Using Applied Quantitative Genetics to Uncover the Potential of Diversity-Enriched Wheat

Tally Wright 04/12/2024

“…there are estimated to be close to 0.8 m accessions in wheat collections worldwide and considerably more in situ—about which relatively little is known for the most part.”

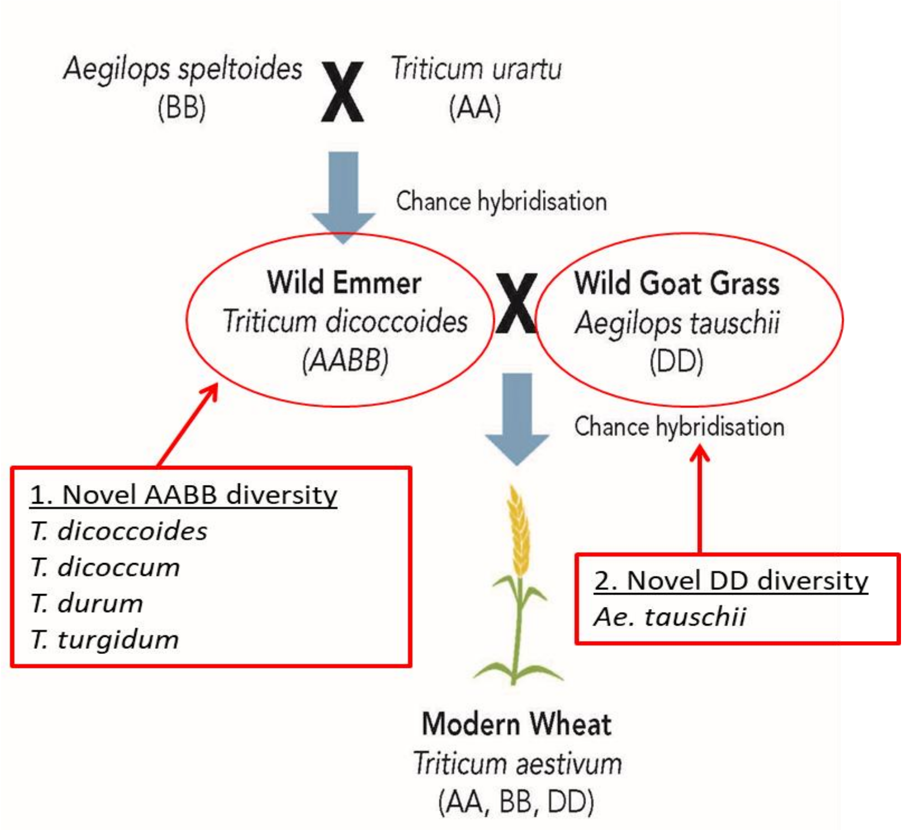
King et al. 2024

ChatGPT: “Create a picture showing wheat genetic diversity”

WISP DFW

DSW
DELIVERING
SUSTAINABLE
WHEAT

UKRI
Biotechnology and
Biological Sciences
Research Council



Aegilops speltoides (BB) X *Triticum urartu* (AA)

Chance hybridisation

Wild Emmer
Triticum dicoccoides
(AABB)

X

Wild Goat Grass
Aegilops tauschii
(DD)

Chance hybridisation

Modern Wheat
Triticum aestivum
(AA, BB, DD)

1. Novel AABB diversity
T. dicoccoides
T. dicoccum
T. durum
T. turgidum

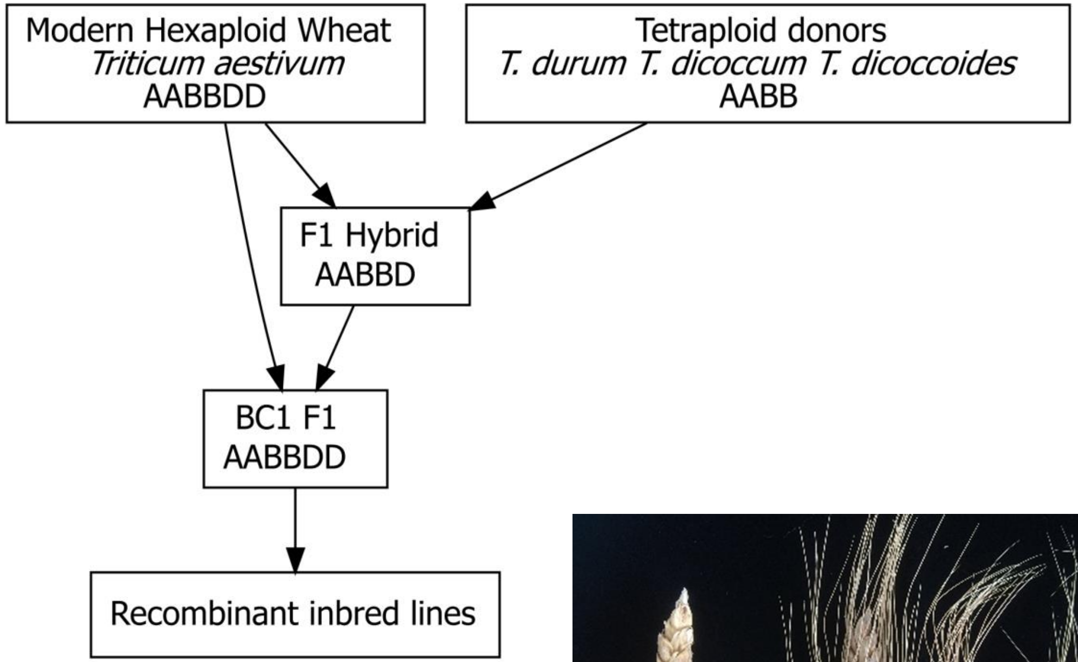
2. Novel DD diversity
Ae. tauschii

Niab has a large collection of Plant Genetic Resources:

- 130 *Triticum dicoccoides* accessions (AABB)
- 257 *Triticum dicoccum* landraces (AABB)
- 20 *Triticum durum* varieties (AABB)
- 300+ *Triticum durum* landraces (AABB)
- 200 *Aegilops tauschii* accessions (DD)
- 100 Synthetic Hexaploid Wheats

+ variety collections we use for cross-referencing e.g. WAGTAIL GWAS panel

Tetraploid diversity captured via direct crossing



Modern Hexaploid Wheat
Triticum aestivum
AABBDD

Tetraploid donors
T. durum T. dicoccum T. dicoccoides
AABB

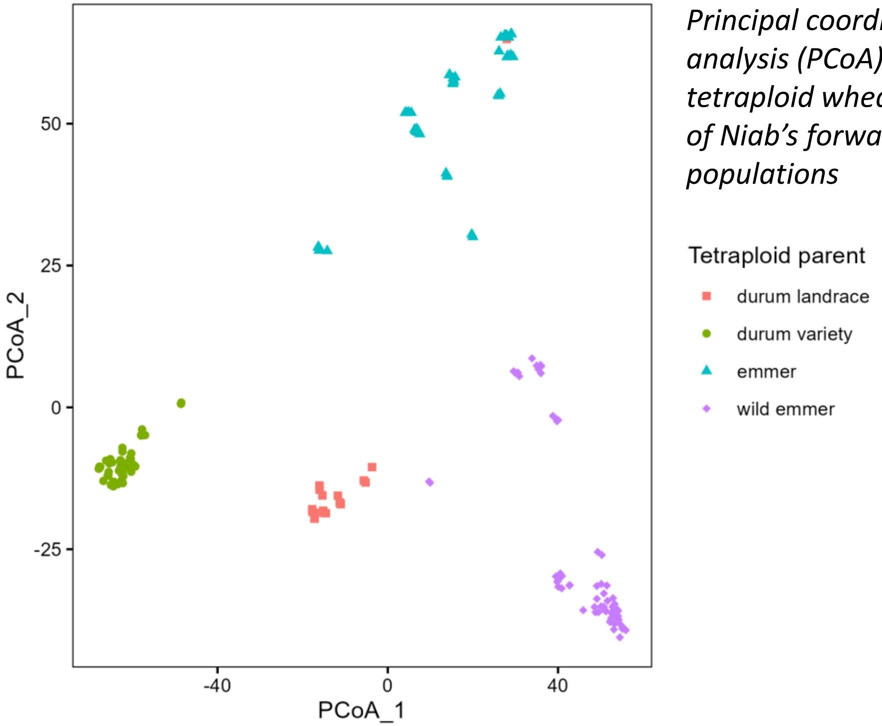
F1 Hybrid
AABBD

BC1 F1
AABBDD

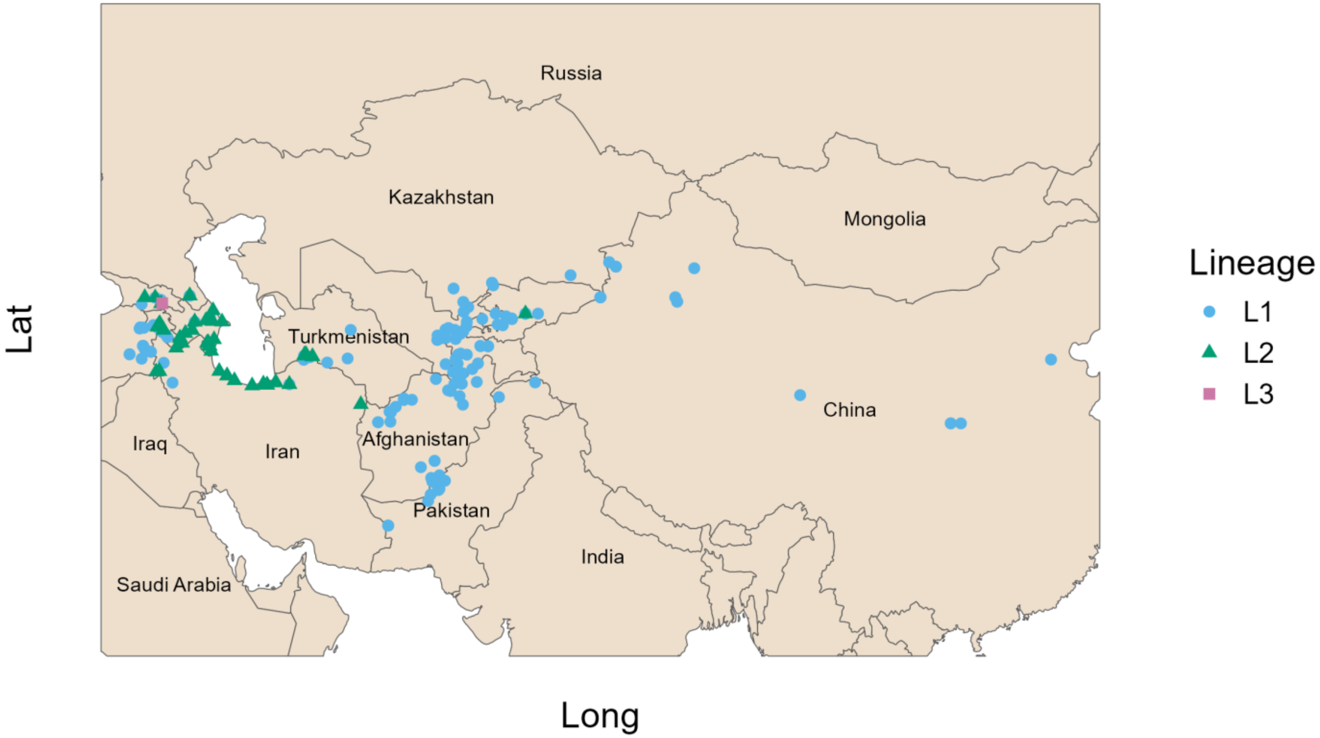
Recombinant inbred lines

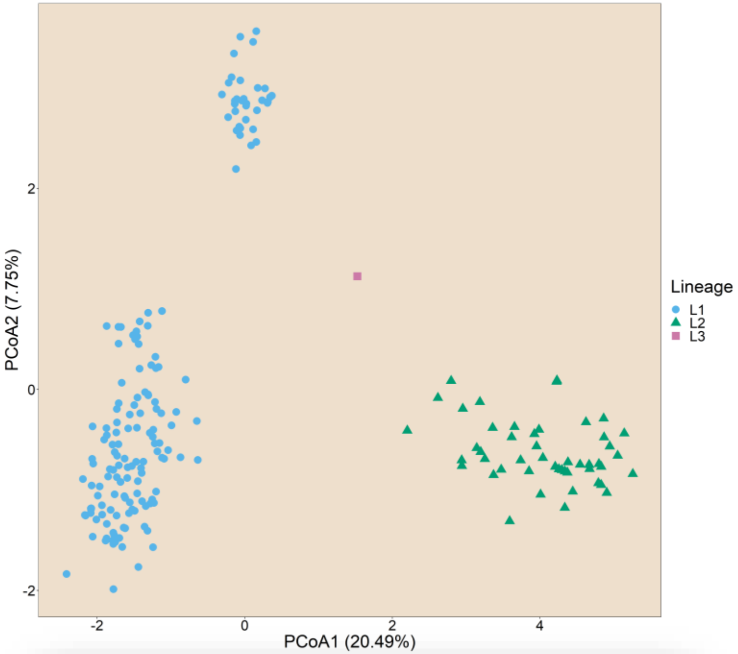
Backcross scheme to capture tetraploid diversity via direct crossing

