Phenotypes in Einkorn introgressed Wheat

Michael Hammond-Kosack

Rothamsted Research

20th WGIN Stakeholders' Meeting

John Innes Centre, Norwich, February 6th 2023

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Why?

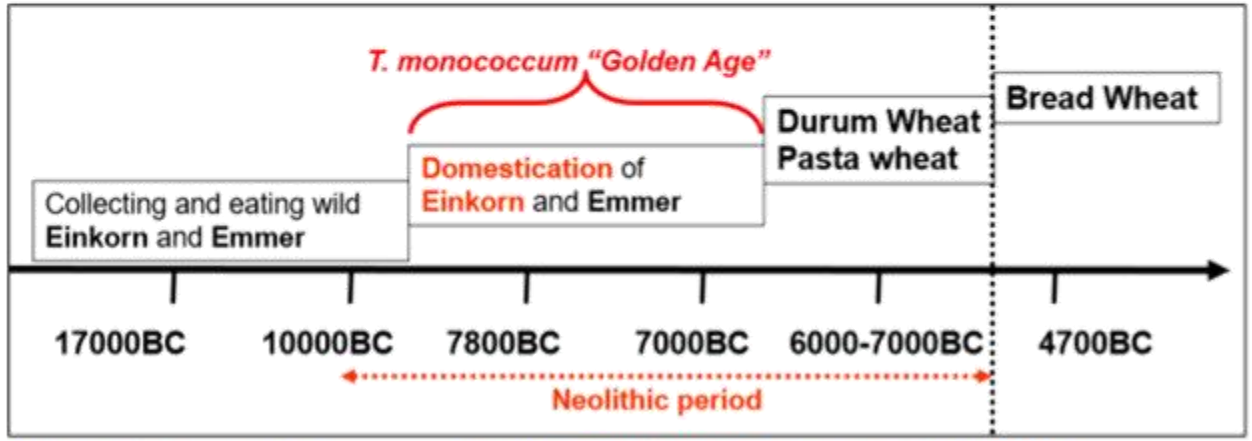
- Commercial hexaploid bread wheat (*Triticum aestivum*) grown in monoculture **highly susceptible to a large number of diseases**.
- This necessitates frequent **spraying with fungicides and insecticides**.
- Many diploid ancestors, including **Einkorn (*Triticum monococcum*)** and related species exhibit **strong natural resistance** to most of these diseases.
- transferring (introgressing) these natural resistance traits from *T.mon* into bread wheat using conventional crossing strategies would **result in bread wheat partially or fully resistant** to multiple diseases.

What is *Triticum monococcum*?

- **Einkorn**, "Nature's First And Oldest Wheat"

- originates from the **Karacadağ Mountains** (Eastern Türkiye) within the Fertile Crescent

- domesticated between **6500 – 9000BC**



- **diploid** wheat species – only has **A genome (A^mA^m)**

  [nb: this is not the actual A genome donor of modern wheat]

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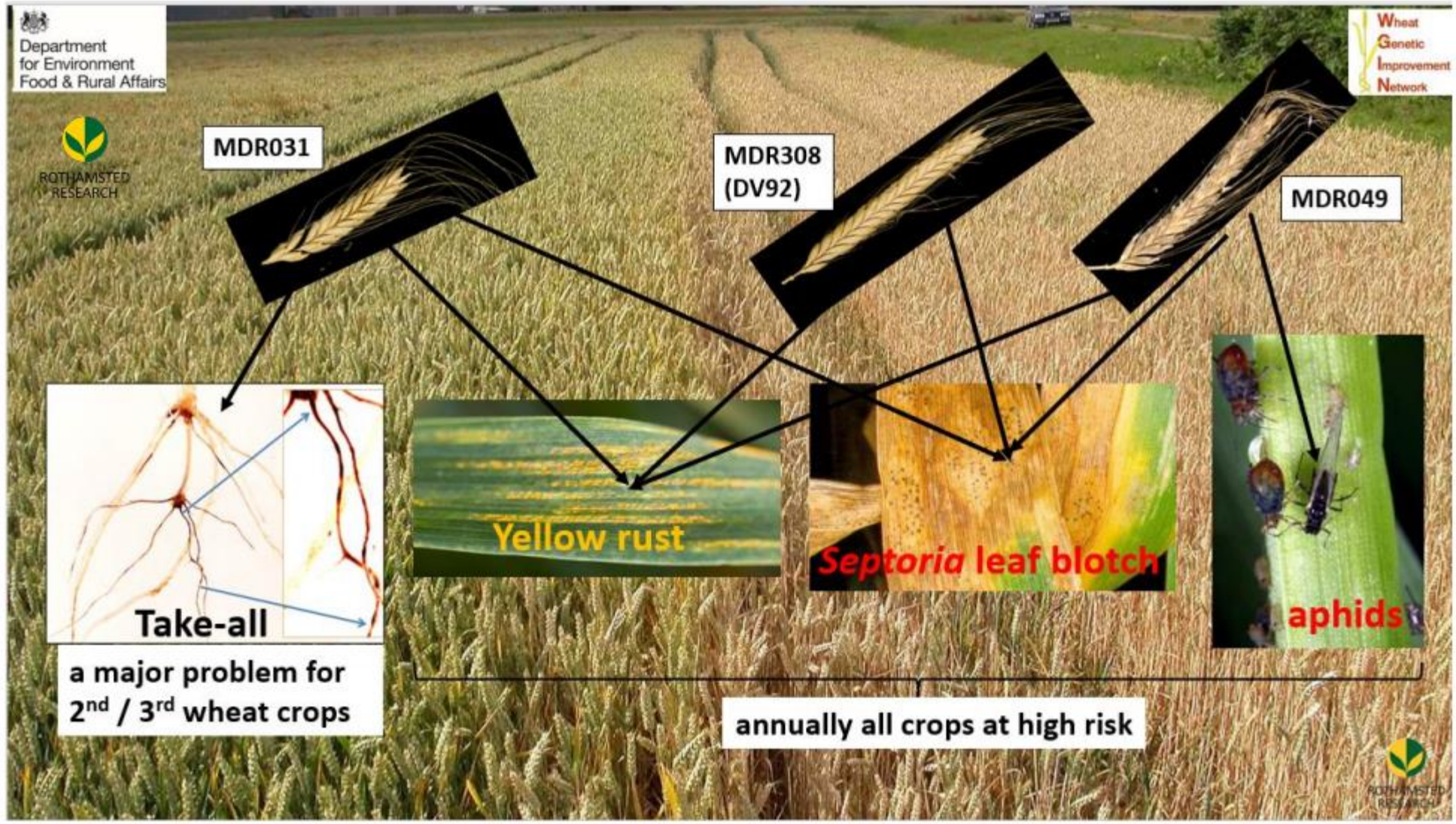
Triticum monococcum (diploid species with AA genome) a good source of resistance to various pathogens and pests



