Final Project
Genetic Improvement of Wheat

Presented by: Fizza and Leonid

Contents of Presentation

Breeding Targets

Following are the breeding targets which we as a plant breeder wants to achieve

1. Identify genetically resistant wheat varieties against barley yellow dwarf virus (BYD)
2. Propose a strategy to create an improved variety for HMWG content
3. Develop FHB resistant wheat variety using chromosome engineering and mapping
4. Variety Certification

Barley Yellow Dwarf resistance

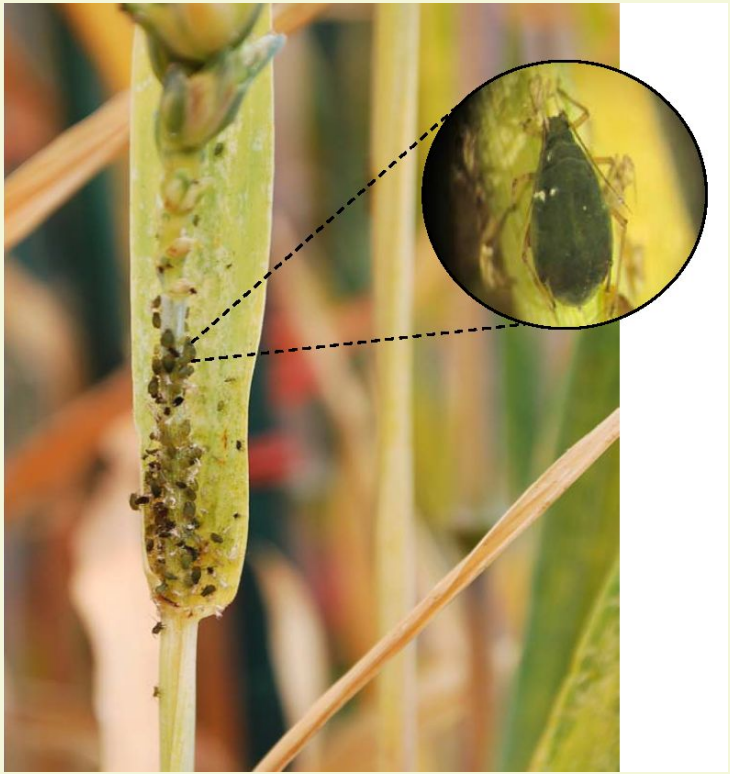


Fig. 1 – Aphid-transmitter

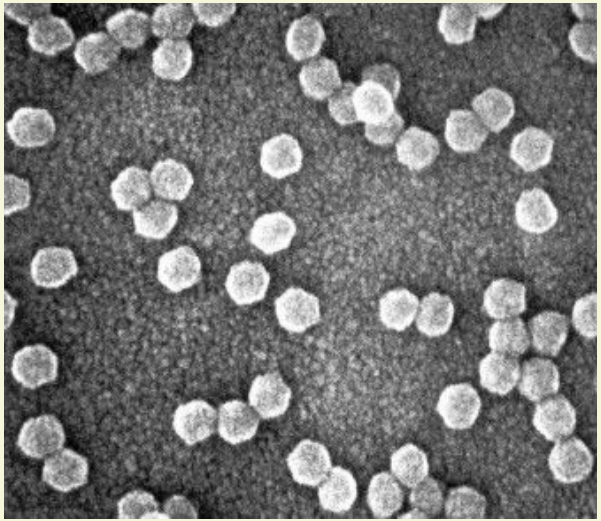


Fig. 2 – Scanning electron micrograph of barley yellow dwarf virus-PAV particles, magnified 200 000×
https://doi.org/10.1016/B978-0123744410-4.00637-3

Experimental Design

- A total of 381 different wheat genotypes were characterized for BYD resistance
- Split-plot field design with 2 or 3 replications
- Main plot was insecticide treatment
- Split-plot was the wheat genotype.

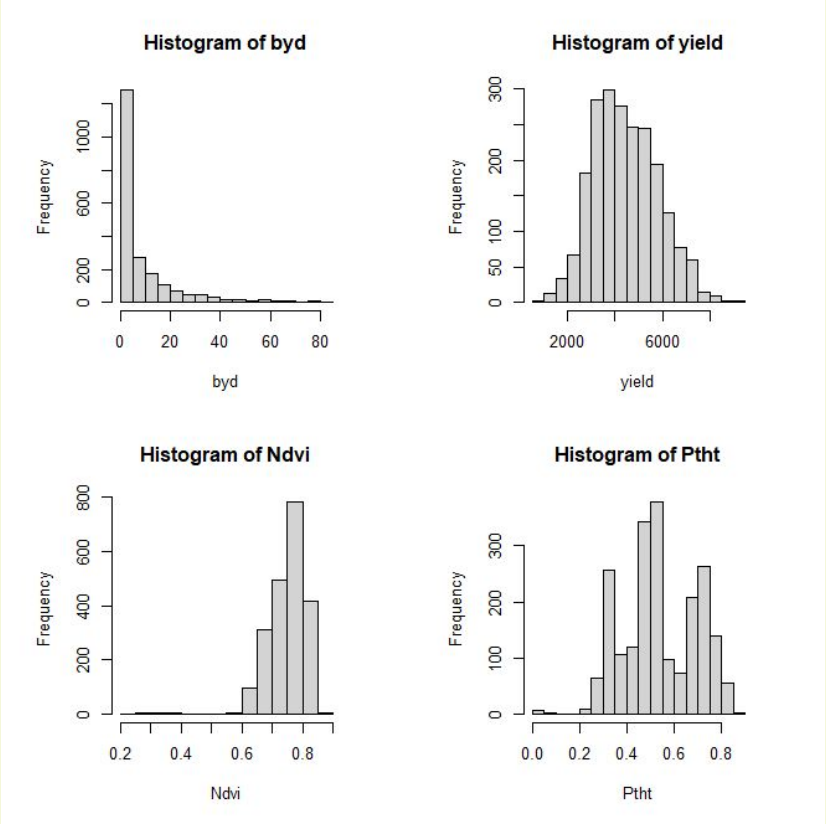
Individual plots were assessed for:

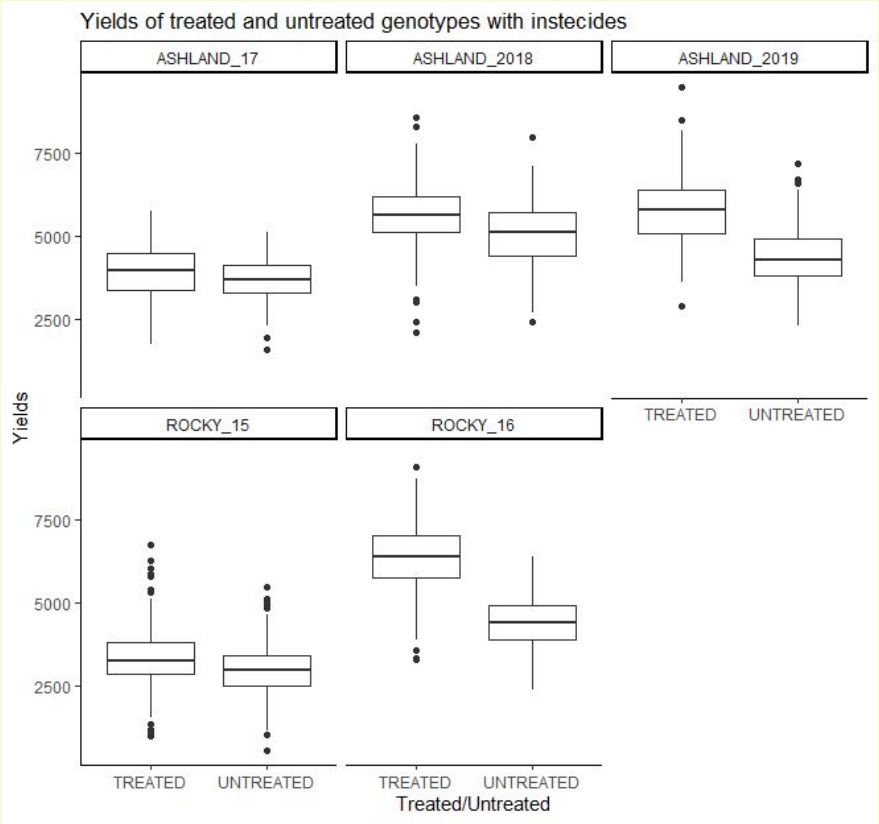
- BYD severity
- Manual plant height
- Grain yield
- Digital plant height, and NDVI

Table 1. Field experimental details for the 5 wheat nurseries.

