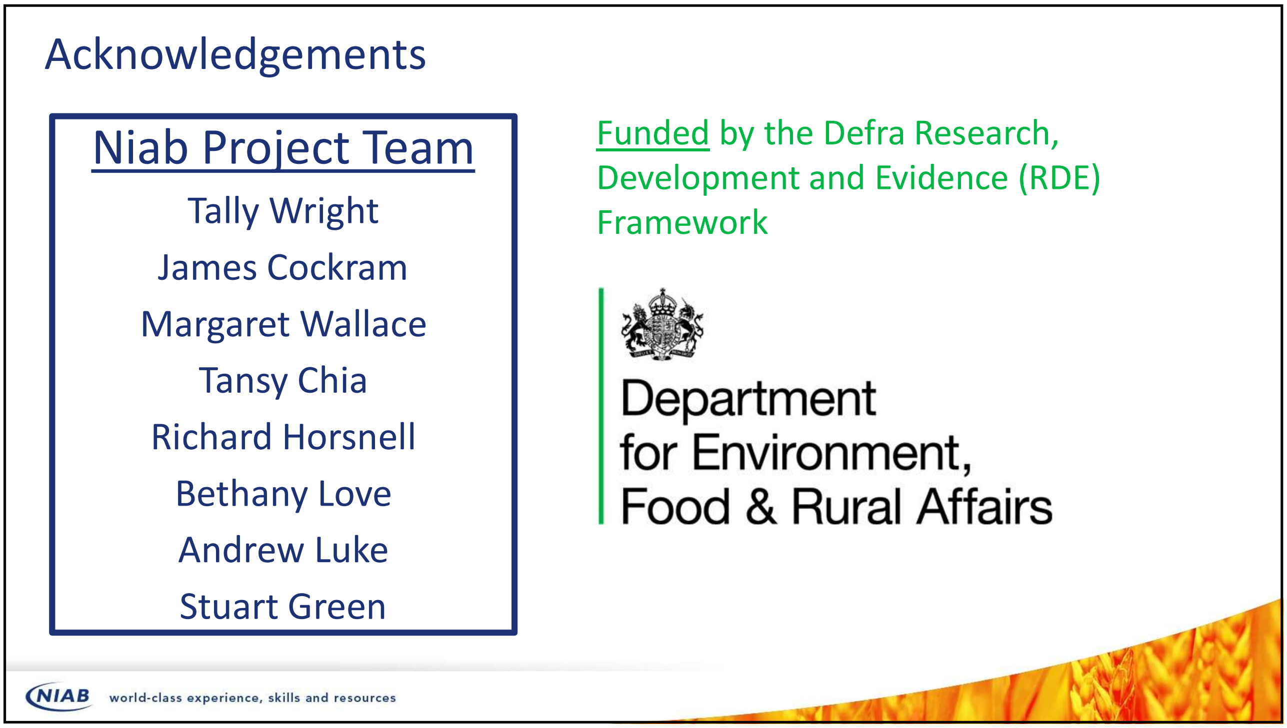
- Sequence-specific primers designed for each marker and tested against a subset of 46 barley genotypes
- Not all of the markers initially selected passed the validation testing
- Re-iterative runs of the genetic marker selection were carried out to select additional markers



- Final validated marker set = 22 markers that can completely distinguish between over 1000 varieties from the United Kingdom barley variety collection

Summary

- Project is providing proof of concept for the use of molecular markers within the United Kingdom barley DUS testing system
- Recommendation for future research is to focus on refinement and validation of the approaches developed, and real-world testing scenarios



Acknowledgements

Niab Project Team

Tally Wright
James Cockram
Margaret Wallace
Tansy Chia
Richard Horsnell
Bethany Love
Andrew Luke
Stuart Green

Funded by the Defra Research, Development and Evidence (RDE) Framework

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for Environment,
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