Emmer wheat ear diversity



Principal coordinate analysis (PCoA) for tetraploid wheat donors of Niab's forward genetic populations

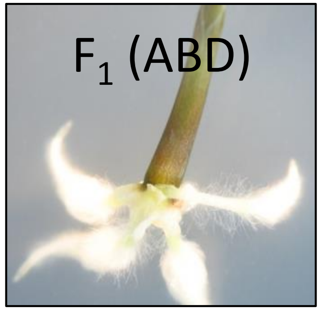




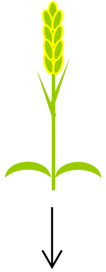
- 200 *Aegilops tauschii* (DD) covering all lineages of the species

Richard Horsnell
Dr Bethany Love
Bright Fiakeye

Durum
(AABB) **x** *Ae. tauschii*
(DD)



F_1 (ABD)



Chromosome doubling

Synthetic wheat
(AABBDD)

Process of forming new
Synthetic Hexaploid
Wheats at Niab

Magical SHW machine

@BrandeWulff
www.davidhurtado.com

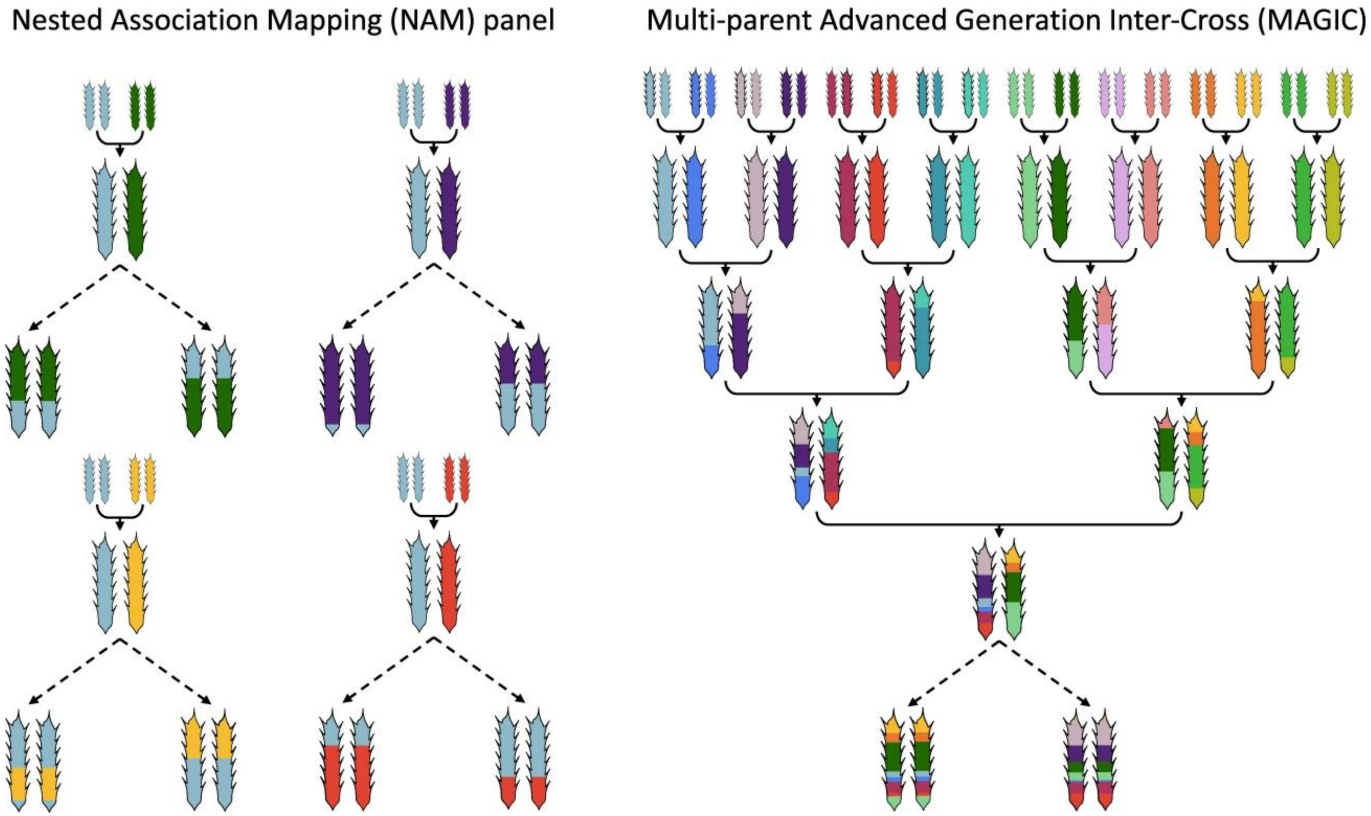
- 64 NIAB Synthetic Hexaploid Wheat
- 36 SHW sourced from NARDI Fundulea and CIMMYT.

Parental genetic resources

Diversity Donor	Genotyping	Sequencing
T. dicoccoides ***T. dicoccum*** ***T. durum***	• 60+ accessions genotyped with the Breeders' 35K Axiom SNP array	• *T. dicoccoides* = 2 with chromosome level assemblies pending via Tetraploid Pangenome + 1 with NIAB DFW 15x Illumina coverage. • *T. durum* = 1 existing reference genome, 3 chromosome level assemblies pending via TetPanG + 1 chromosome level pending via NIAB
Aegilops tauschii	• 200+ accessions genotyped with KASP marker data (~300 markers)	• 33 accessions with 10-30x Illumina sequencing (via: Gaurav et al. 2022).
Synthetic Hexaploid Wheat	• 50+ accessions genotyped with the Breeders' 35K Axiom and *Triticum aestivum* 'Next Generation' SNP array	• 2 accessions sequenced to the chromosome-level via the Wellcome Sanger Institute.
Triticum aestivum **(MAGIC Founders)**	• 8 and 16 founder accessions genotyped with the 90K Infinium iSelect SNP array and Breeders' 35K Axiom array.	• 8 accessions with chromosome-level genome sequence assemblies. • 16 accessions with exome and promotor capture sequences

Parents of each population are either genotyped or sequenced with a range of technologies.

How to capture this diversity for forward genetics?



Scott & Ladejobi et al. 2020

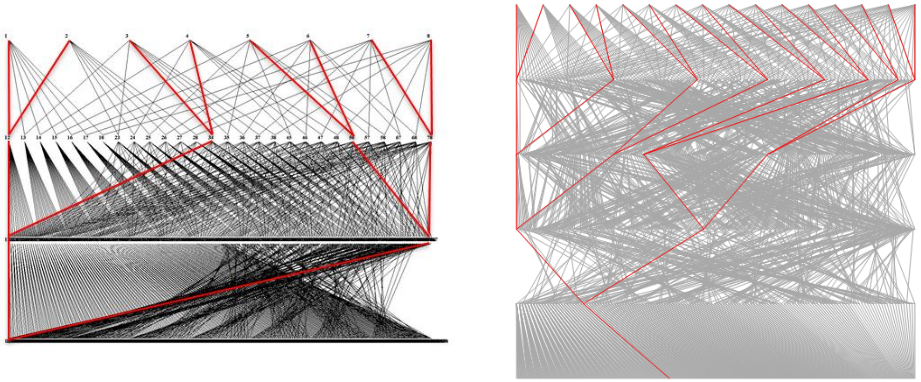
Multi-founder populations are powerful forward genetic resources:

- **High power** and **high resolution** for mapping QTL
- Captures **wide genetic diversity** through **multiple founders** (especially in NAM panels)

MAGIC

Multi-parent Advanced Generation Inter-Cross

- Capture of diversity and phenotypic variation in one population
- High recombination – greater mapping precision



MAGIC **Elite** (*Mackay et al. 2014*)

- Population genotyped with 90k SNP data
- 8 varieties selected by breeders
- 81% UK SNP polymorphism

MAGIC **Diverse** (*Scott et al. 2021*)

- Population has been skim-sequenced
- 16 varieties selected to maximize elite diversity capture
- 92% UK SNP polymorphism

Example studies from **MAGIC Elite:**

Several BBSRC projects: incl. LINK, IPA

- *Yield and yield components*
- *Photosynthesis*
- *Leaf morphology*
- *Disease traits (tan spot, STB, SNB)*

PhD/postdoc projects:

- *Zymoseptoria tritici, Parastagnospora nodorum*
- *Fusarium*
- *Yellow rust (Puccinia striiformis f.sp. tritici)*
- *Grain quality*
- *Circadian rhythm*

Internal NIAB/collaborations:

- *Flowering time, height*
- *Tiller and ear counts*
- *Various root projects*
- *Growth and development in phenomics platforms*
- *Micronutrients*
- *And more....*

New MAGIC:

MAGIC **Wilder:**

- Eight high performing BC1-derived RIL selections in a spring elite wheat background
- Founders were formed using: Synthetic Hexaploid Wheat, *T. dicoccoides*, *T. dicoccum* and *T. durum.*
- After 3 rounds of intercrossing, >1000 RILs currently at F5 generation

Name	NIAB Sel Code	Recurrent Parent	Wild Donor	Species of Donor
1710	DFW SEL 035	Paragon	NIAB SHW-008	Re-synthesised *T. aestivum*
1726	DFW SEL 036	Paragon	Tios	*T. dicoccum*
1727	DFW SEL 037	Paragon	Tios	*T. dicoccum*
1729	DFW SEL 038	Paragon	27996	*T. dicoccum*
1736	DFW SEL 041	Paragon	dic050	*T. dicoccoides*
1747	DFW SEL 045	Paragon	Karim	*T. durum*
1749	DFW SEL 046	Paragon	Karim	*T. durum*
1762	DFW SEL 048	Paragon	NIAB SHW-023	Re-synthesised *T. aestivum*

Nested Association Mapping

Nested Association Mapping panels (typically 50-60 wild donors) crossed into elite spring and winter wheat hexaploid backgrounds.

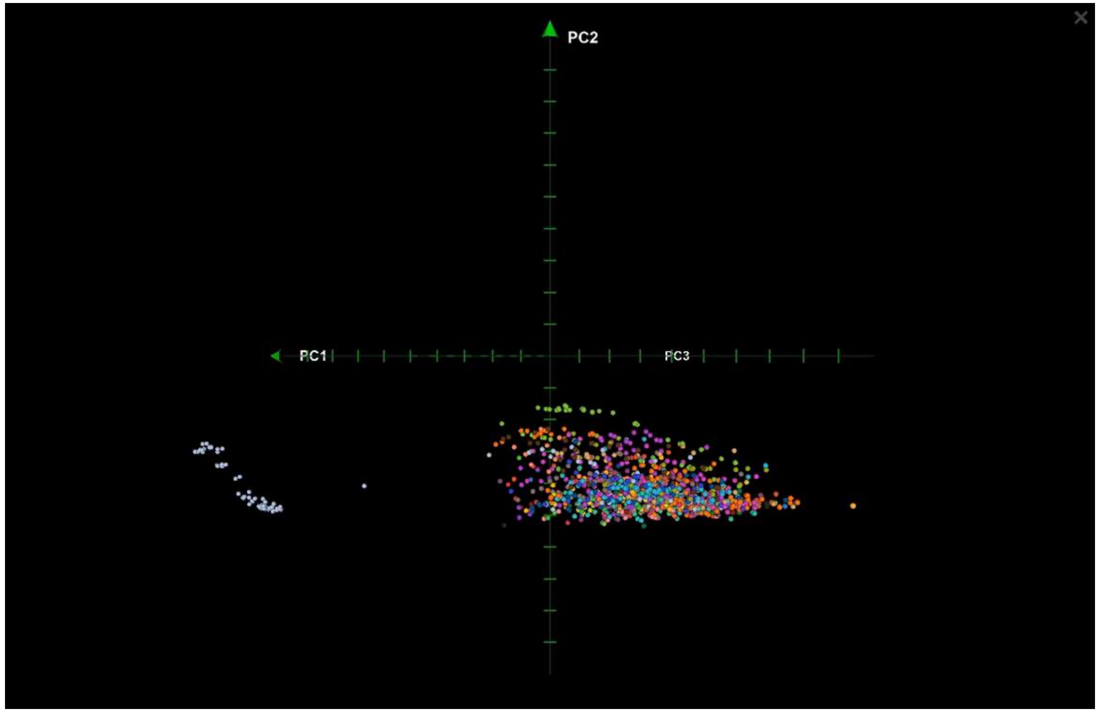
- Combines **high statistical power** of QTL mapping with **large diversity capture** through multiple donors

UK Elite Wheat (Robigus and Paragon)

x

Synthetic diversity

Tetraploid diversity



PCoA space of typical NIAB NAM population.
Created using CurlyWhirly (JHI).