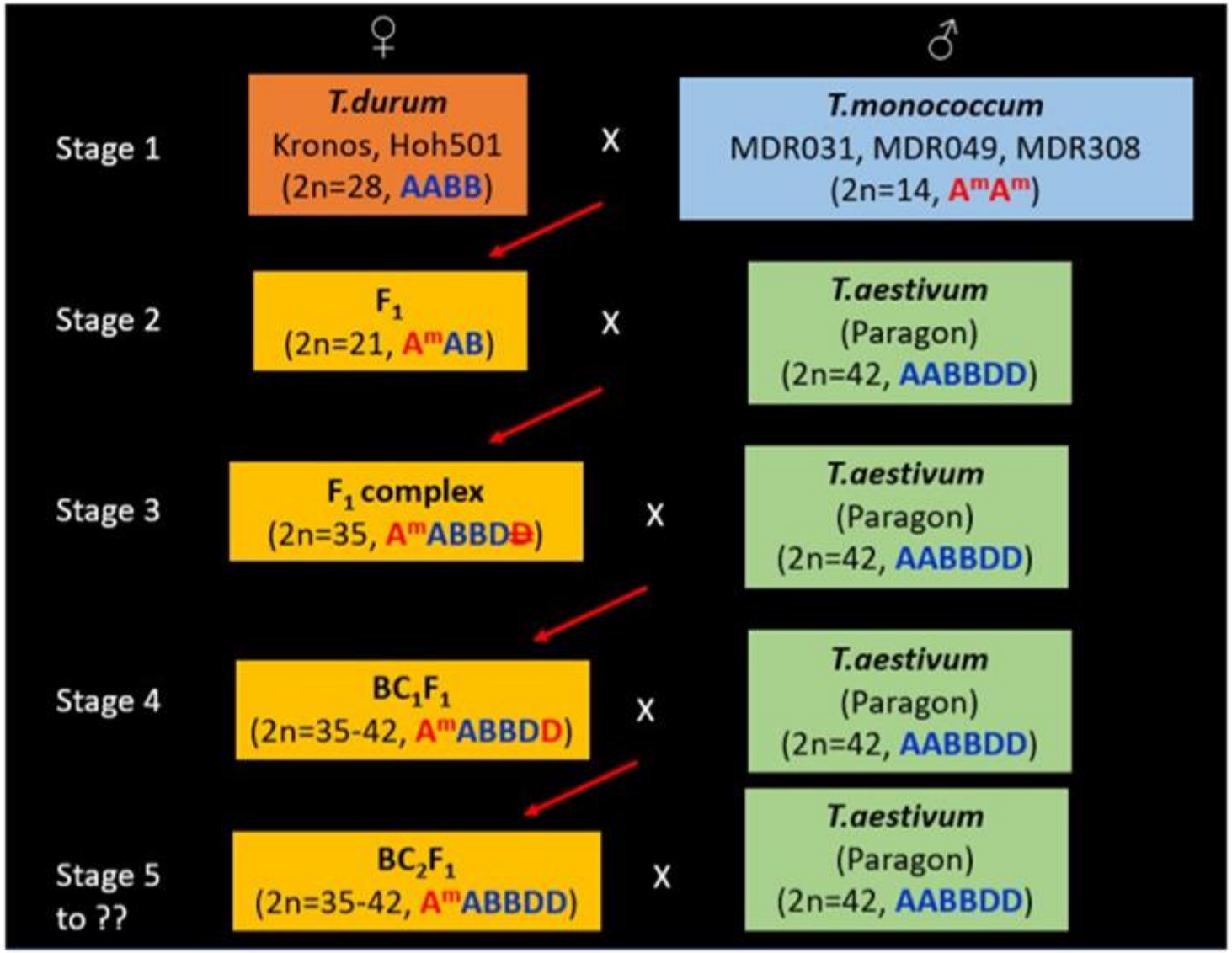
Einkorn Introgression into Breadwheat using Pasta Wheat as a Bridging Species

3 T.mon accessions chosen:

- MDR031 – Take-all
- MDR049 – aphid
- MDR308 (DV92) – Zymoseptoria

most Tmon accessions also exhibit broad spectrum resistance against fungi

[MDR = molecular disease resistance]