Season	2015–2016	2016–2017	2017–2018	2018–2019	2019–2020
Location	Rocky Ford farm		Ashland Bottoms farm		
	39°13′45.60″N, 96°34′41.21″W		39°07′53.76″N, 96°37′05.20″W		
Planting date	2015 September 17	2016 September 12	2017 September 19	2018 September 17	2019 September 17
Number of entries	68	52	81	81	107
Number of plots	504	360	400	392	476
Field design	Split-plot with insecticide treatment as main factor effect and wheat genotype as secondary factor				
Replications	3	3	2	2	2
Plot size	6 rows plots—1.5 m × 2.4 m				
BYD evaluation	2016 April 28	2017 May 12	2018 May 19	2019 May 13	2020 May 19
Harvesting date	2016 June 20	2017 June 19	2018 June 23	2019 June 28	2020 June 25

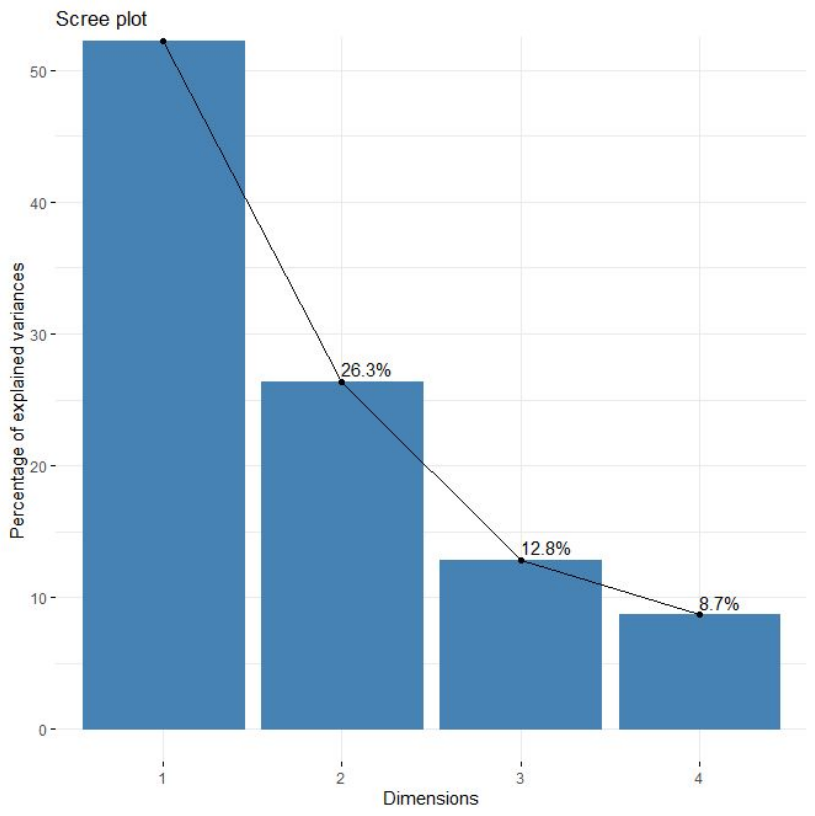
What is inside?

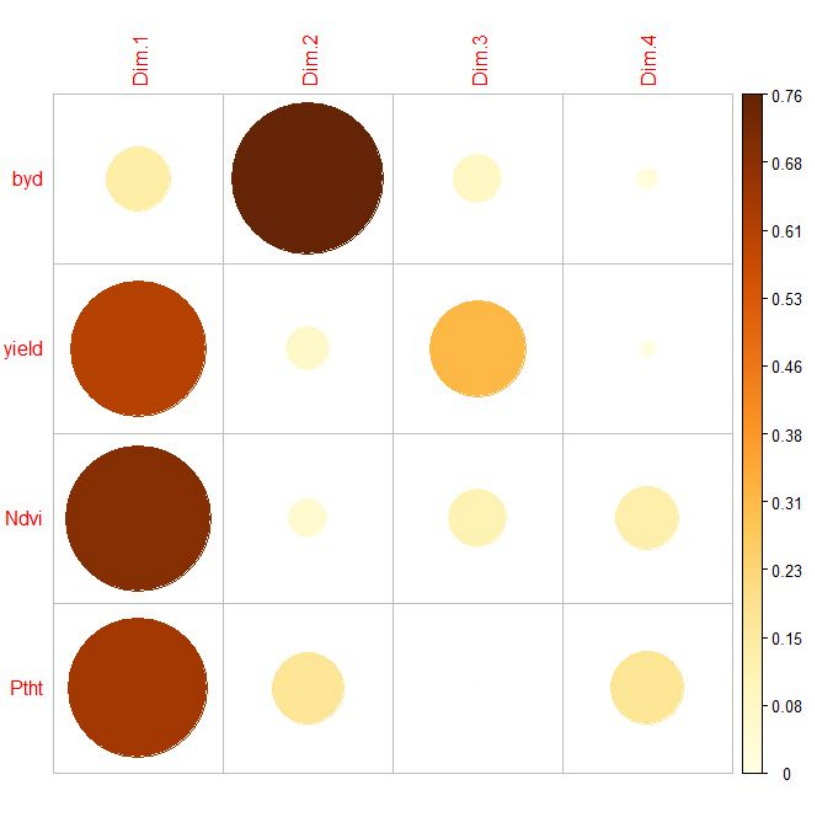




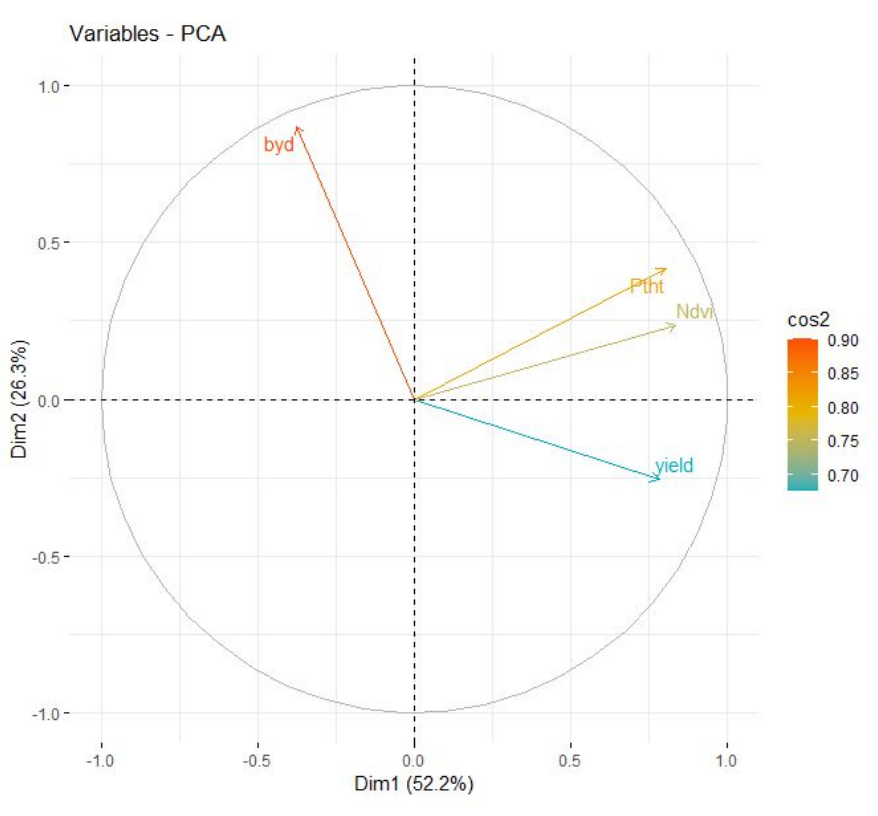
BYD – barley yellow dwarf severity;
Yield – yield;
Ndvi – normalized difference vegetation index;
Ptht – plant height.