NIAB diversity resources

DFW

Biotechnology and
Biological Sciences
Research Council

NIAB_WW_SHW_NAM ☆★

- 54 bi-parental populations; 3,137 RILs; 54 SHW donors; winter recurrent elite.

NIAB_SW_SHW_NAM

- 64 bi-parental populations; 4,200 RILs; 64 SHW donors; spring recurrent elite.

NIAB_SW_TET_NAM ☆

- 58 bi-parental populations; 1,784 RILs; 58 tetraploid donors; spring recurrent elite.

NIAB_WW_TET_NAM

- 21 bi-parental populations; 646 RILs; 21 tetraploid donors; winter recurrent elite.

NIAB_WISH_SHW_NAM ☆

- 24 bi-parental populations; 282 RILs; 24 SHW donors; spring/winter recurrent elite.

MAGIC WILDER

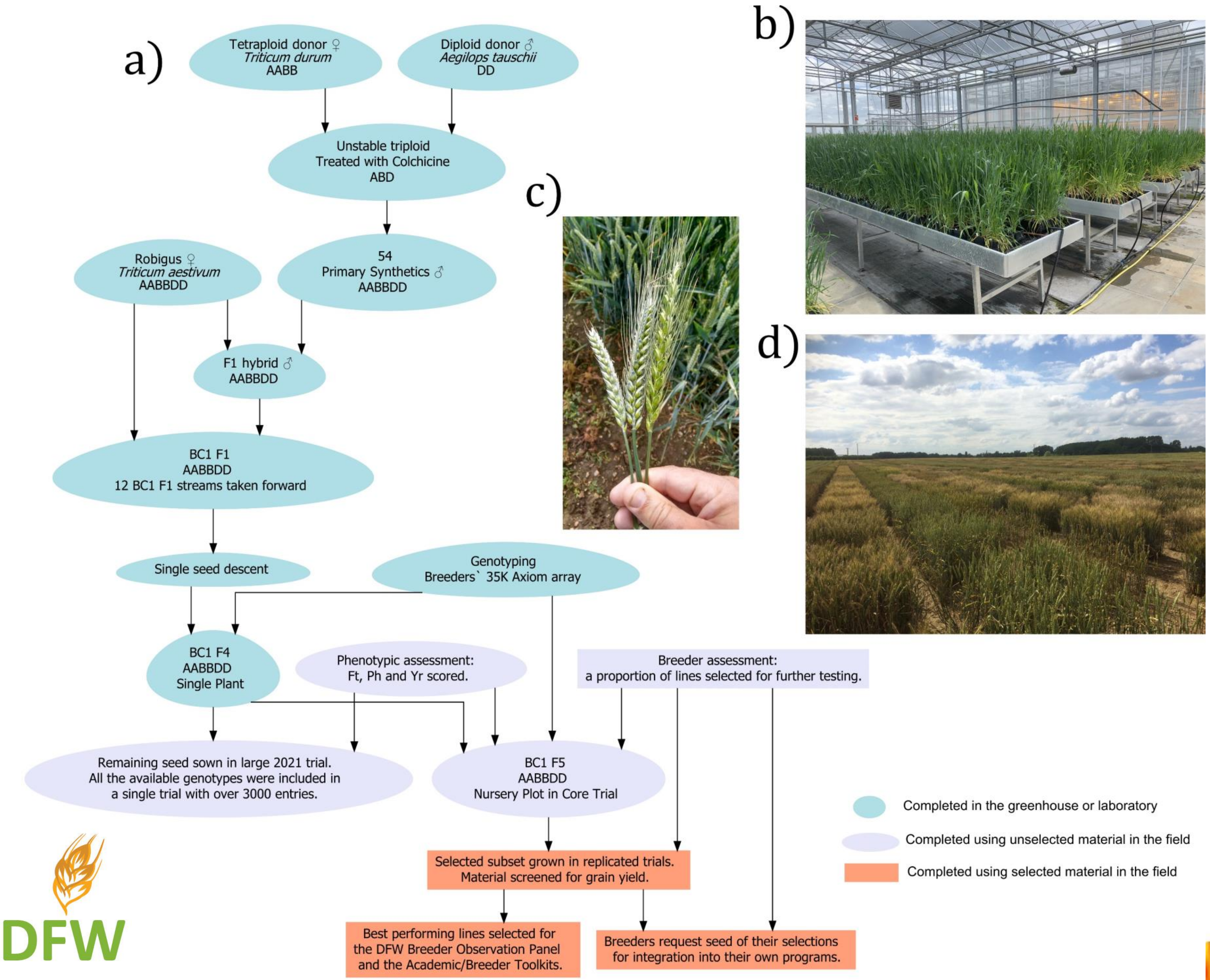
- 8 founders diversity enriched donors; 3 intercross stages; 1,000 RILs.

Chromosome segment substitution lines (CSSLs) ☆★

- 109 lines with introgressions from SHW and wild emmer spanning the Paragon genome.

☆ Genotyped on SNP array

★ Associated publication



Tetraploid donor ♀
Triticum durum
AABB

Diploid donor ♂
Aegilops tauschii
DD

Unstable triploid
Treated with Colchicine
ABD

Robigus ♀
Triticum aestivum
AABBDD

54
Primary Synthetics ♂
AABBDD

F1 hybrid ♂
AABBDD

BC1 F1
AABBDD
12 BC1 F1 streams taken forward

Single seed descent

Genotyping
Breeders` 35K Axiom array

BC1 F4
AABBDD
Single Plant

Phenotypic assessment:
Ft, Ph and Yr scored.

Breeder assessment:
a proportion of lines selected for further testing.

Remaining seed sown in large 2021 trial.
All the available genotypes were included in
a single trial with over 3000 entries.

BC1 F5
AABBDD
Nursery Plot in Core Trial

Selected subset grown in replicated trials.
Material screened for grain yield.

Best performing lines selected for
the DFW Breeder Observation Panel
and the Academic/Breeder Toolkits.

Breeders request seed of their selections
for integration into their own programs.

● Completed in the greenhouse or laboratory
● Completed using unselected material in the field
▬ Completed using selected material in the field

a). The development pipeline and workflow of how the NIAB Winter Wheat SHW NAM population was created.

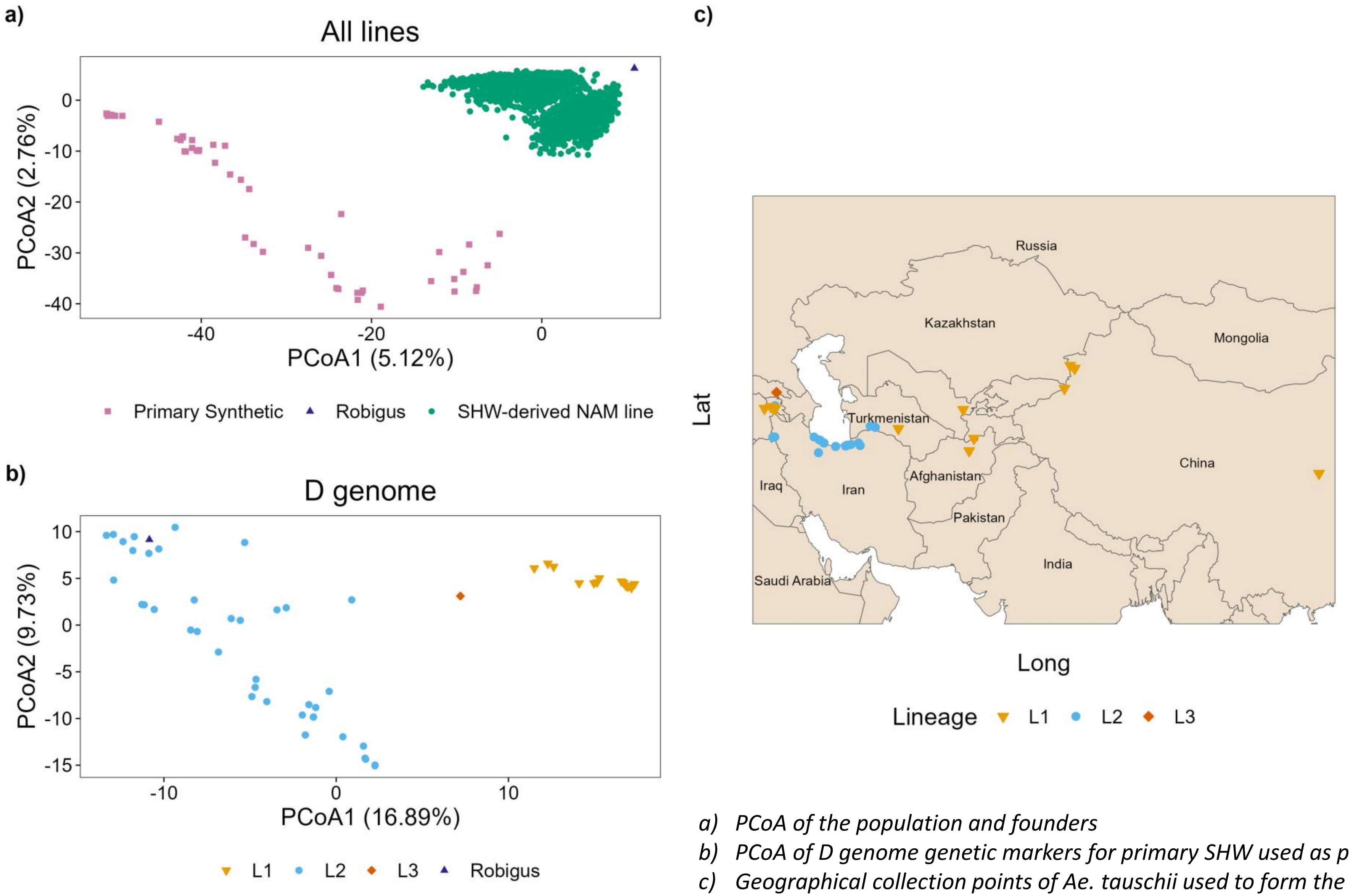
b). Generation advancement of NAM material at the BC1 F4 stage in the greenhouse.

c). A field sample of wheat ear diversity showing from left to right: a Robigus, SHW derivative and a primary SHW ear.

d). The material in a field assessment nursery from 2018.