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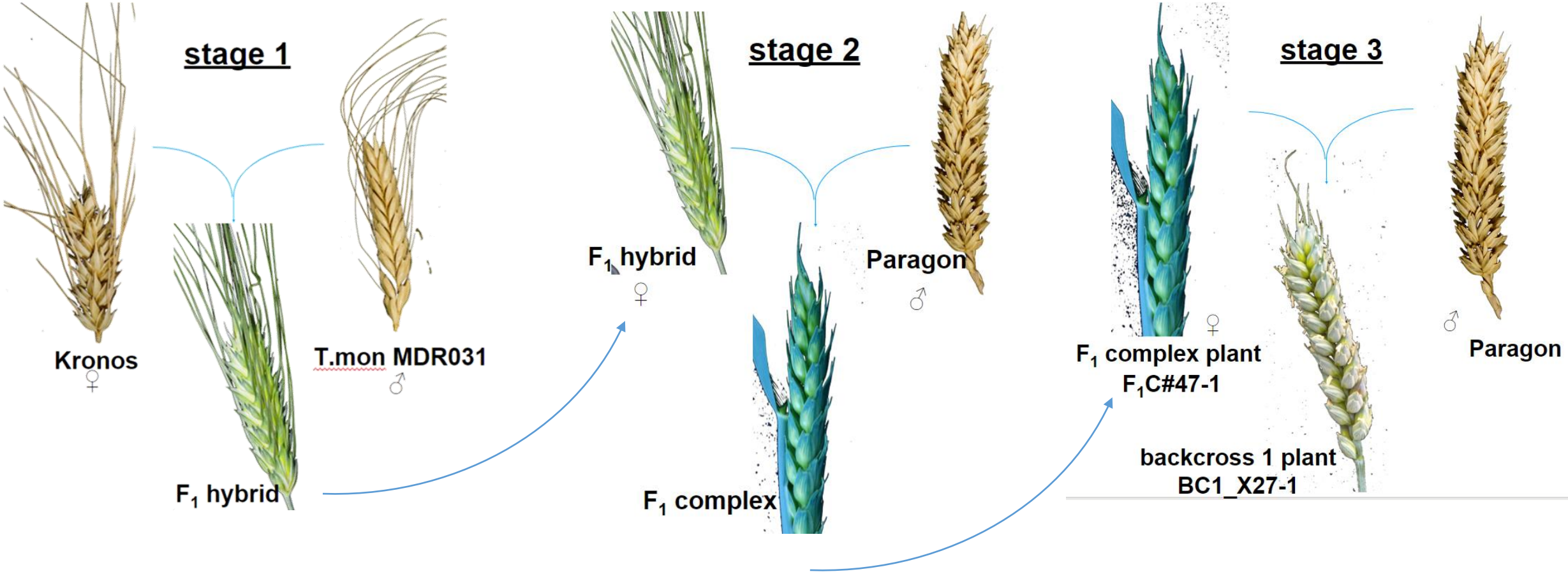
Einkorn Introgression into Breadwheat using Pasta Wheat as a Bridging Species



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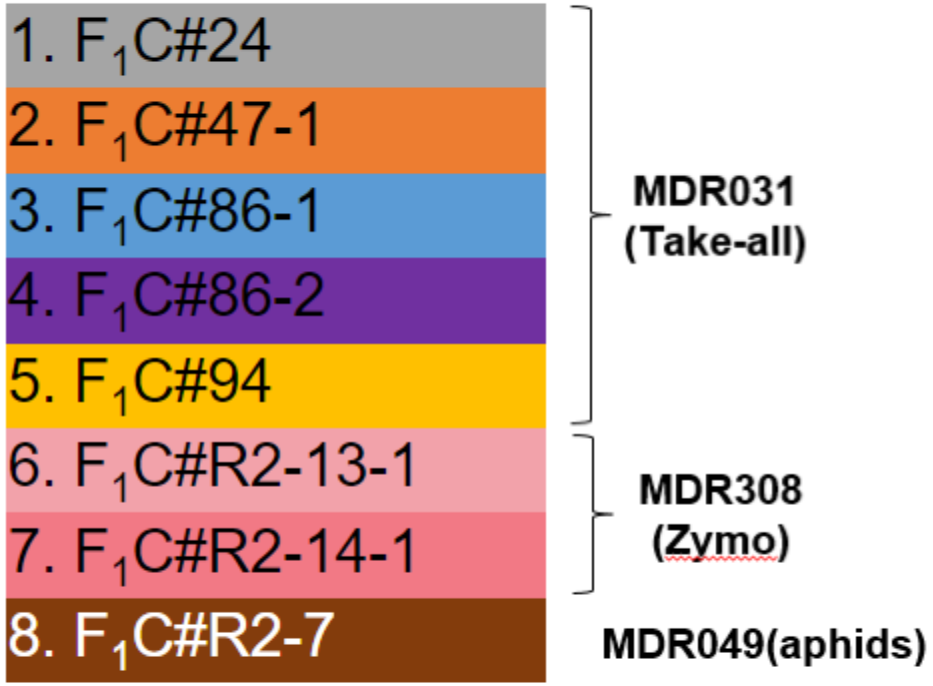
8 F1C complexes generated...



F_1C = F_1 Complex
(pentaploid)

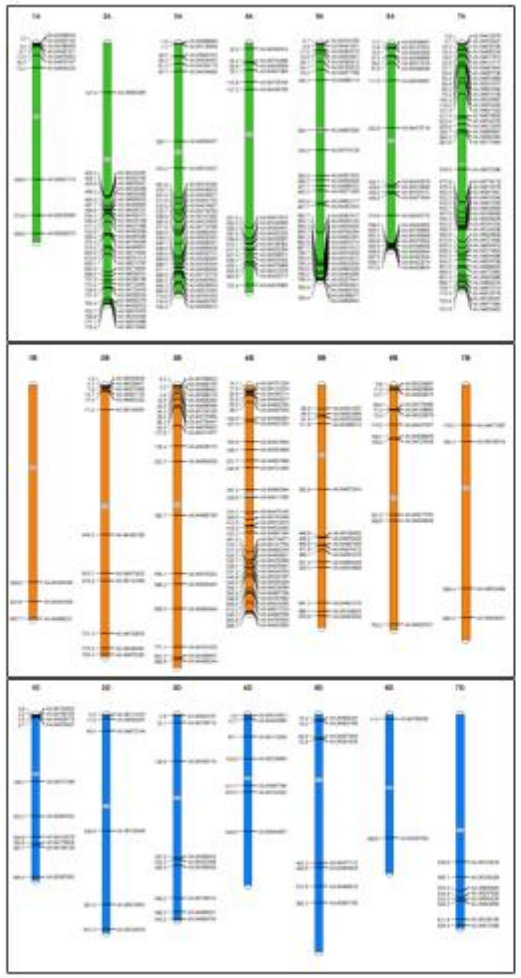
...approx. 5000 F1 hybrid and F1C crosses later