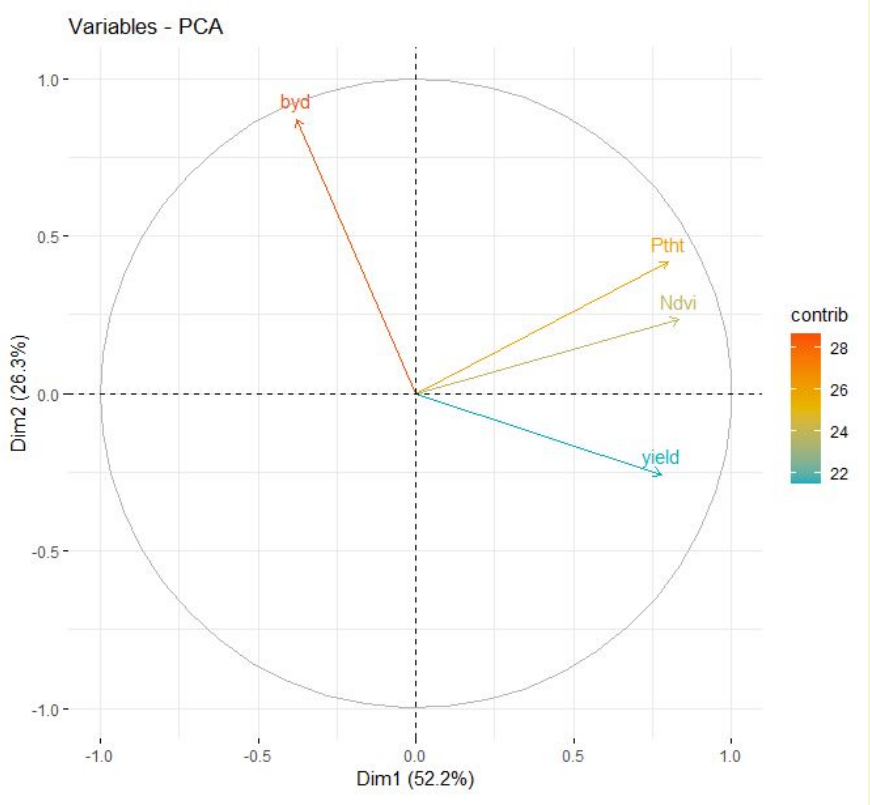
Variances of the principal components



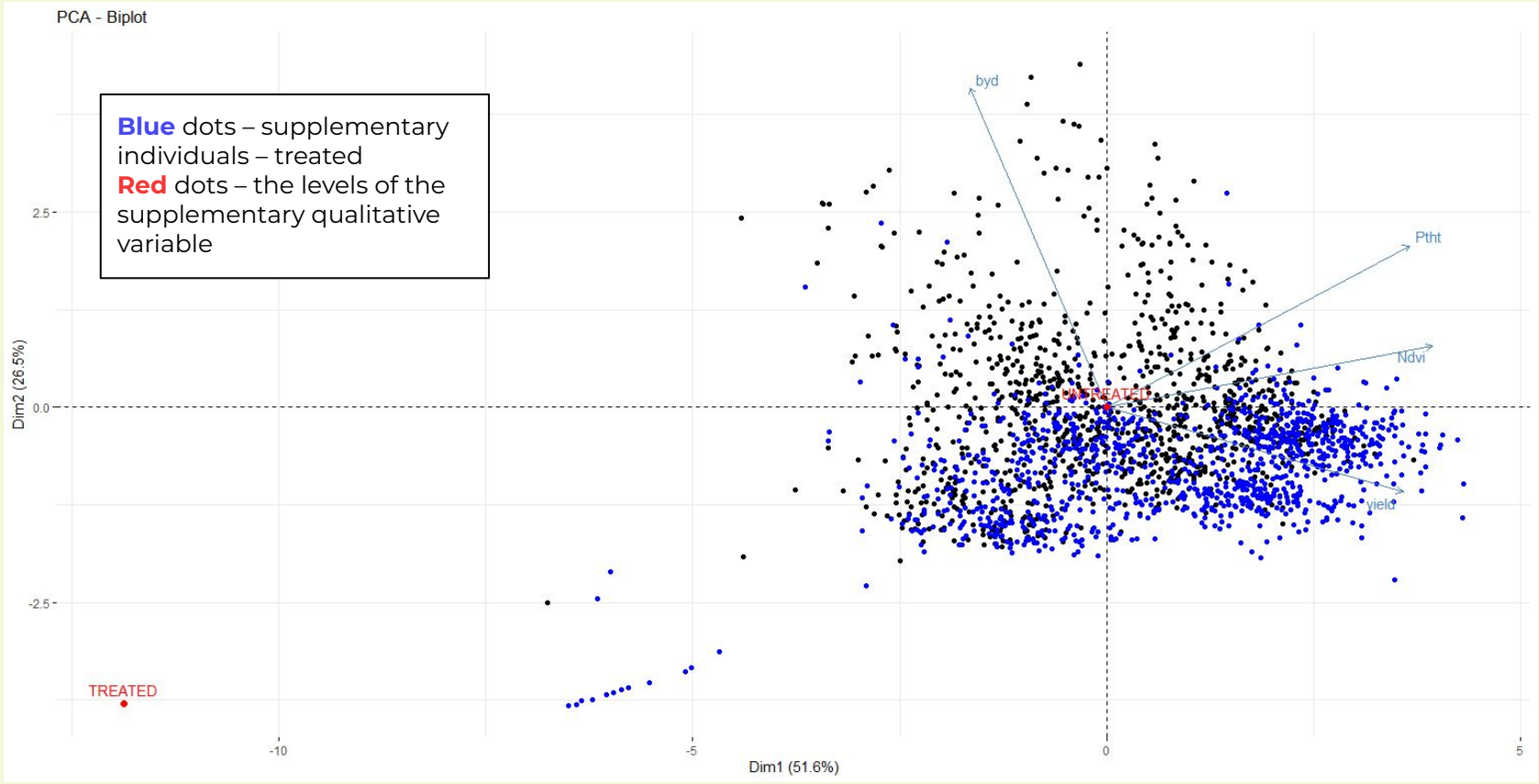


The importance and of a principal component

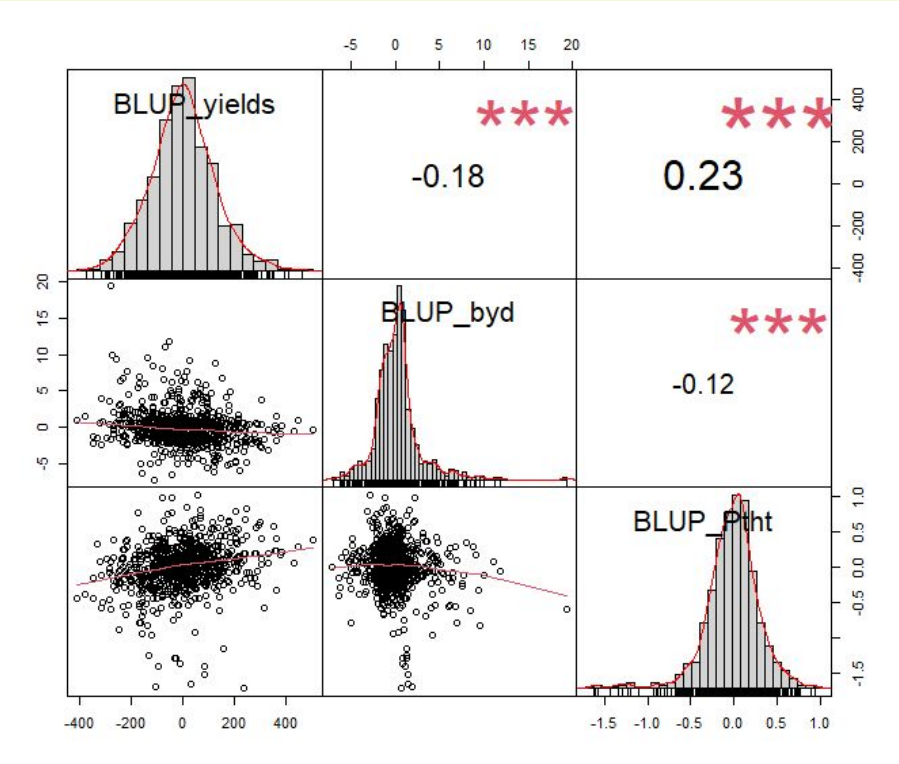




PCA with supplementary individuals: dim 1:2



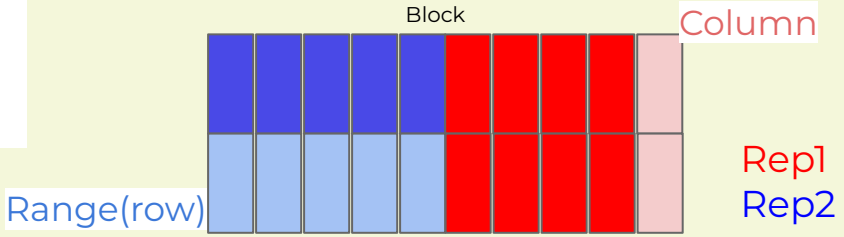
Obtaining the BLUPs from phenotypes



yield ~ year_location + (1|trt) + (1|entry) + (1|trt:entry) + (1|rep/block) + (1|range/rep/block) + (1|column/rep/block)

BYD ~ year_location + (1|trt) + (1|entry) + (1|trt:entry) + (1|rep/block) + (1|range/rep/block) + (1|column/rep/block)