Wright, Horsnell et al. Theor. Appl. Genet. (2024). *https://doi.org/10.1007/s00122-024-04577-1*

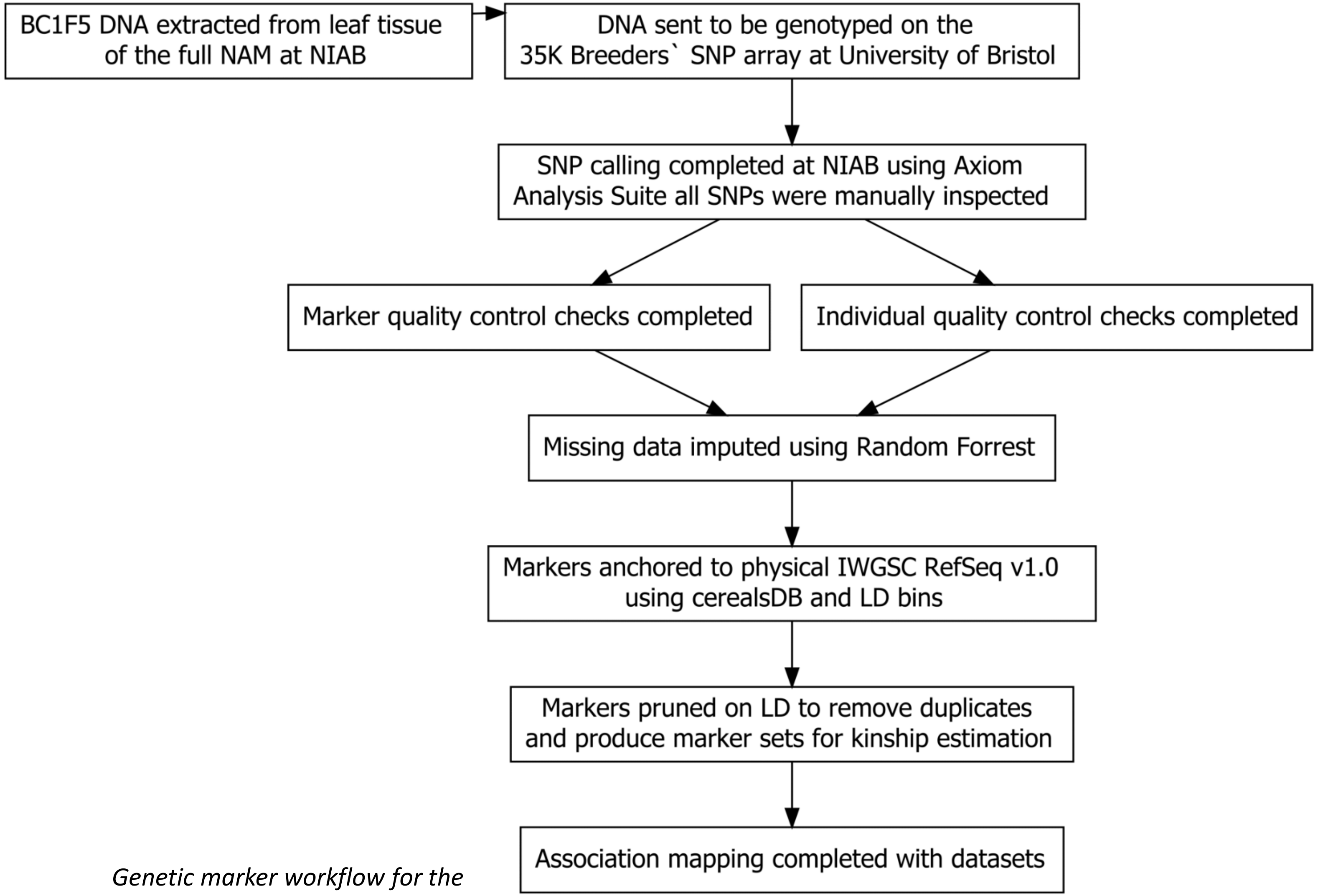
NIAB_WW_SHW_NAM



- 54 bi-parental populations; 3,137 RILs; 54 SHW donors; winter recurrent elite.
- The SHW were formed using 47 unique *Ae. tauschii* and 15 different *T. durum* donors

a) PCoA of the population and founders
b) PCoA of D genome genetic markers for primary SHW used as parents in the NIAB_WW_SHW_NAM
c) Geographical collection points of Ae. tauschii used to form the parental primary SHW

Genetic analysis workflow



BC1F5 DNA extracted from leaf tissue of the full NAM at NIAB

DNA sent to be genotyped on the 35K Breeders` SNP array at University of Bristol

SNP calling completed at NIAB using Axiom Analysis Suite all SNPs were manually inspected

Marker quality control checks completed

Individual quality control checks completed

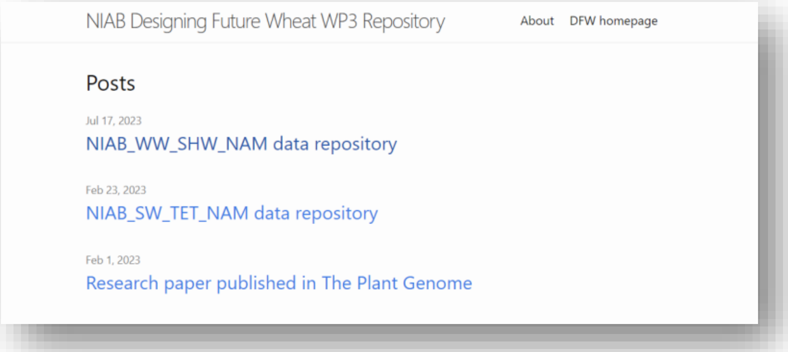
Missing data imputed using Random Forrest

Markers anchored to physical IWGSC RefSeq v1.0 using cerealsDB and LD bins

Markers pruned on LD to remove duplicates and produce marker sets for kinship estimation

Association mapping completed with datasets

Genetic marker workflow for the NIAB_WW_SHW_NAM



NIAB Designing Future Wheat WP3 Repository

Posts

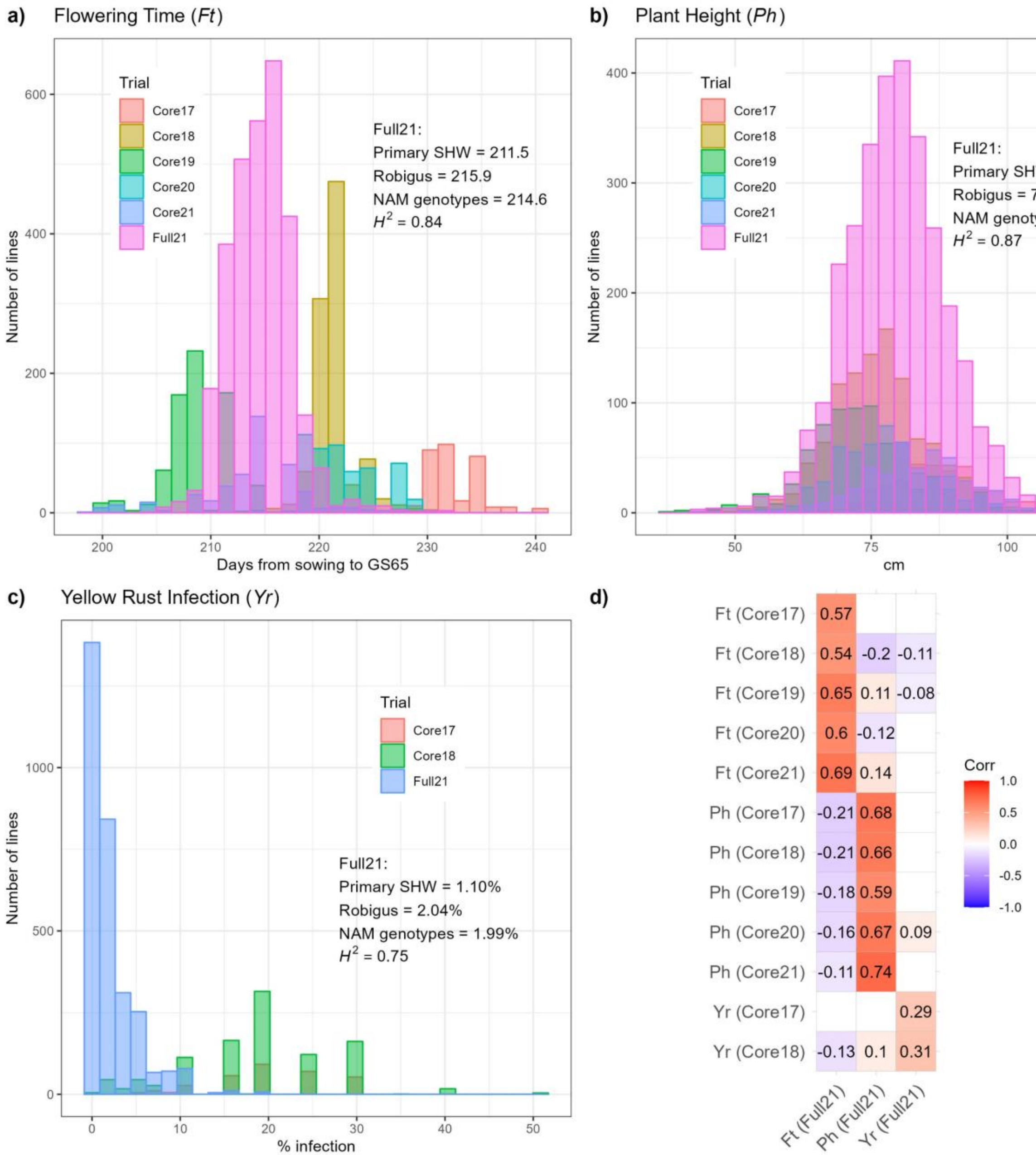
Jul 17, 2023
NIAB_WW_SHW_NAM data repository

Feb 23, 2023
NIAB_SW_TET_NAM data repository

Feb 1, 2023
Research paper published in The Plant Genome

GitHub

https://niab.github.io/niab-dfw-wp3/

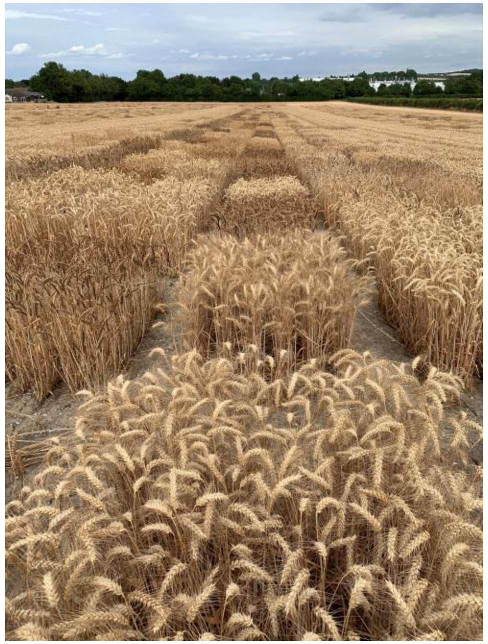


5 x Core Nurseries across five years (with subsets of the NAM)

1 x ‘Mega’ NAM Nursery with the whole population

Primary SHW parents were **earlier flowering**, **taller** but showed **better yellow rust resistance**.

Flowering time (a), plant height (b) and yellow rust infection (c) across all the NIAB_WW_SHW_NAM trials. Correlations between these traits are shown in figure d).



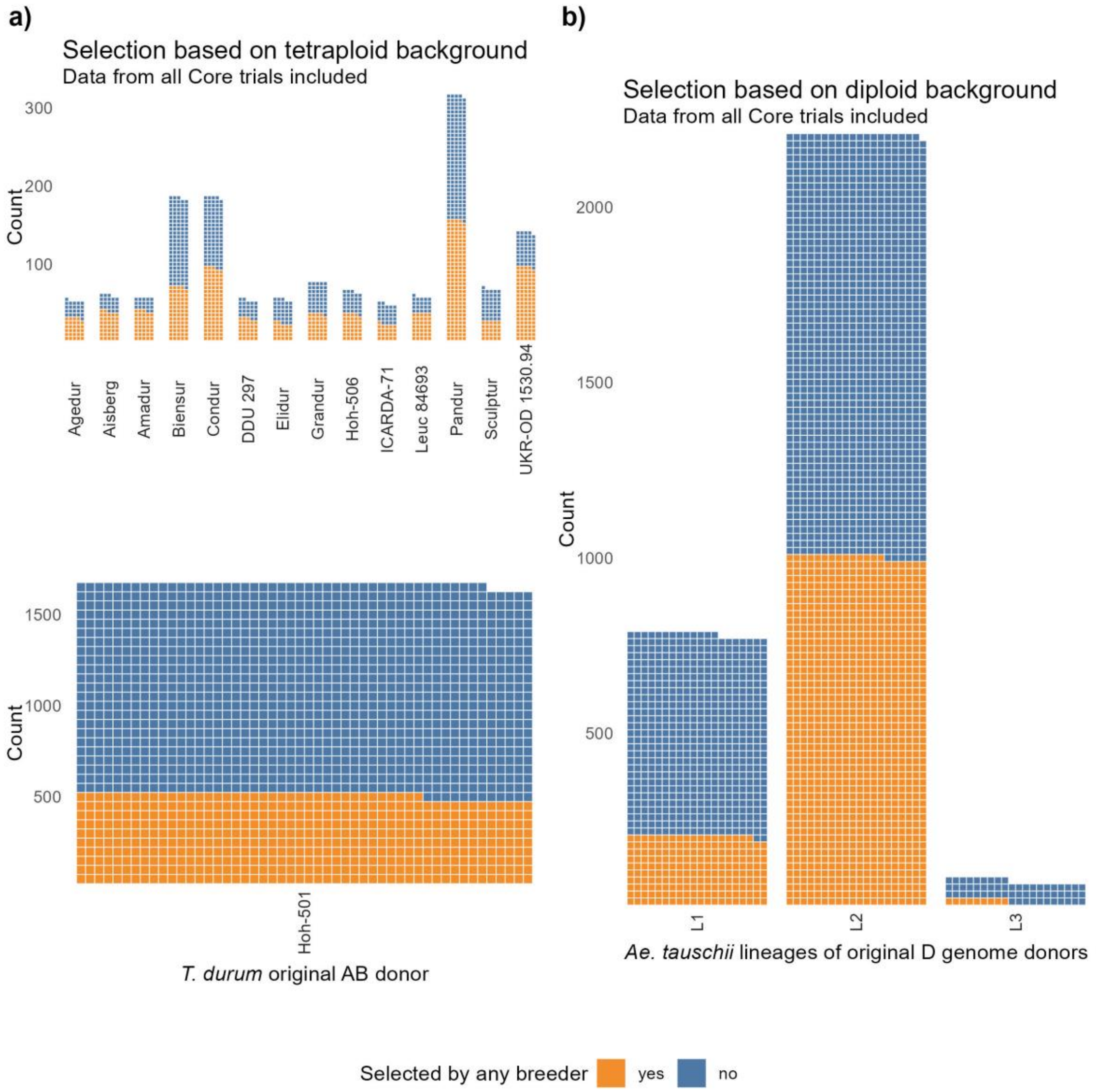
Aerial image from NIAB Data Science Department

Wheat breeders visited the Core trials each year and made selections for future testing. The companies included:

- DSV
- Elsoms
- KWS
- Limagrain
- RAGT
- Syngenta
- NIAB (pre-breeders)

SHW-derivatives from this population have been taken by breeders to **commercial programmes**. DSV UK took one SHW-derived line, called ‘Gandalf’, to National Listing.