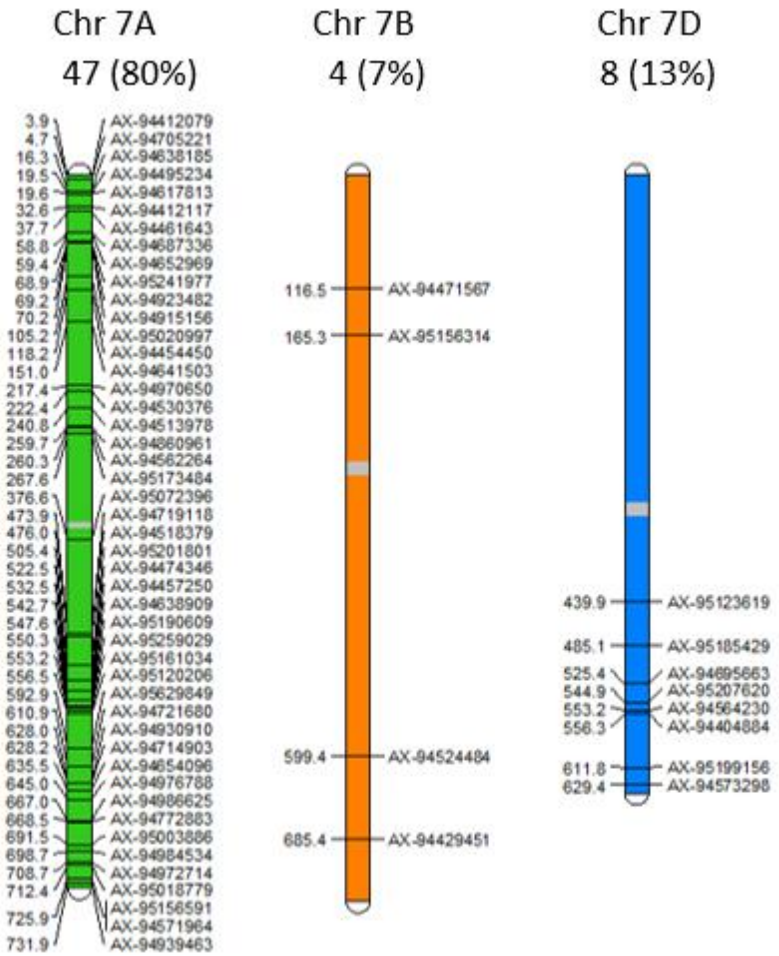
Genotyping on 35K Breeders' Array

- considerably **higher introgression in A genome**: A-55% B-30% D-15% for all chromosomes
- especially high introgression on **chromosome 7A**

hetMarkers total:

- **A Genome** – 182 markers (55%)
- **B Genome** – 99 markers (30%)
- **D Genome** – 50 markers (15%)





Timeline

Field Trial 2020

- **28 introgressed lines** (+ controls)
- **No fungicide treatment** to assess response to naturally occurring fungal disease

4 replicate blocks with up to 6 plants per block each (**∑672 plants**)

selfing 2021

- **1400 individual plants selfed 3x** (glasshouse) to make them **near homozygous / isogenic**
- assessment of phenotypes

planted in triplicate in individual 1 in^2 cells, all ears bagged before anthesis

seed multiplication 2022

- **seed multiplication of 1023 individual plants** (& 6 Paragon pots) in GH
- **phenotypes scored**, including plant and ear length, seed numbers and weight

3 plants grown in
2l, 25 in^2 pots,
∑1029 pots,
bagged before anthesis

Field Trial 2023 Spring

- **Spring 2023 sowing of ~700 lines** (100 seeds in 1m^2 plots)
- **assessments throughout season** by Rothamsted and **other interested parties – let us know if you are**
- September/October 2023: distribution of seed to Breeders' Observation Panel (BOP)

details being worked out right now

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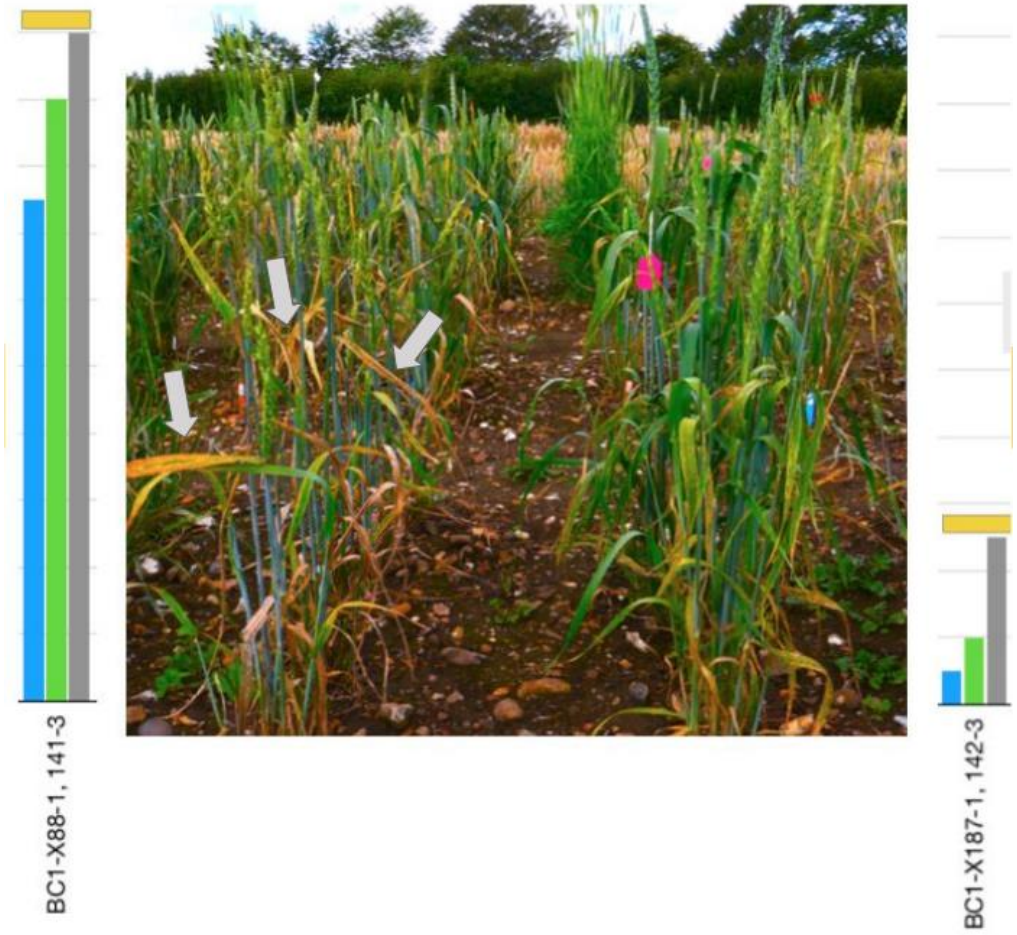
Field Trial 2020