Ptht ~ year_location + (1|trt) + (1|entry) + (1|trt:entry) + (1|rep/block) + (1|range/rep/block) + (1|column/rep/block)



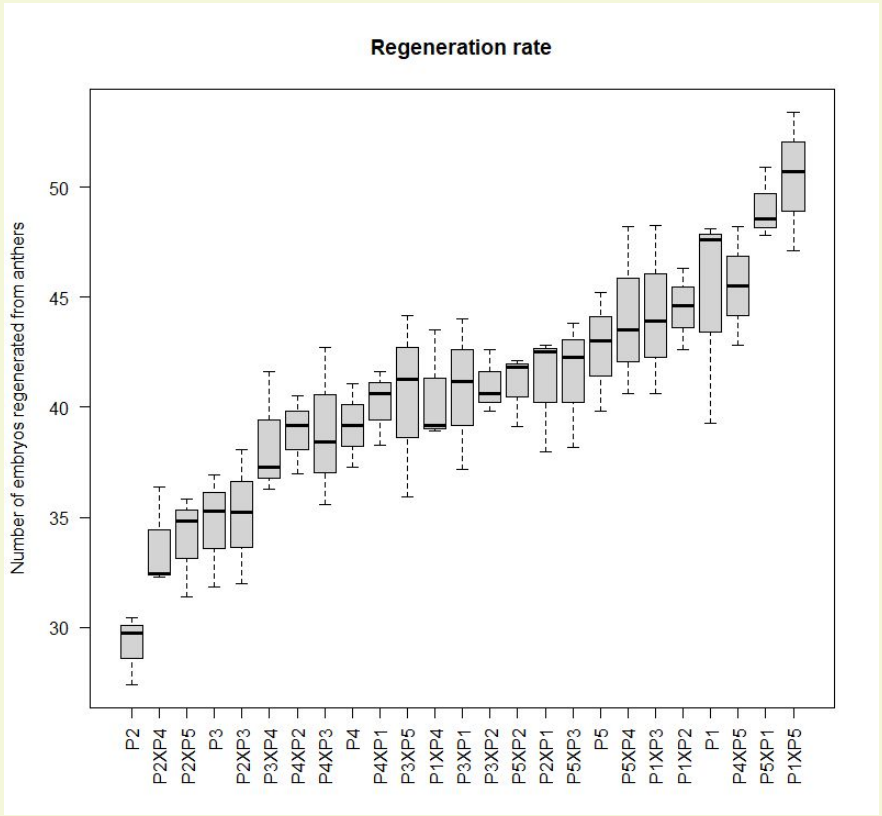
Five genotypes with the highest BLUPs

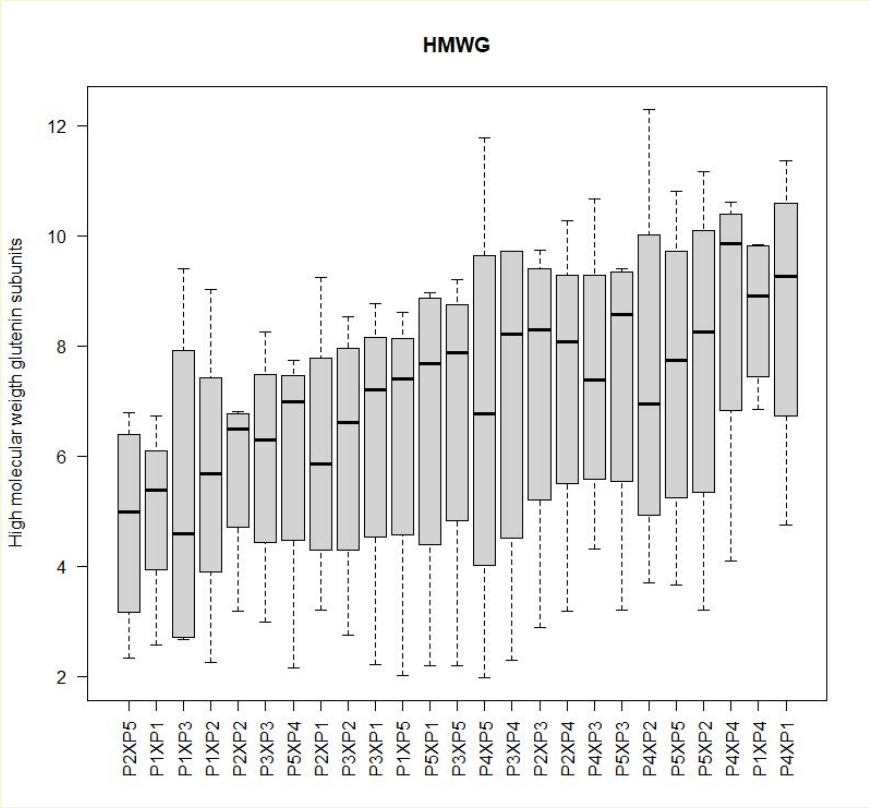
- Unfortunately, there were the troubles with the genotype data file, which did not allow us to use properly GWAS and genomic selection approaches. Even the authors of the initial data set had them!
- Therefore, we chose top five genotypes with the highest yield BLUPs and with the negative BLUPs for BDY:

trt	variety	BLUP_yields	BLUP_byd	BLUP_Ptht
UNTREATED	KS120081M-5	331.0999	-3.1313877	0.0608853
UNTREATED	KS120580M-7	286.7609	-2.0743605	0.5558467
UNTREATED	KS13DH0002-19	278.7560	-0.9676398	0.1968779
UNTREATED	KS100060K-15	276.9403	-1.7807048	-0.1626424
UNTREATED	KS12DH0296-156	269.0164	-2.1583758	-0.2344582

Diallel Analysis

Let's imagine, we did the diallel analysis of previous genotypes for regeneration rate of and High Molecular Weight Glutenin!



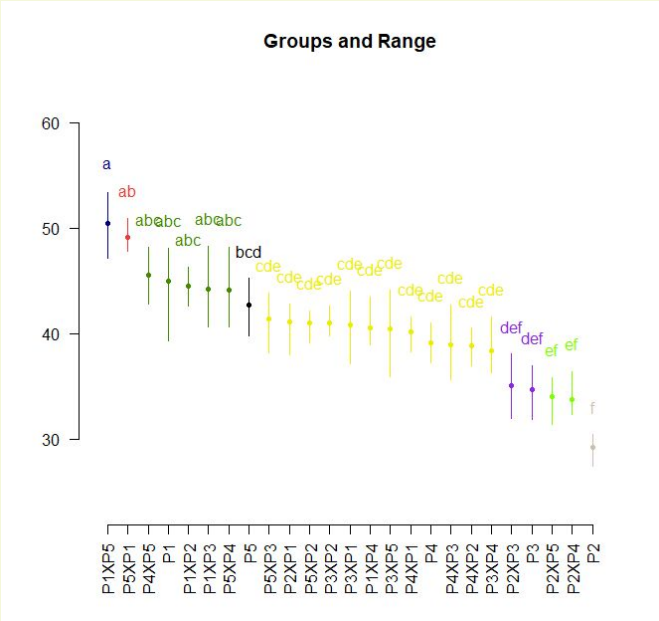


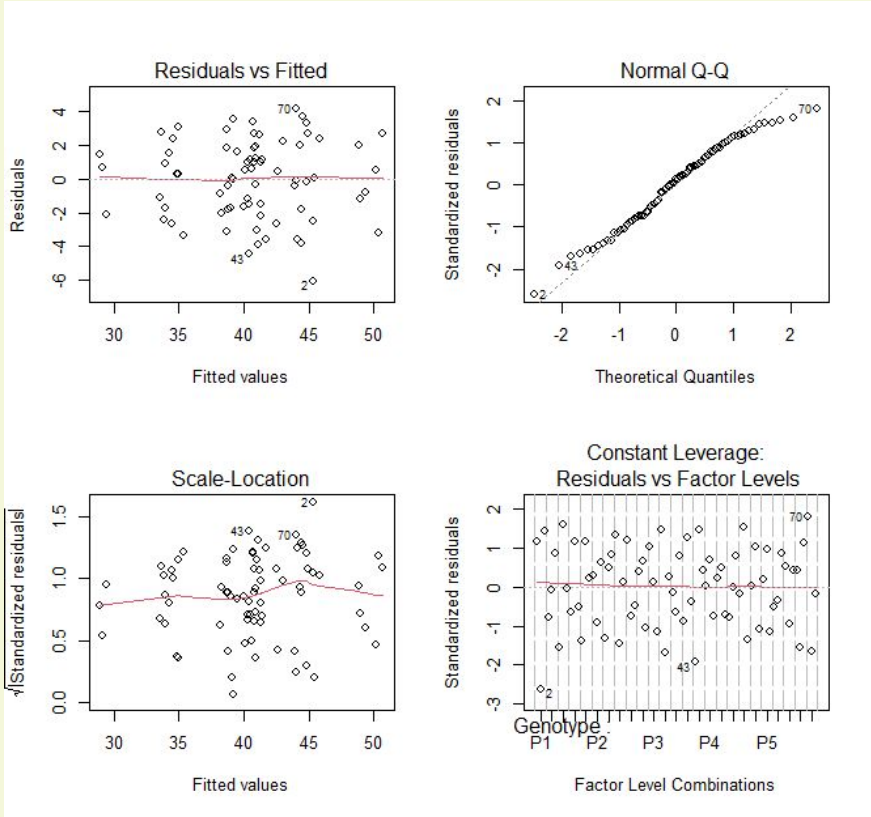
Regeneration Rate

NbEmbryons ~ Genotype + Rep

naive ANOVA

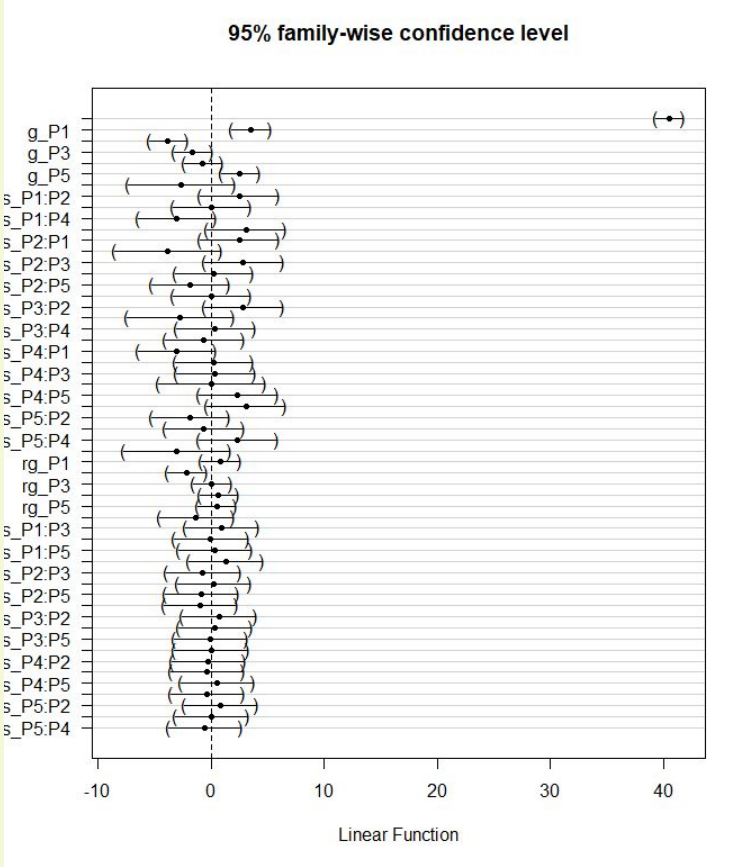
```
            Df Sum Sq Mean Sq F value   Pr(>F)
Genotype    24 1671.9   69.66   8.384 3.01e-10 ***
Rep          2    3.4    1.71   0.205    0.815
Residuals   48  398.8    8.31
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```





Regeneration Rate

The "Hayman" model (type 1)



NbEmbryons ~ Block + GCA(Par1, Par2) + tSCA(Par1, Par2) + RGCA(Par1, Par2) + RSCA(Par1, Par2)