Matt Kerton, DSV UK, personal communication.



Breeders' selection of NIAB_WW_SHW_NAM material during the DFW project.

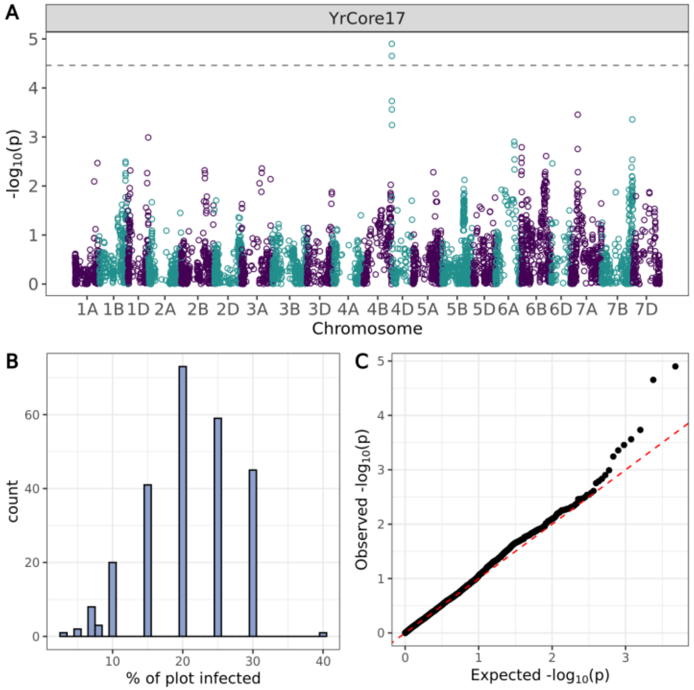
Certain **tetraploid and diploid backgrounds** appeared to be **favoured by breeders in selection**.

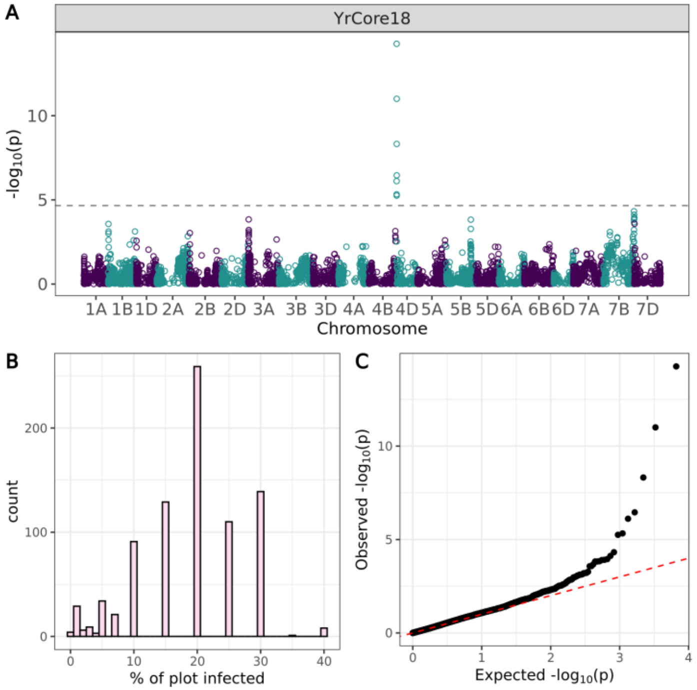
Hoh-501 was the most frequent used durum parent but others had a **higher selection proportion**.

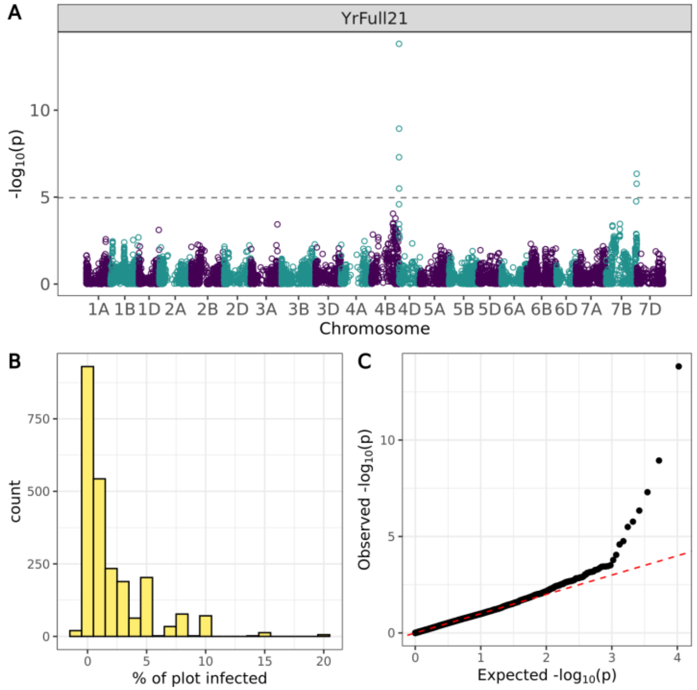
Lineage 2 *Ae. tauschii* had a higher selected proportion than lineage 1.

Genetic Mapping

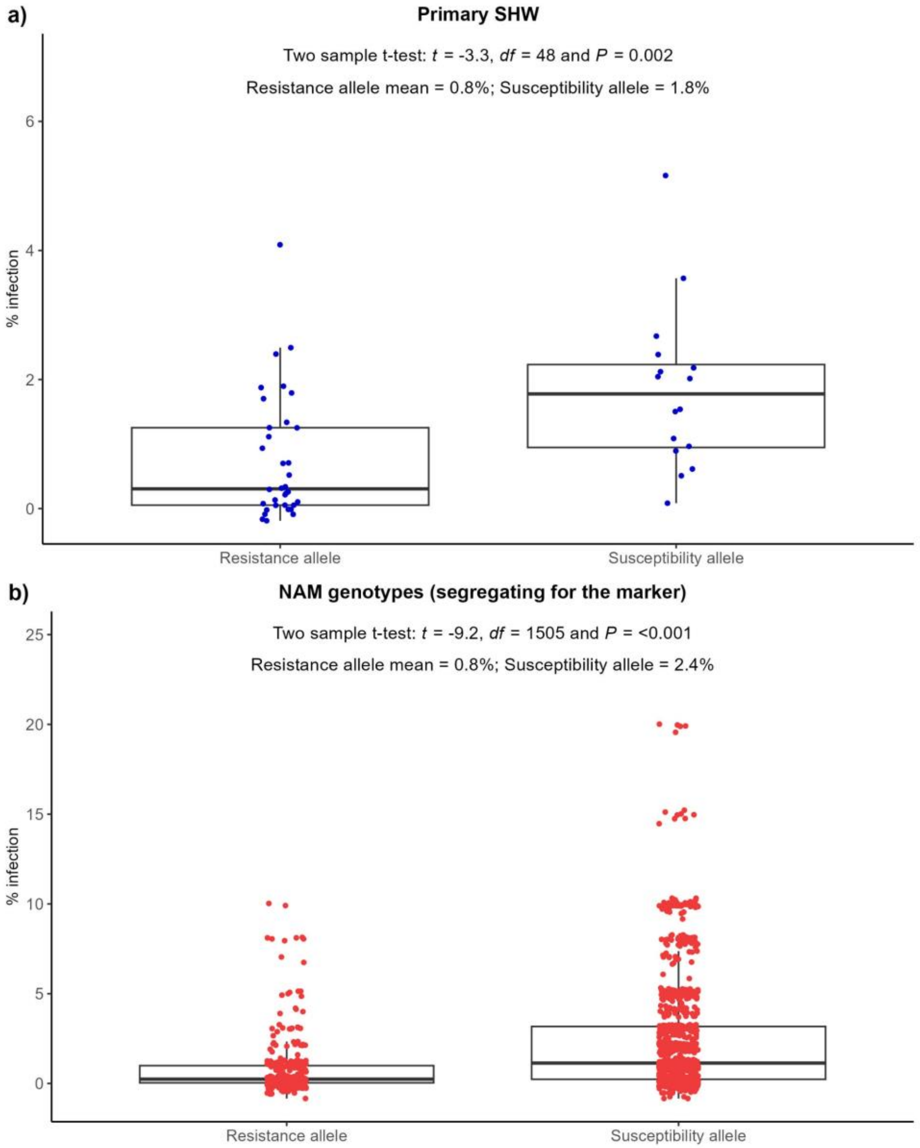
For Ft, *Ph* and *Yr* across **6 different trials** there were **26 QTL mapped**, with consistent QTL across year for each trait.







- Peak hits BLAST to 1.4 Mb on 4D. ***Yr28*** first identified on 4DS (Singh et al. 2000) and is around 1.8 Mb in Chinese Spring (Zhang et al. 2019).
- Not very common in many landraces or cultivars. No evidence of gene success in UK wheat background.

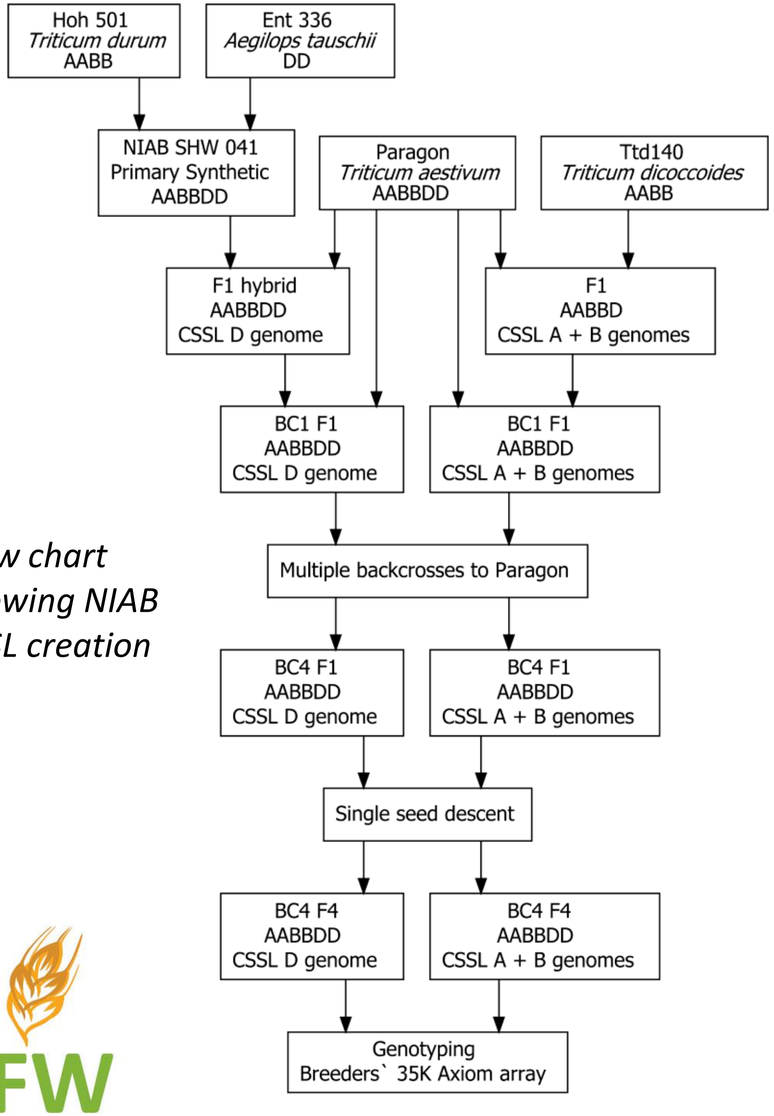


Yellow rust infection in fungicide treated 'Mega' NAM nursery for primary SHW (a) and NAM genotypes (b).