- **696** individual introgressed seedlings planted
- all plants scored for **Yellow Rust**, height, senescence, flowering, awns, waxiness
- growth stages recorded twice a week
- individual plants from the same line segregated for various traits both within rows as well as blocks
- thus decided to harvest ears and grain from all plants individually
- ears from a **total of 509 introgressed plants** were harvested.



example of high Yellow Rust resistance:

- plant 3 in row 142 (142-3, red flag) had **less than 5% YR**
- neighbouring row 141 – all plants highly susceptible (white arrows)
- also neighbours in same row highly susceptible

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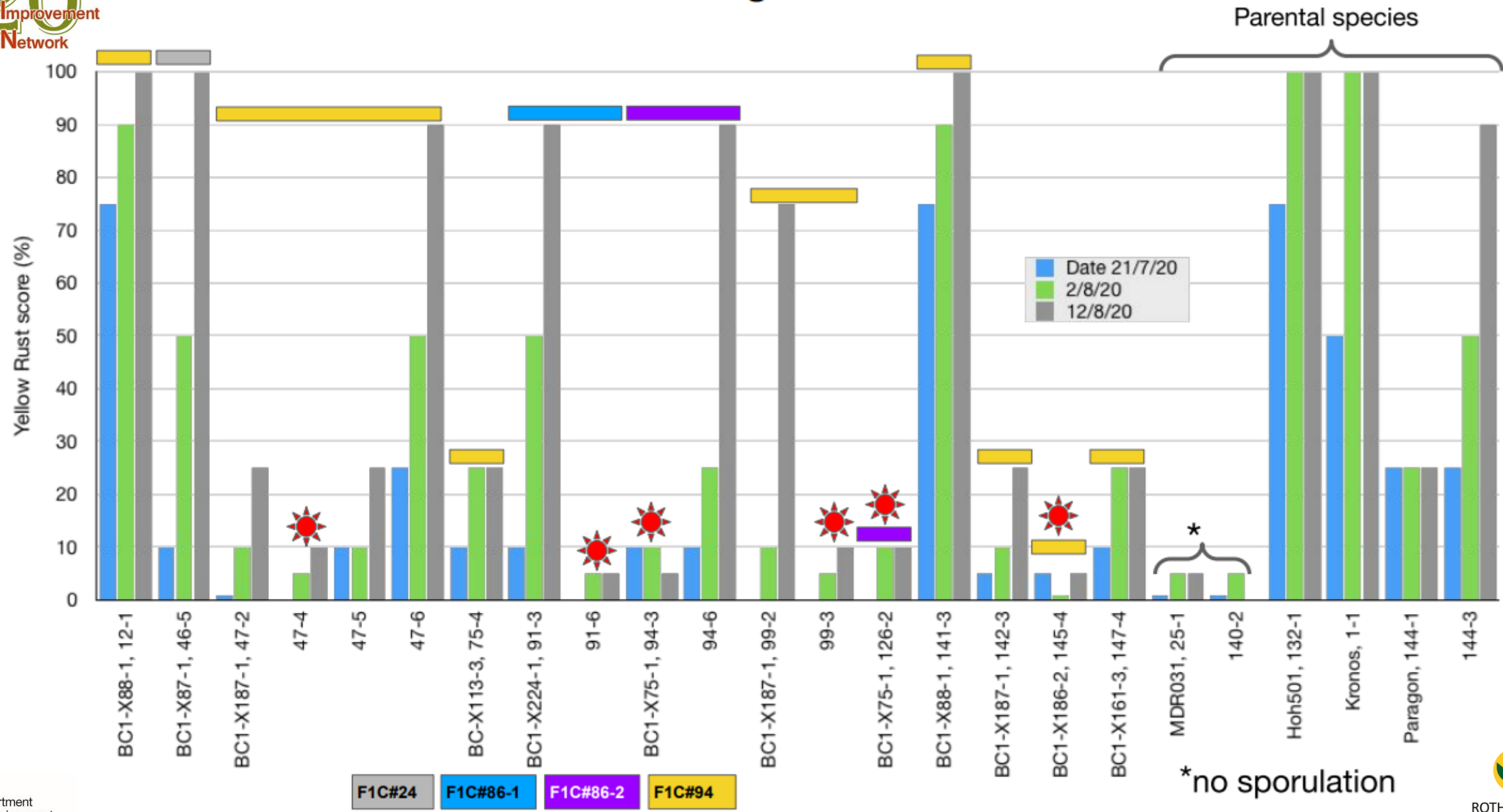
Yellow Rust Scoring of Field Trial