```
Analysis of Variance Table

Response: NbEmbryons
           Df  Sum Sq Mean Sq F value    Pr(>F)
Block       2    3.41   1.706  0.2053 0.8151259
GCA         4 1093.58 273.396 32.9029 3.273e-13 ***
tSCA       10  369.56  36.956  4.4476 0.0001916 ***
RGCA        4  182.37  45.592  5.4869 0.0010144 **
RSCA        6   26.39   4.399  0.5294 0.7831642
Residuals 48  398.84
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

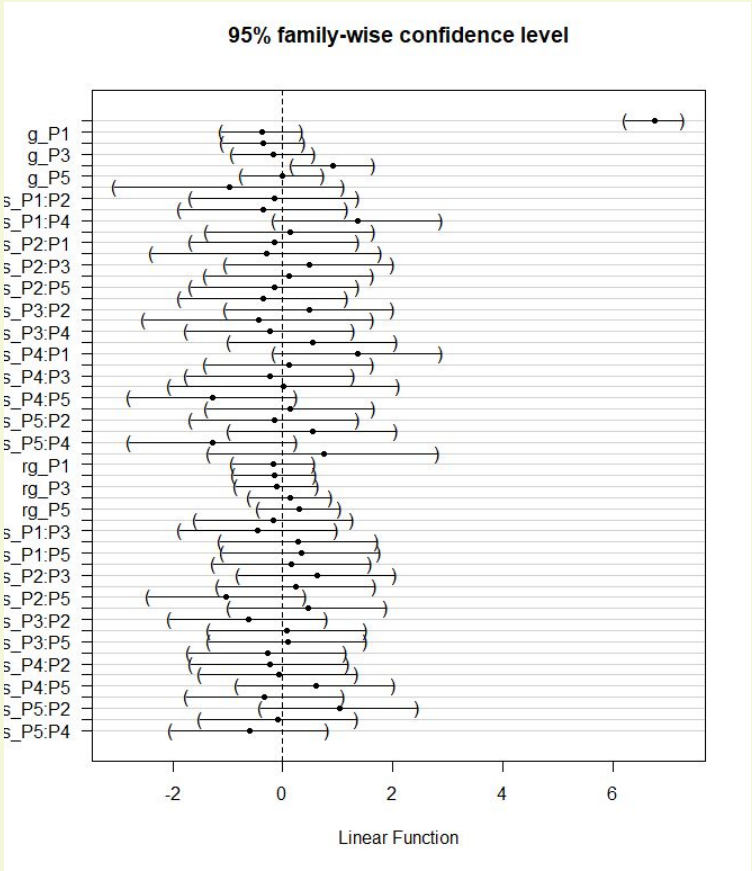
```
        Shapiro-Wilk normality test

data:  residuals(dMod2_H1_reg)
W = 0.97563, p-value = 0.1567
```

Conclusions: for GCA **all** the parental lines are significantly different from each other, **except** for P5; for SCA the most interesting genotypes will be: **P1xP5, P2xP3.** For RCA no genotype has the interest for us.

HMWG

The "Hayman" model (type 1)

HMWG ~ Block + GCA(Par1, Par2) +
tSCA(Par1, Par2) +
RGCA(Par1, Par2) + RSCA(Par1, Par2)




```
Analysis of Variance Table

Response: HMWG
          Df Sum Sq Mean Sq F value    Pr(>F)
Block      3 456.25 152.082 69.5581 < 2.2e-16 ***
GCA        4  45.93  11.482  5.2514 0.0009081 ***
tSCA      10  41.20   4.120  1.8844 0.0613910 .
RGCA       4   6.85   1.714  0.7837 0.5394666
RSCA       6  18.42   3.070  1.4042 0.2248497
Residuals 72 157.42
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
        Shapiro-Wilk normality test

data:  residuals(dMod2_H1)
W = 0.96663, p-value = 0.01228
```

Conclusions: at the 0.1% sign. level we have an evidence that GCA is different only for **P4**.

We could also detect that at 10% there is an evidence for tSCA that it has the difference within the group. LHS showed us that **P4xP1** is the most different from others.

Strategy to create an improved variety for HMWG content

Overall conclusion:

- **P4** and **P1** genotypes during the analysis of HMWG showed the best tSCA;
- But we would also combine the good content of HMWG with regeneration rate!
- Also, 1% significance level if the null hypothesis is true by LHS it was shown that for regeneration rate SCA was different for lines **P1xP5, P2xP3.**

We would propose to cross the lines **P1xP4** and **P1xP5**, and probably, due to the common parental line it would be possible to combine in one genotype good HMWG content and high regeneration rate of embryons!

Chromosome engineering, mapping, and transferring of resistance to Fusarium head blight disease from *Elymus tsukushiensis* into wheat

Fusarium head blight (FHB) caused by the fungus Fusarium graminearum Schwabe [telomorph = Gibberella zeae (Schwein. Fr.) Petch].

- Resistance to initial infection (type-1)
- Resistance to spread of infection within the spike (type-2)

Genetic Resources

Fhb1 and Fhb2

- Type-2 FHB resistance
- Present in wheat cultivar Sumai 3
- Mapped to the short arms of wheat chromosomes 3B and 6B

Fhb3

- Type-2 FHB resistance
- Derived from a tetraploid wheat relative Leymus racemosus
- Compensating Robertsonian translocation T7AL. 7Lr#1S.

Fhb4 and Fhb5

- Conferring type-1 resistance
- Chinese cultivar Wangshuibai
- Mapped to the long arm of chromosome 4B and the short arm of 5A

Production and characterization of wheat-*E. tsukushiensis*

Elymus tsukushiensis Honda (2n = 6x = 42)

- Perennial cross-pollinating hexaploid species
- Native to China, Korea, and Japan
- Source of resistance to FHB

Plant Material

- Wheat-*E. tsukushiensis* disomic chromosome addition line **DA1Ets#1 (TA7684)**
- Derived wheat-E. tsukushiensis disomic addition/translocation line **DATW.1Ets#1S (TA5655)**
- Wheat cultivars **'Chinese Spring', 'Everest', 'Karl 92', and 'Overley'**

Step 1

Line **TA5655 with TW.1Ets#1S** added to the wheat complement (2n = 44) was crossed twice with the ph1b mutant stock (TA3809).

Step 2

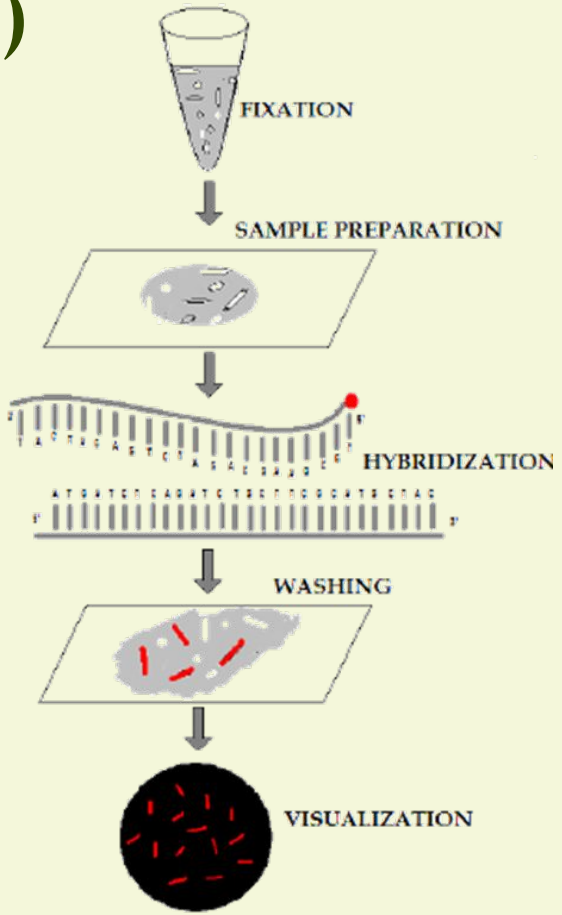
Plants with 2n = 43 and homozygous ph1b/ph1b and hemizygous for DATW.1Ets#1S were selected and their progenies screened by molecular markers to identify putative recombinants

Cytological Procedure (GISH & FISH)

- Oligonucleotide probe pAs1 hybridized to all D-genome
- BAC clone 676D4, paints all A-genome chromosomes of wheat

- Squash preparations were made after staining with acetocarmine.
- After hybridization at 37 °C overnight, the slides were washed

- A drop (25–30 µl) of Vectashield mounting medium containing 1 µg/ml of PI was added to each slide, and then covered with a 24 × 30 cm glass cover slip.