- The allele associated with 4DS *Yr28* resistance was present in 37 out of 54 of the SHW.

- Breeder selected genotypes from the Core nurseries were enriched with the SHW *Yr28* allele linked to improved resistance.

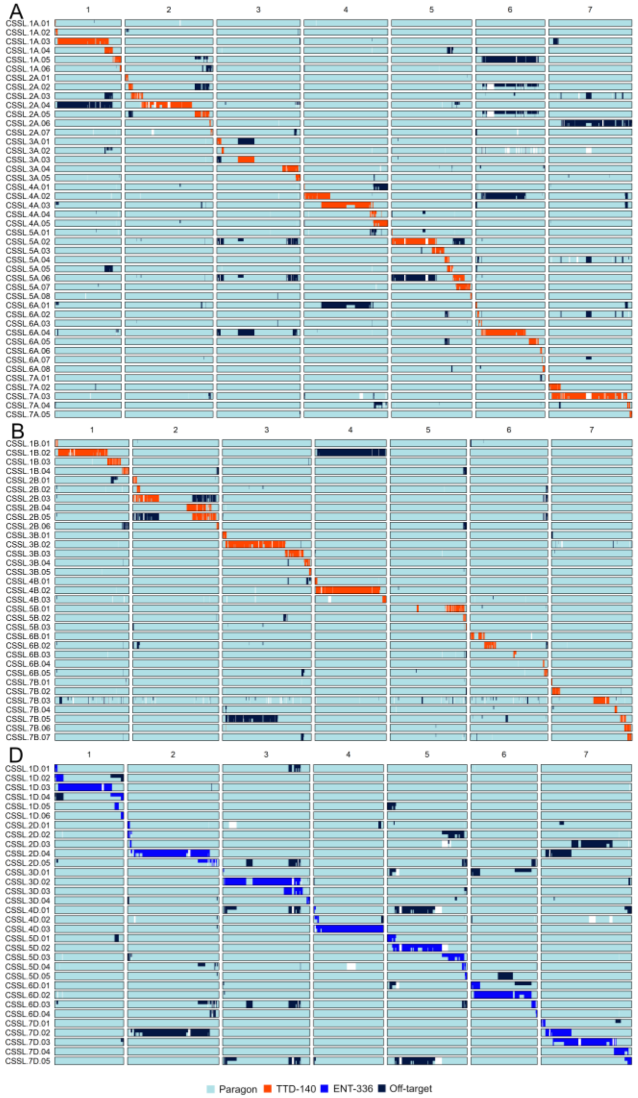
Chromosome Segment Substitution Line (CSSL)



Flow chart showing NIAB CSSL creation

AB-CSSL: 77 lines that contain introgressions from *T. dicoccoides* across the A and B genome of Paragon (spring wheat).

D-CSSL: 32 lines that contain introgressions from *Ae. tauschii* across the D genome of Paragon (spring wheat).



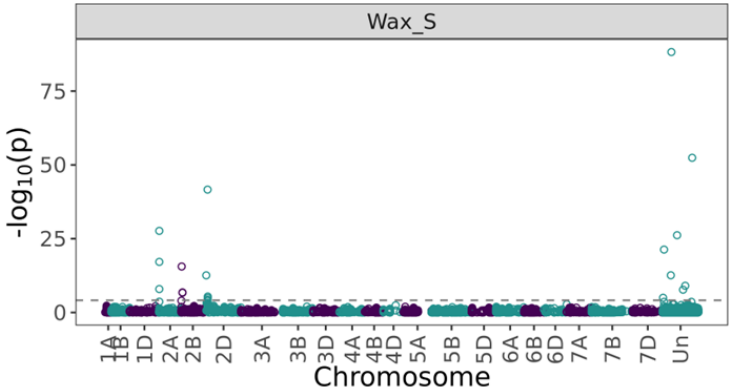
Horsnell, Leigh, Wright et al. (2023), The Plant Genome
https://doi.org/10.1002/tpg2.20288

QTL Discovery with NAM and CSSL

1. Trait assessment

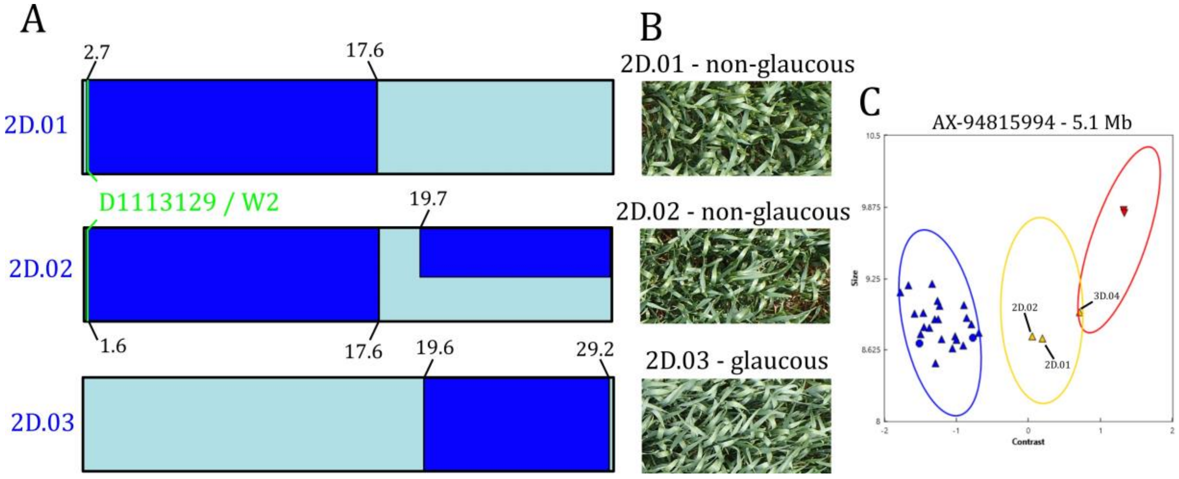
Field assessment of targeted trait responses.

2. QTL mapping



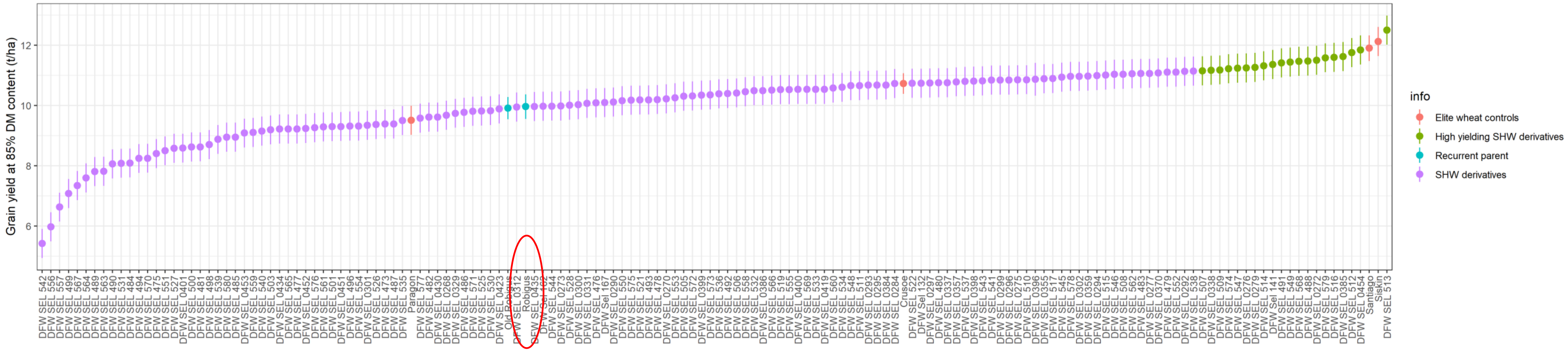
Using NAM genomic resources, complete QTL mapping to explore trait responses and identify beneficial alleles from wild backgrounds.

3. Search introgression libraries: CSSLs



Horsnell et al. 2023, Plant Genome

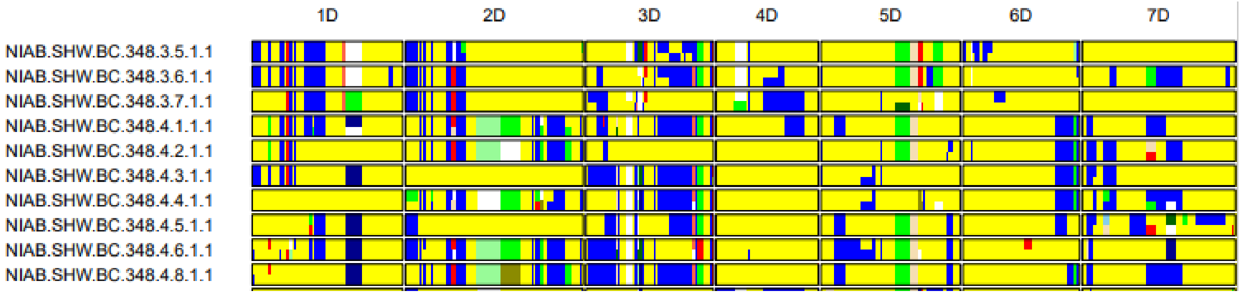
Search for wild introgressions that contain candidate QTL and use CSSL as a fine mapping resource.



Data source: Rothamsted 2022 Academic Toolkit (NIAB material). Estimated means and standard errors (BLUEs).

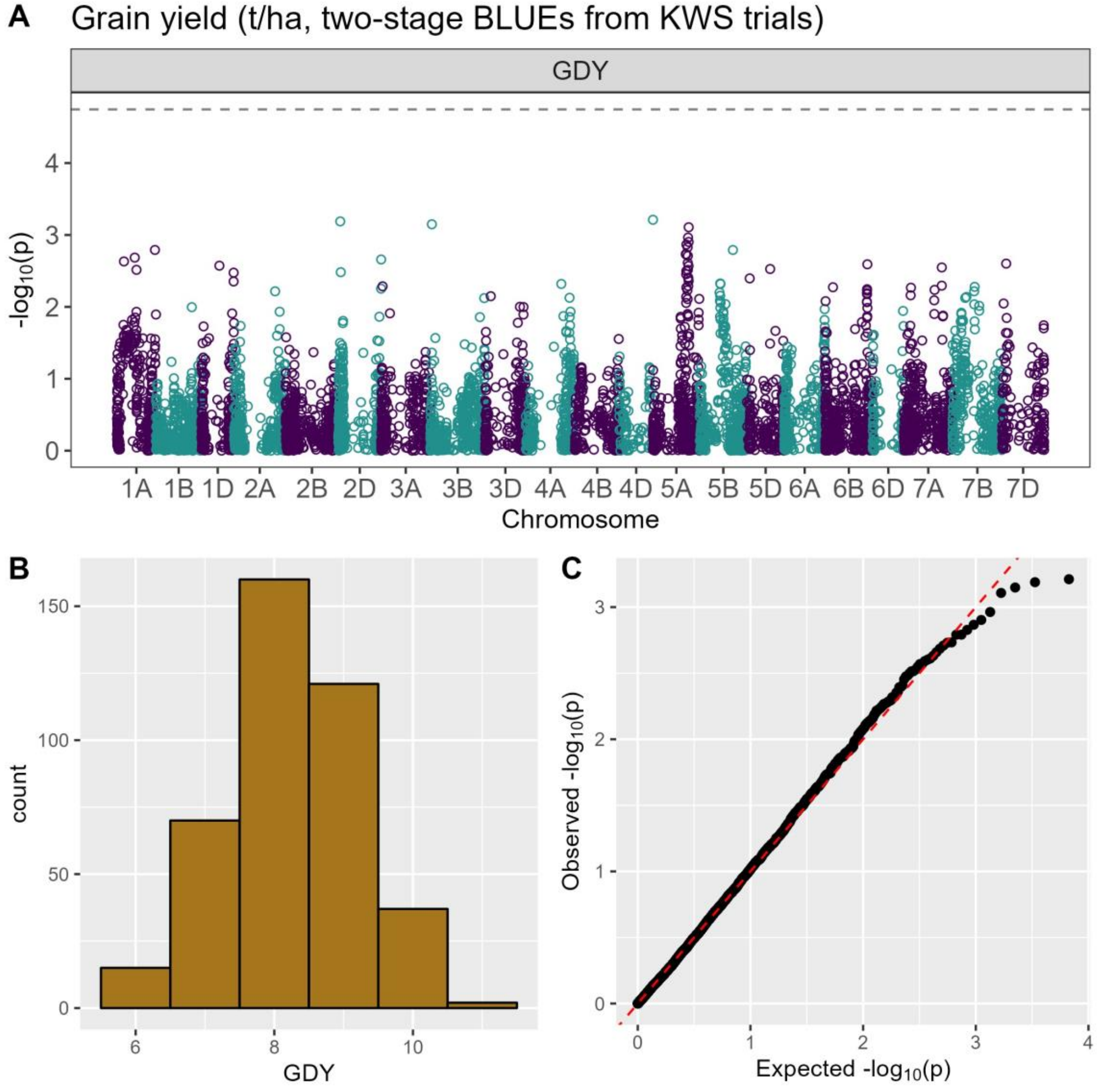
SHW-derivatives combine competitive grain yields and high genetic diversity.