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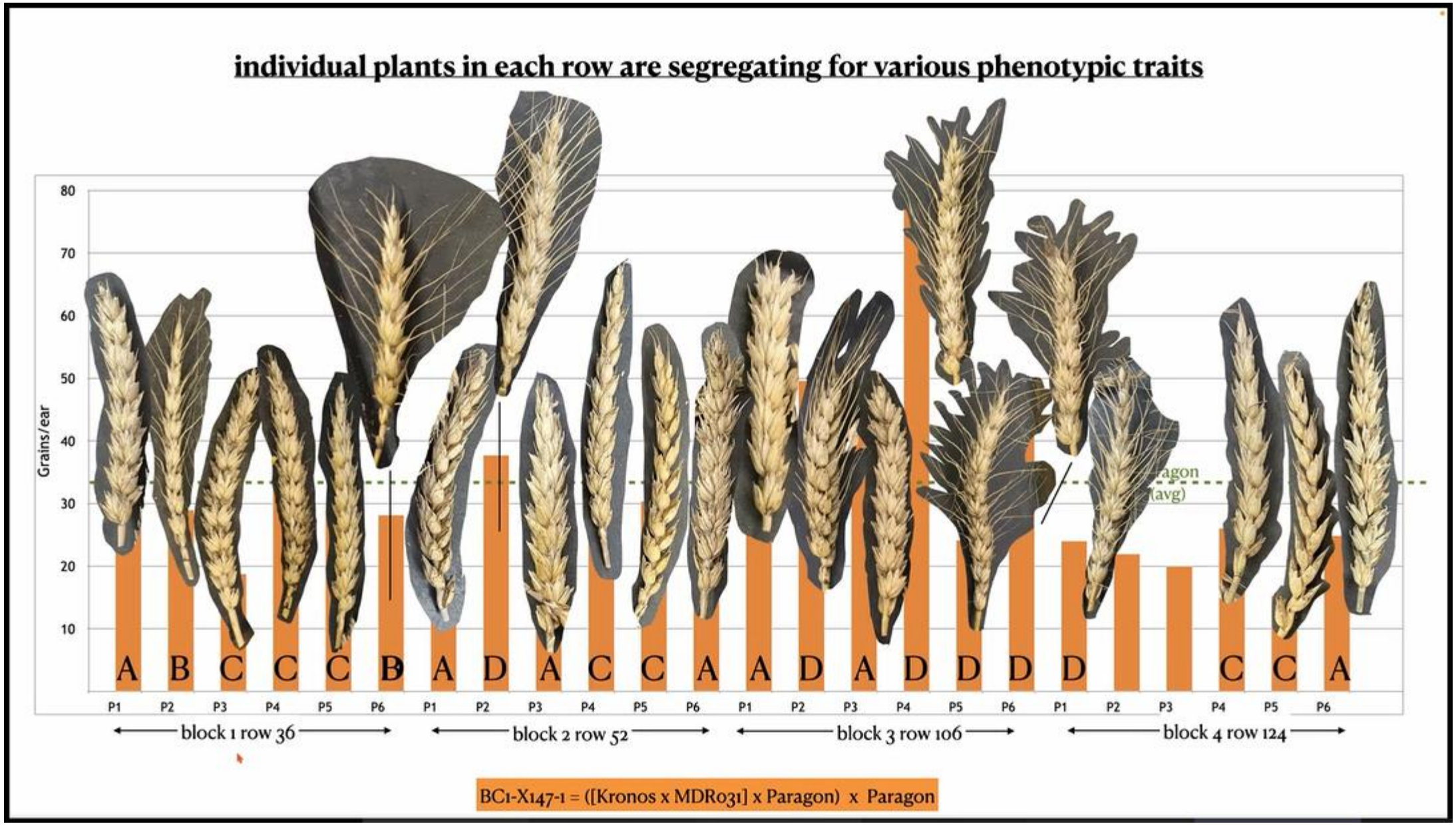