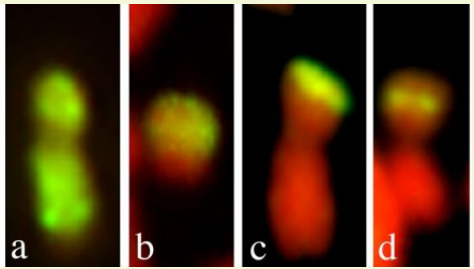
C-banding and chromosome identification

https://www.researchgate.net/publication/282879897_Chapter_18

Marker Screening

PCR-based markers (WPG90 and PSR2120) and two new EST–STS markers **(BF202643/HaeIII and BE591682/HaeIII)** specific to the TW.1Ets#1S translocation

GISH



#107 exhibited a heterozygous distal translocation on the long arm of a wheat chromosome, distal segment of 1Ets#1S

#74 had a heterozygous interstitial translocation, a small segment derived from 1Ets#1S, and a distal wheat chromosome part

Screen 488

1. DATW.1Ets#1S translocation x the ph1b mutant

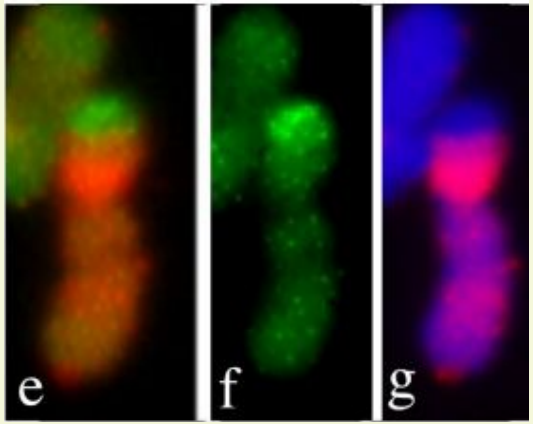
23 plants homozygous for the ph1b mutation (ph1b/ph1b) and hemizygous for the TW.1Ets#1S translocation.

Two plants (#74 and #107) were identified as recombinants (proximal and distant recombinant, respectively)

Cytology Study for Genome Identification

- Two-color GISH with the D-genome-specific oligonucleotide probe pAs1 and total genomic DNA of E. tsukushiensis - NO Detection!
- Simultaneous detection using A-genome-specific probe BAC676D4 labeled with Texas Red and total genomic E. tsukushiensis DNA labeled with fluorescein in green revealed

Presence of A genome in rec 107



The size and arm ratio of the A-genome chromosome involved in rec107 identified this chromosome as 1A of wheat and, thus, this recombinant chromosome can be described as **T1AL. 1AS1Ets#1S.**

FHB Resistance

Spring Entries

5 seeds were sown in a 15 cm diameter plastic pot with four replications, and incubated in a greenhouse (25 ± 4 °C) with supplemental light.

Winter Entries

3 seeds were sown in a plastic cone (2.5 × 13 cm) with four replications and the resulting seedlings were grown in the greenhouse for 10 days.

Flowering

The seedlings were then vernalized at 4 °C for 7 weeks and transplanted into 15 cm diameter pots, 3 tubes per pot with four replications.

Inoculation

A single floret on the tenth spikelet from the bottom of each head was inoculated. A conidial suspension (10 µl) with about 10^5 spores per ml of Fusarium was introduced between the lemma and palea with a pipette

FHB Severity Scoring

Only The Inoculated Floret Blighted = 3 %;

Two Of The Three Florets In The Inoculated Spikelet Blighted = 7 %;

Only The Inoculated Spikelet Blighted = 10 %

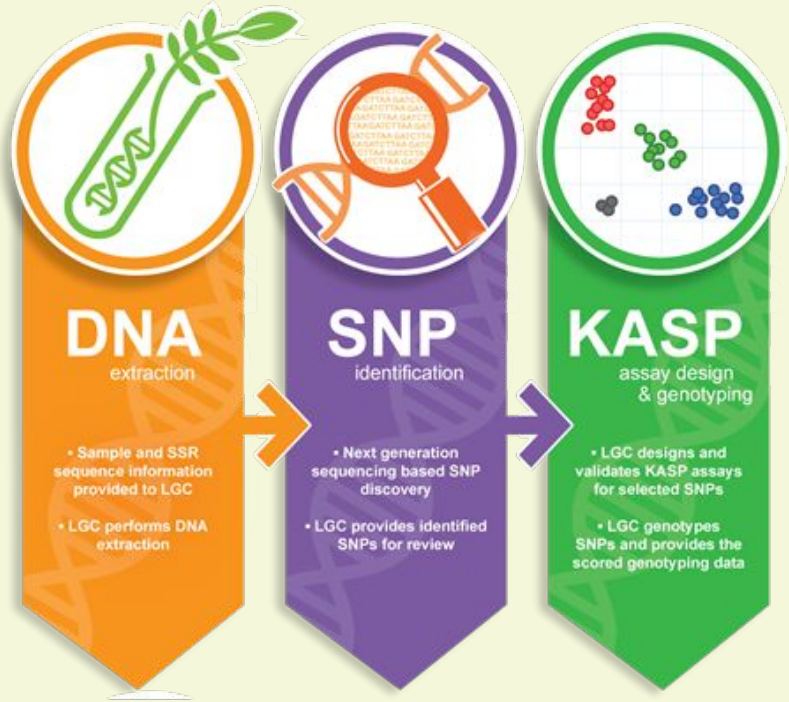
The Inoculated and the Spikelet Immediately Below The Inoculated One blighted =20 %;

Three Spikelets Blighted = 30 %

If The Inoculated Spikelet And All 9 Spikelets Below The Inoculated One Were Blighted, Severity = 100 %.