Making them desirable candidates for use as PGRs in pre-breeding programs.



D-genome introgressions in SHW-derivatives

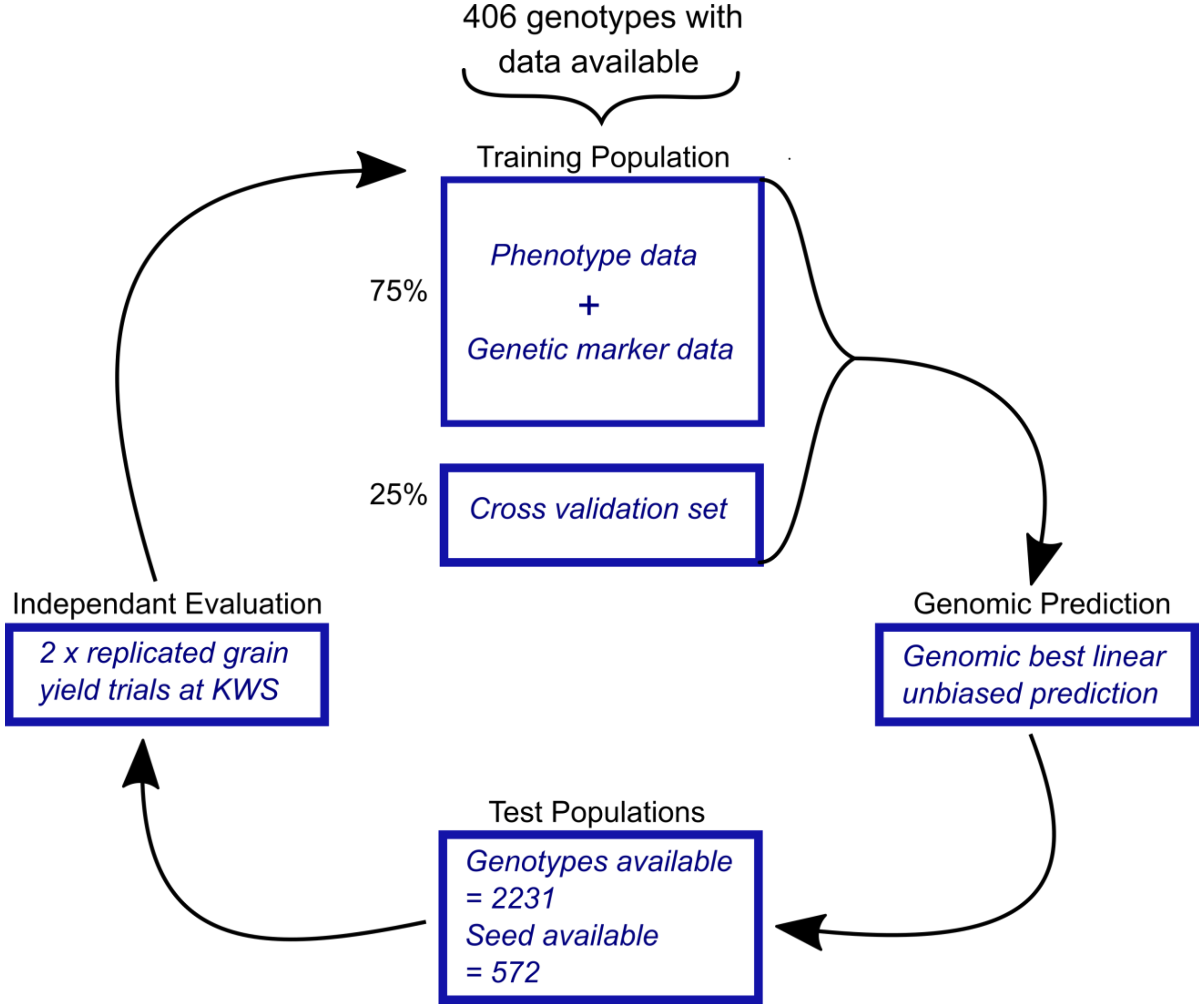
Dr. Carus John-Bejai



GWAS for grain yield in selected SHW-derivatives

- Selected genotypes grown at KWS over four consecutive years of yield trials.
- There were 406 lines with phenotype and genotype data.
- No standout QTL for grain yield.

Genomic Selection



406 genotypes with data available

Training Population

75%

Phenotype data
+
Genetic marker data

25%

Cross validation set

Genomic Prediction

Genomic best linear unbiased prediction

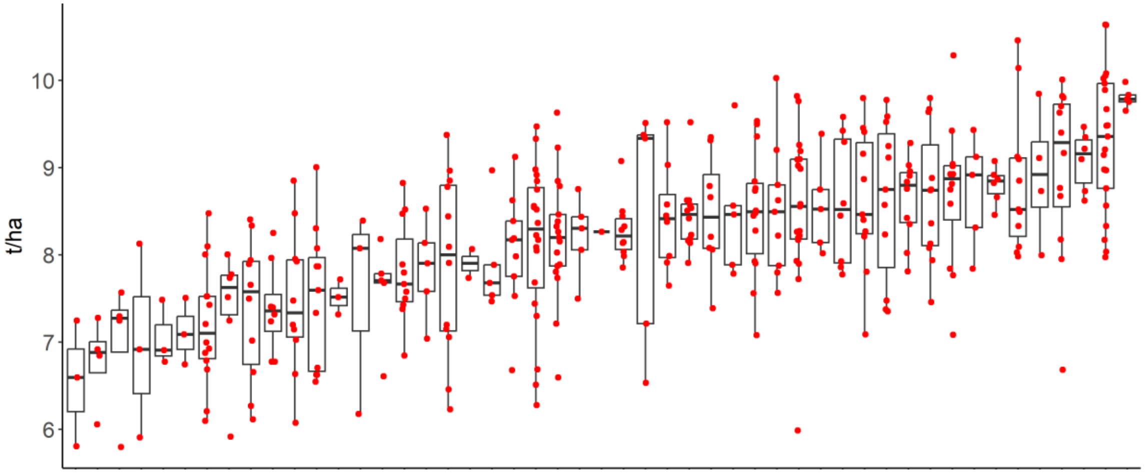
Test Populations

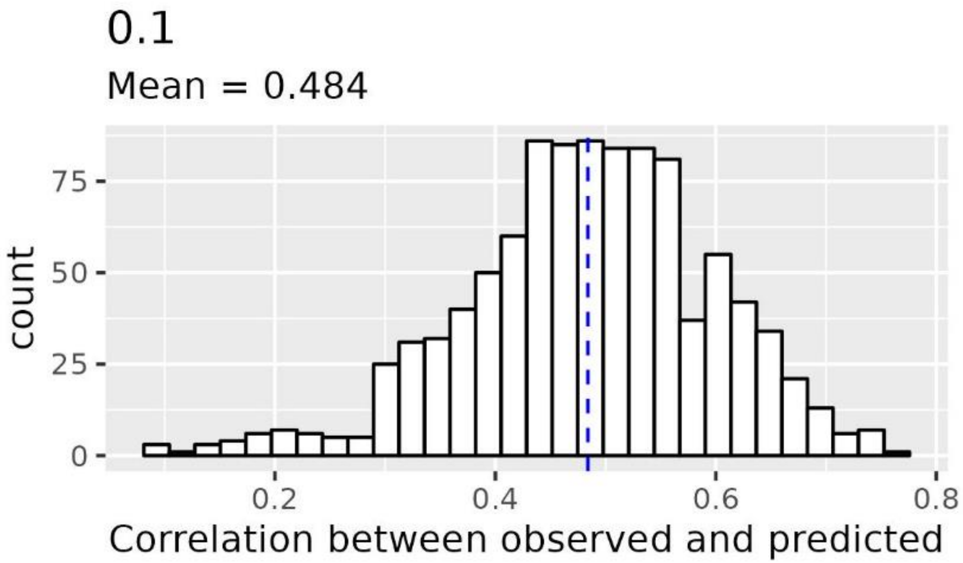
Genotypes available = 2231
Seed available = 572

Independant Evaluation

2 x replicated grain yield trials at KWS

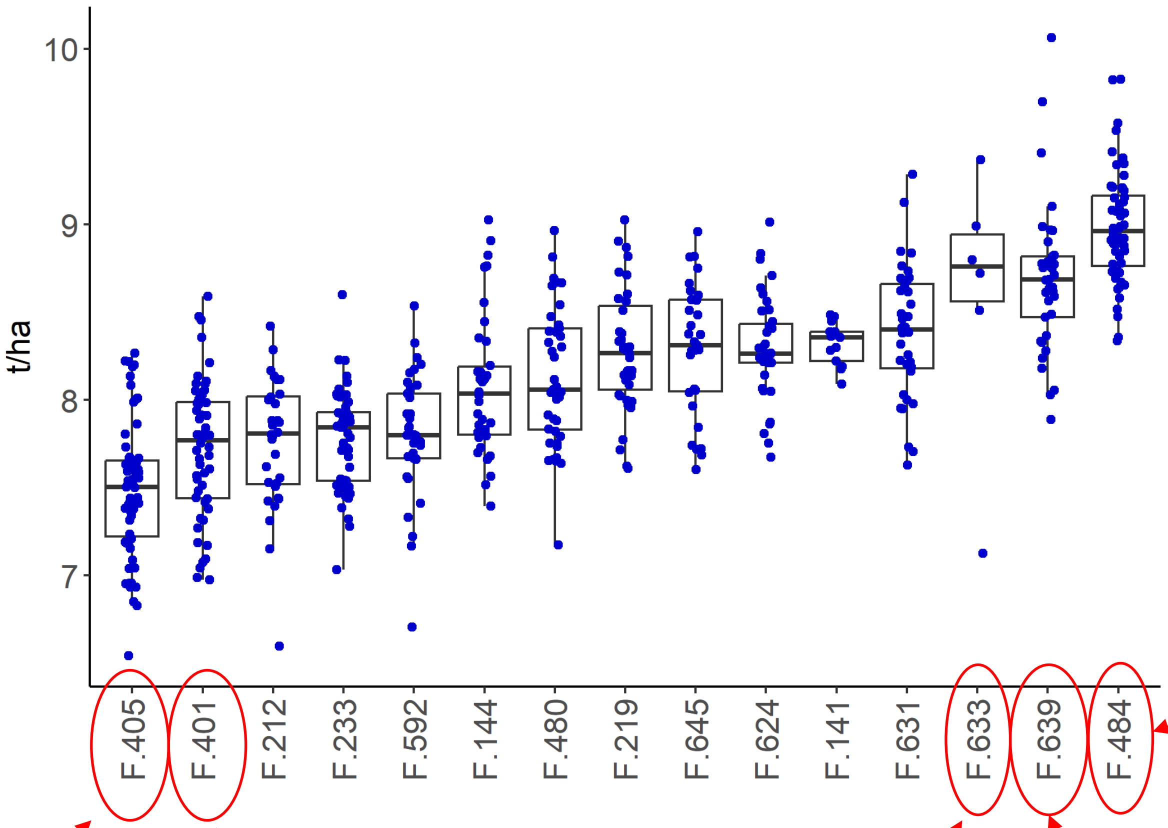
Genomic selection workflow. Ongoing collaboration between NIAB and KWS.





- Observed grain yield for each nested population in the training population
- Some nested populations are better represented than others

- Average prediction accuracy of 0.48 for grain yield
- Prediction accuracy dependent on representation in training population?



Predicted grain yield per nested-population.

L2 donor, very high selection from breeders, 68% of genotype with this tetraploid background were selected.

L3 donor, very low selection from breeders. Hoh-501 background.