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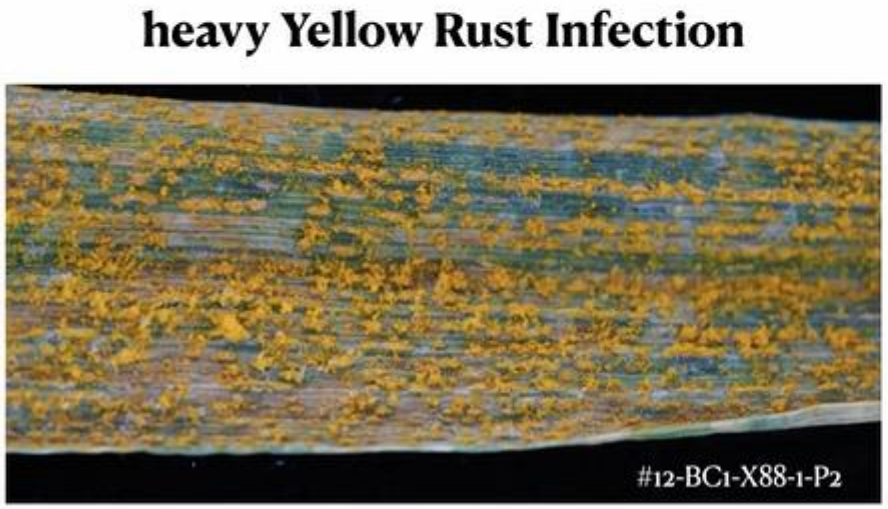
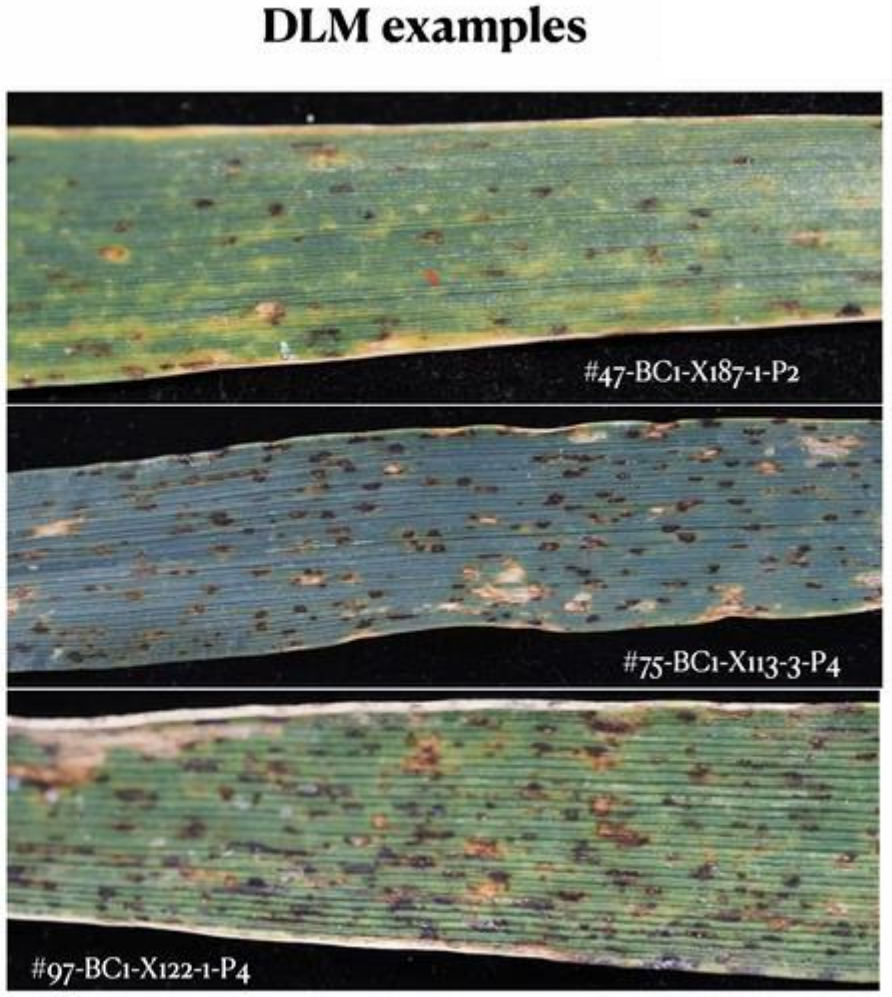
Disease Lesion Mimics (DLM) – they mimic Disease without fungal spores present on leaves

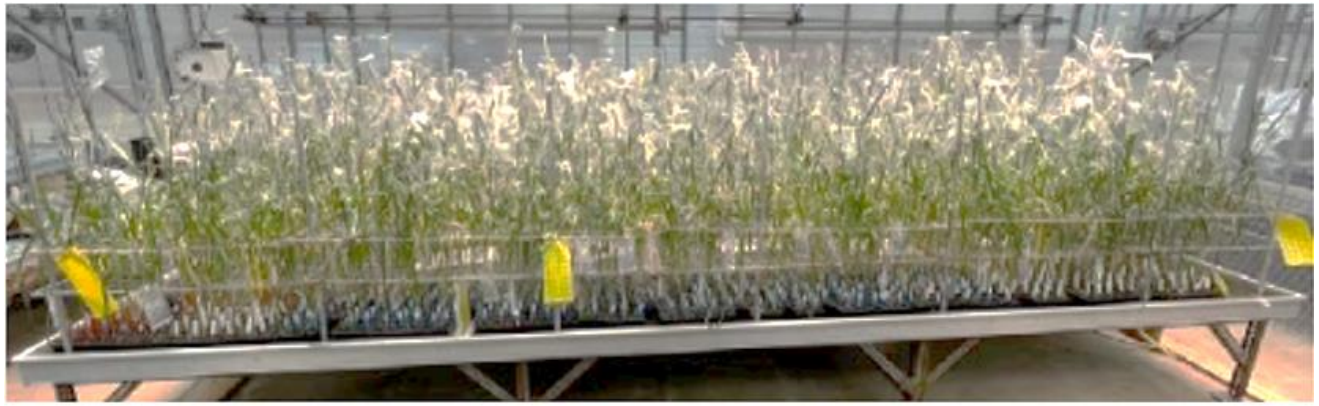
- 41 individual plants in Field Trial exhibited DLM