KASP Assay

- SNP based KASPar™ markers were designed from 20 SNPs from the 10 K infinium chip developed
- Samples were genotyped for the SNP markers using KASPR
- DNA from the parents and recombinants was amplified using two allele-specific primers and a common primer
- Normalized signals from each SNP allele (x and y) were plotted in two dimensions under the allelic discrimination mode.



https://www.lifomics.com/kasp.html

Scoring for FHB Resistance

Table 2 Fusarium head blight ratings of wheat-*Elymus tsukushiensis* introgression lines

Name	Chromosome constitution	Cultivar or genetic background	Average FHB rating (%)	No. of heads in inoculated
Everest	–	Everest	27.7	40
TA2923	–	Karl 92	32.7	40
TA9107	–	Overley	54.6	40
TA3008	–	Chinese Spring	35.1	42
TA7684-2	DA1E^{ts}#1	Chinese Spring	12.5	41
TA5655	TW·1E^{ts}#1S	Chinese Spring	6.2	51
2011-55-5	rec74, hom TiWL·WS-1E^{ts}#1S-WS	Chinese Spring	13.3	53
2011-55-12	rec74, hom TiWL·WS-1E^{ts}#1S-WS	Chinese Spring	14.7	41
2011-55-14	rec74, hom TiWL·WS-1E^{ts}#1S-WS	Chinese Spring	12.5	39
2011-55-13	no *E. tsukushiensis* chromatin	Chinese Spring	39.3	41
2011-56-3	rec107: hom T1AL·1AS-1E^{ts}#1S	Chinese Spring	4.2	40
2011-56-10	rec107: hom T1AL·1AS-1E^{ts}#1S	Chinese Spring	13.3	42
2011-56-13	rec107: hom T1AL·1AS-1E^{ts}#1S	Chinese Spring	8.9	51
2011-60-5-1	rec107: hom T1AL·1AS-1E^{ts}#1S	Chinese Spring	8.6	40
2012-56-4	no *E. tsukushiensis* chromatin	Chinese Spring	31.7	40
2012-60-5-2	no *E. tsukushiensis* chromatin	Chinese Spring	42.5	39

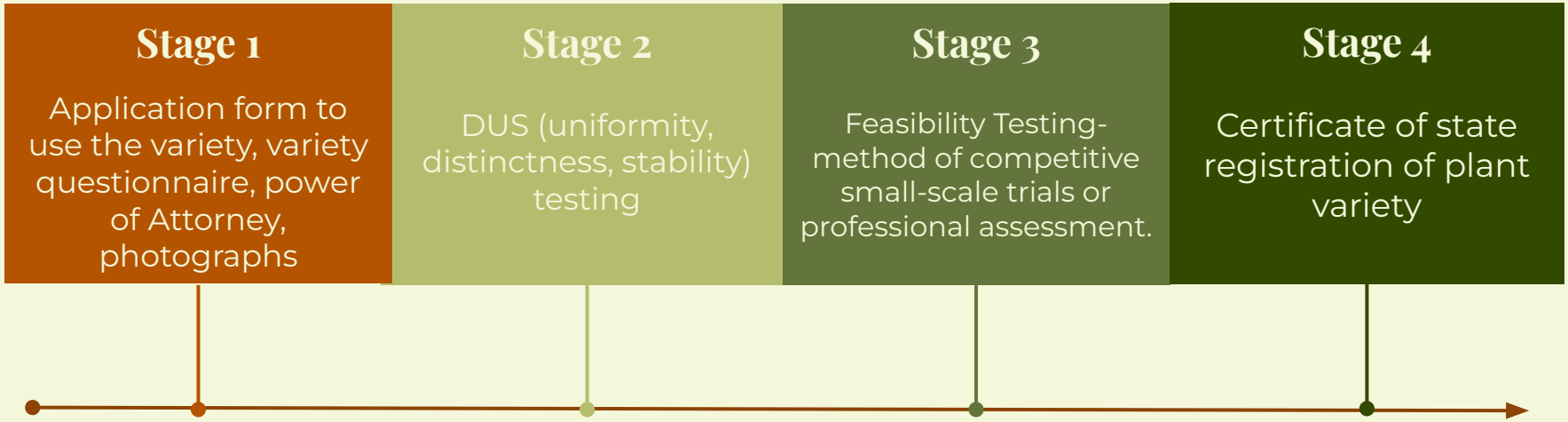
LSD ($P = 0.05$) 10.85

The FHB rating is the percentage blighted spikelets from plants inoculated under greenhouse conditions

Certification Procedure

Application Filling

Application is submitted at the Federal Service for Intellectual Property (Rospatent) registers patents in Russia.



Conclusion

What we achieved?

1. Identified 5 potential resistant wheat genotypes for BYD
2. Proposed a strategy to create an improved wheat variety for HMWG content
3. Illustrated a detailed protocol for developing FHB resistant wheat varieties using biotechnological approaches
4. Variety Certification

What needs to be done?

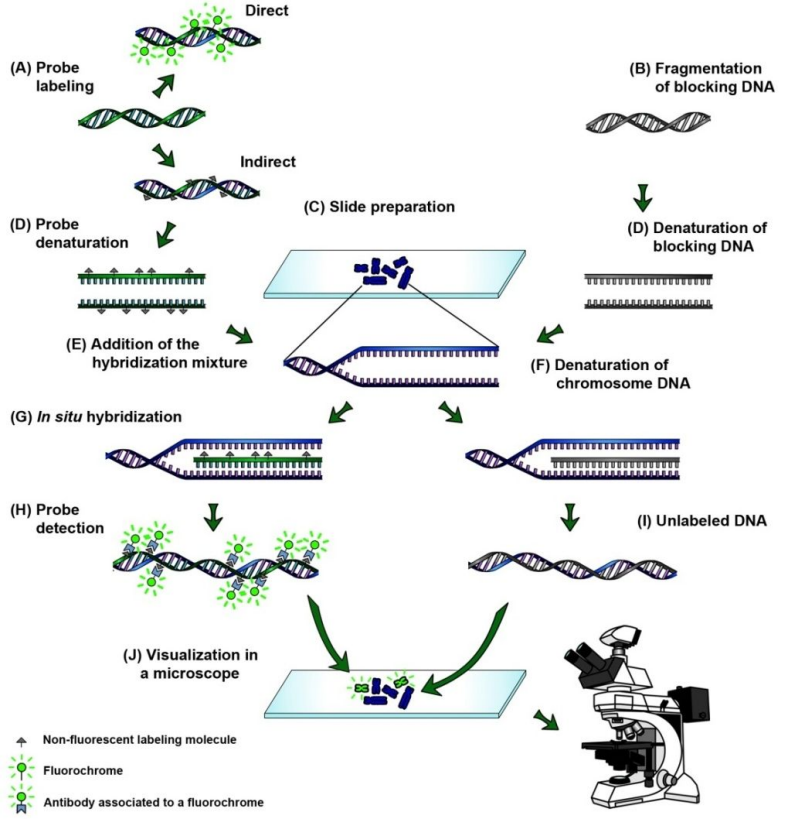
GxE analysis of the resistant wheat varieties

Detailed genomic analysis of the varieties

Thank you for your attention!

Our source code you can find in our GitHub repo!
still under construction

Supplementary



Chromosome designations follow the nomenclature where ‘T’ indicates a terminal translocation, ‘Ti’ indicates an interstitial translocation, ‘. ’ marks the centromere, ‘-’ marks an interstitial translocation breakpoint, the number indicates the homoeologous group, followed by the genome symbol, and the chromosome arm designation ‘S’ for short and ‘L’ for long arms, the ‘#’ sign is used to distinguish between the

Marker Development and Screening

01

- 96 wheat expressed sequence tags (ESTs)
- Primer design using Primer 3 software
- ESTs markers were developed to identify the resistant genes in the hybrids.

02

Screening of progenies of homozygous ph1b and heterozygous TW.1Es#1S

- Four polymorphic EST–STS markers to screen for putative recombinants.
- Putative recombinants characterized by GISH

03

For tagging the E. tsukushiensis segment

- Markers - Comparative sequence analysis of wheat with rice chromosome 5, Brachypodium chromosome 2 and barley chromosome 1.
- Sorted chromosome arm sequence of wheat chromosome 1AS was also used

04

- Design 50 conserved primers using Wheat ESTs/flcDNA
- Amplicons from Chinese Spring, Everest and TW. 1Ets#1S (TA5655) were digested with seven enzymes, HaeIII, AluI, EcoRI, RsaI, MspI, MseI, MboI
- The polymorphic markers were used to screen the recombinant progenies.