L2 donor, Hoh-501 background

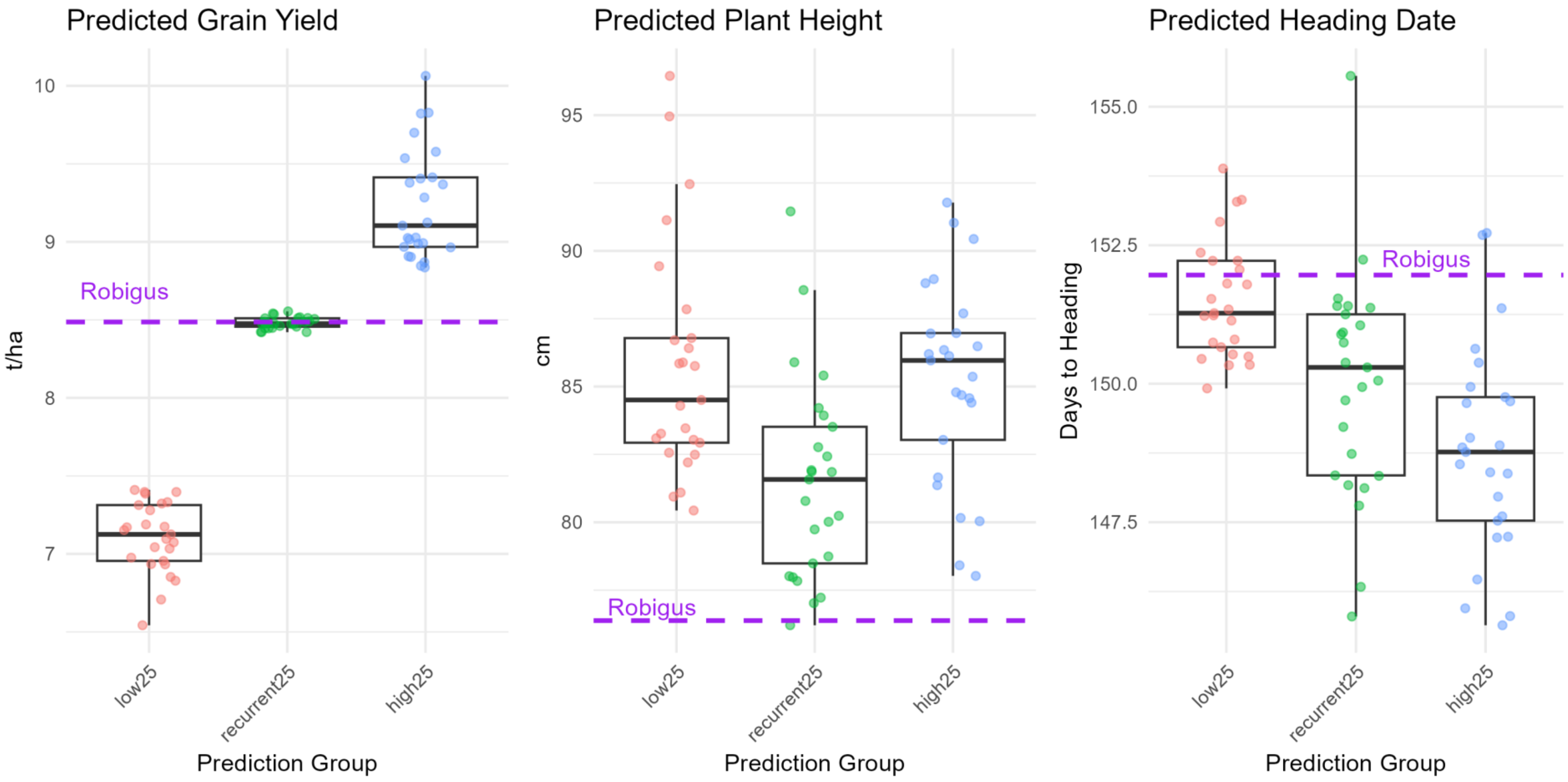
L2 donor, most selected tetraploid background (69% selected)

L1 donor, a few lines selected by every breeder.

Independent evaluations

Images from independent evaluation trials at KWS this summer.

- Independent evaluation of predicted yield at two locations.
- Testing the SHW-derivatives with the predicted highest, lowest and most similar grain yield to the recurrent parent ‘Robigus’.
- 25 genotypes in each prediction group.



Predicted grain yield, plant height and heading date per evaluation group

- Crosses with SHW-derivatives disrupt local adaptation in the recurrent elite wheat background

Optimising diversity capture from PGRs

BBSRC Doctoral Training Partnership
PhD studentship (ICase):
Maegan Green; 2024 to 2028.

Harnessing plant genetic resources without compromising local adaptation in hybrid and inbred wheat breeding programs.

(1) Optimisation through simulation - **capturing diversity** from PGRs via backcross populations while **maintaining local adaptation**.

(2) Improving estimation of combining ability with PGRs - assessing the **utility of testcross derived F2 populations** in determining the genetic merit of PGRs.

(3) Establishing a training set for **predicting combining ability** for local adaptation in an elite gene pool.

Direct D-genome crossing

Developing adapted crossability lines for direct crossing with *Ae. tauschii* to increase wheat D-genome diversity:

1. Establishing the success rate of direct crossing between a representative collection of *Ae. tauschii* and a genotype carrying the recessive alleles at two crossability genes (Kr1/Kr2; Molnár-Láng et al. 2010).
2. Backcrossing the crossability alleles into more adapted local elites.
3. Developing assay for marker assisted selection of *kr1kr1* genotypes.

Seed set in the crossability genotype after pollination with Ae. tauschii.

What’s next for Niab’s "diversity-enriched" material:

1. Stress tolerance

LeasyScan assessment: NIAB_SW_SHW_NAM and NIAB_SW_TET_NAM is being screened for Heat Stress Tolerance in ongoing trials at Hyderabad in collaboration with ICRISAT and Sigrid Heuer.

- Establishing international partnerships to evaluate "diversity-enriched" material in different environments to explore novel phenotypic variation and potentially useful stress tolerance

2. International, interdisciplinary research projects

Wheat Alliance: boosting sustainable wheat nutrition through microbes

Funded by the Novo Nordisk Foundation. Led by: Professor Simona Radutoiu.

Partners:
- Niab
- Aarhus University
- University of Copenhagen
- University of Utrecht
- North Carolina State University
- University of Cambridge.

Niab trial site at Hixton

Harnessing the potential of **plant-microbe interactions** for exploring wheat genetics for **improved nitrogen and phosphorus uptake**.

Investigating how **'diversity-enriched' wheat** genetics contributes to the selection of a **beneficial root microbiome.**

3. PhD studentships exploring more disease resistance

PhD project: Anisa Blower
Exploration of NIAB synthetic wheat as a new source of disease resistance for effective genetic control of Septoria tritici blotch

- SHW show strong resistance to Septoria
- Three nested-populations are being used for genetic mapping in both controlled environments and field trials
- Resistance lines are being advanced for fine mapping

Septoria observed in the field.

Clean SHW wheat

PhD project: Bright Fiakeye
Exploiting wheat tetraploid relatives to enhance brown rust resistance in bread wheat.

- Looking for brown rust resistance in tetraploid backgrounds and then testing effectiveness in hexaploid wheat.
- Exploring novel genes for wheat improvement via looking at the genes, pathways, and cellular processes that are altered during *Pt* infection.

Image source: www.ahdb.org.uk

Clean tetraploid wheat

4. Establishing community-based genetic mapping resources.

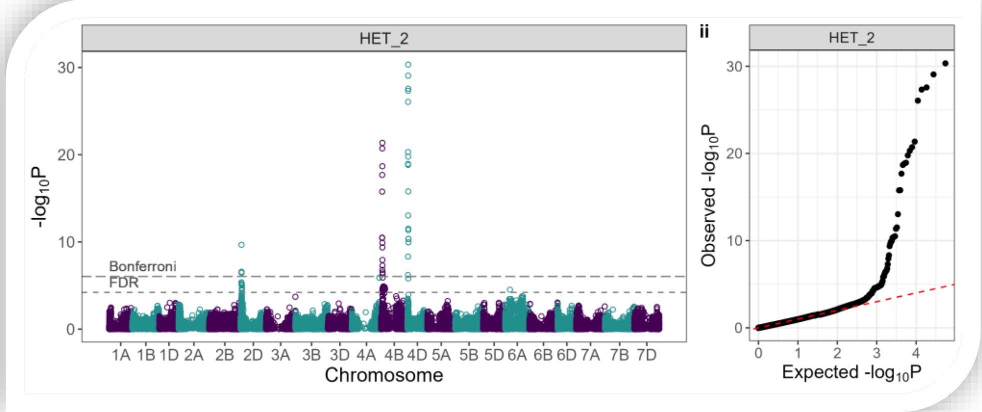
Ensembl Plant Populations

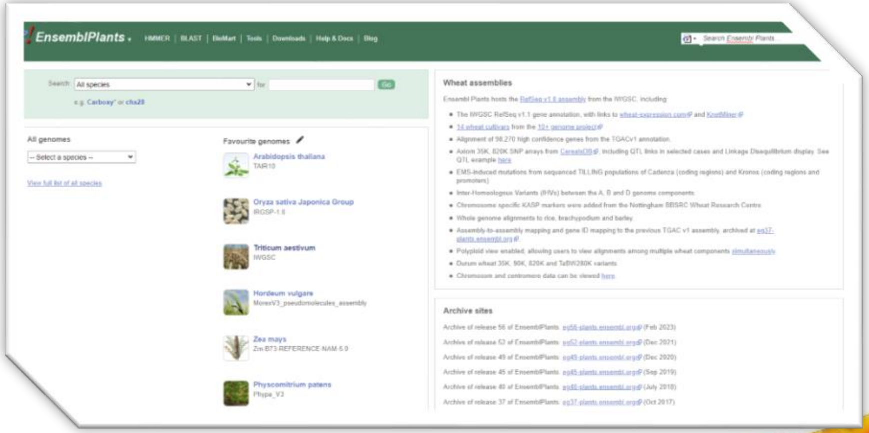
- On the Ensembl Plants genome browser, provide an **open-source genomic tools** for genetic mapping with representative mapping populations from seven crop/plant species.

- Integrating statistically-sound trait mapping approaches with pre-loaded quality-controlled sequence data into a browsable genomic context

Several populations will be included from each of:

- Arabidopsis, Barley, Oat, Oilseed rape, Rice, Tomato and Wheat





e.g. Carboxy* or chs20

All genomes

-- Select a species --

View full list of all species

Favourite genomes

Arabidopsis thaliana
TAIR10

Oryza sativa Japonica Group
IRGSP-1.0

Triticum aestivum
IWGSC

Hordeum vulgare
MorexV3_pseudomolecules_assembly

Zea mays
Zm-B73-REFERENCE-NAM-5.0

Physcomitrium patens
Phypa_V3

Wheat assemblies

Ensembl Plants hosts the RefSeq v1.0 assembly from the IWGSC, including:

- The IWGSC RefSeq v1.1 gene annotation, with links to wheat-expression.com and KnetMiner
- 14 wheat cultivars from the 10+ genome project
- Alignment of 98,270 high confidence genes from the TGACv1 annotation.
- Axiom 35K, 820K SNP arrays from CerealsDB, including QTL links in selected cases and Linkage Disequilibrium display. See QTL example here
- EMS-induced mutations from sequenced TILLING populations of Cadenza (coding regions) and Kronos (coding regions and promoters)
- Inter-Homoeologous Variants (IHVs) between the A, B and D genome components.
- Chromosome specific KASP markers were added from the Nottingham BBSRC Wheat Research Centre
- Whole genome alignments to rice, brachypodium and barley
- Assembly-to-assembly mapping and gene ID mapping to the previous TGAC v1 assembly, archived at eg37-plants.ensembl.org
- Polyploid view enabled, allowing users to view alignments among multiple wheat components simultaneously
- Durum wheat 35K, 90K, 820K and TaBW280K variants
- Chromosom and centromere data can be viewed here

Archive sites

Archive of release 56 of EnsemblPlants: eg56-plants.ensembl.org (Feb 2023)
Archive of release 52 of EnsemblPlants: eg52-plants.ensembl.org (Dec 2021)
Archive of release 49 of EnsemblPlants: eg49-plants.ensembl.org (Dec 2020)
Archive of release 45 of EnsemblPlants: eg45-plants.ensembl.org (Sep 2019)
Archive of release 40 of EnsemblPlants: eg40-plants.ensembl.org (July 2018)
Archive of release 37 of EnsemblPlants: eg37-plants.ensembl.org (Oct 2017)

5. Germplasm availability

Germplasm Resources Unit

.... *a national capability supported by the BBSRC at the John Innes Centre*

SeedStor2

- Many collections discussed are available via the GRU + we are still in the process of submitting the complete NIAB_WW_SHW_NAM population

GitHub

- The population’s genetic marker and field phenotype data is available from: www.niab.github.io/niab-dfw-wp3/.

NIAB

www.niab.com/research/agricultural-crop-research/resources

Perusing new partners from different wheat-growing regions that want to explore diversity resources in their elite genotypes.
Please reach out to us if you are interested.

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