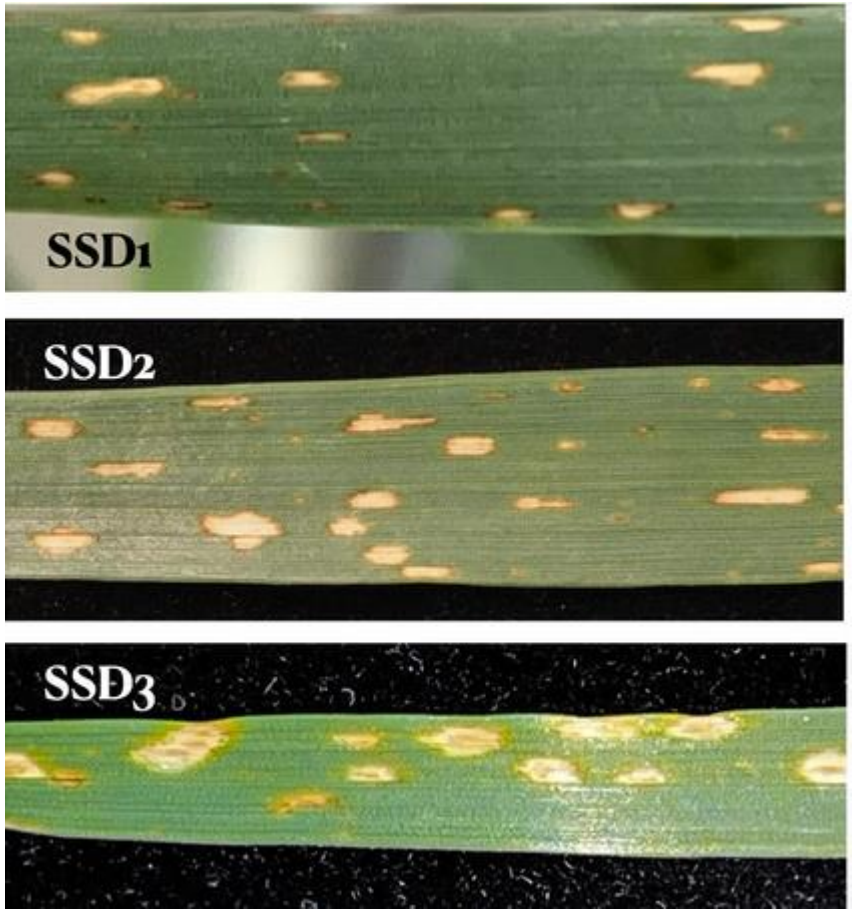


selfing 2021

- **1400 individual plants selfed 3x** (glasshouse) to make them **near homozygous / isogenic**
- assessment of phenotypes: **DLM present**, in several lines throughout all 3 rounds of SSD



3 rounds of selfing (**Single Seed Descent**) to create near **homozygous lines**



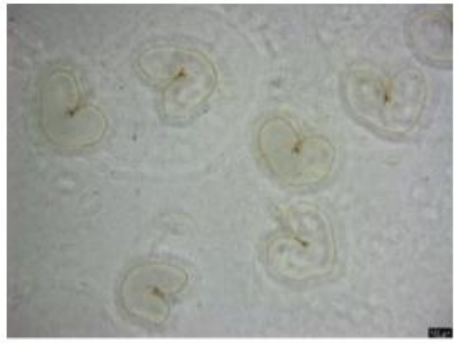
Dark Wheat?!

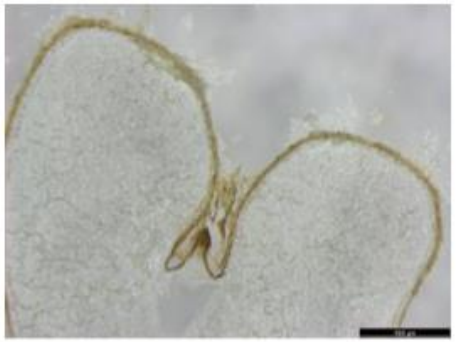

0_Paragon_contol
1_257-2_white_ctrl
2_257-1_BK_grey_01a
3_347-1_white_ctrl
4_347-3_D;BK
5_359-3_white_ctrl
6_359-2_BK
7_359-1_BK

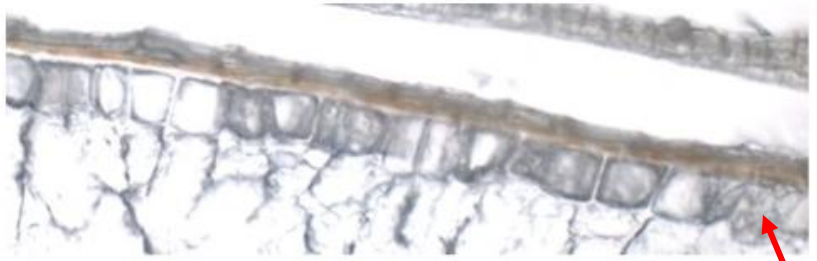

8_262-2_white_ctrl
9_262-1_BK
10_249-1_BK_grey
11_249-2_BK_grey
12_249-3_BK_grey
13_350-1_D_BK
14_350-2_BK


15_391-2_D_BK
16_391-3_BK_grey

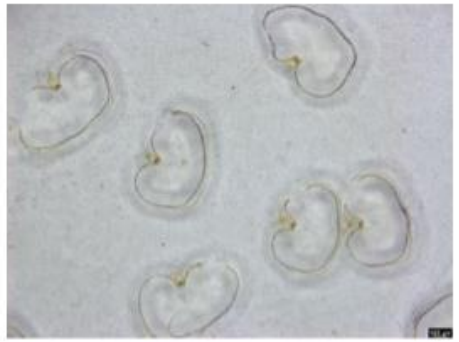

5_359-3_white_ctrl

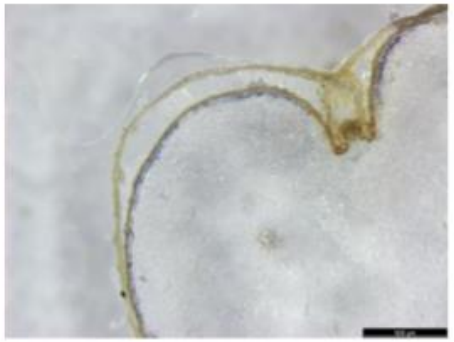






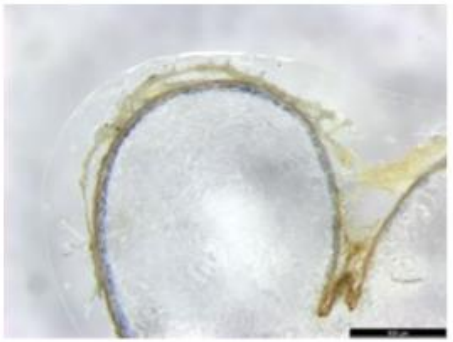

6_359-2_BK





aleurone layer


7_359-1_BK



- dark colour caused only by **blue anthocyanin in aleurone layer**
- no purple colour observed in pericarp

many thanks to Kirstie Halsey (Bioimaging, Rothamsted) for sample preparations and imaging

seed multiplication 2022

- seed multiplication of 1023 individual plants in GH
- phenotypes recorded including plant and ear length, seed numbers and weight

3 plants grown in
2l, 25 in^2 pots,
∑1029 pots,
bagged before anthesis

traits scored:

- primary tiller length
- ear length
- presence of awns
- ear shape
- number of tillers
- grain numbers per line (each pot)
- extrapolated Thousand Grain Weight
- grain shape and colour

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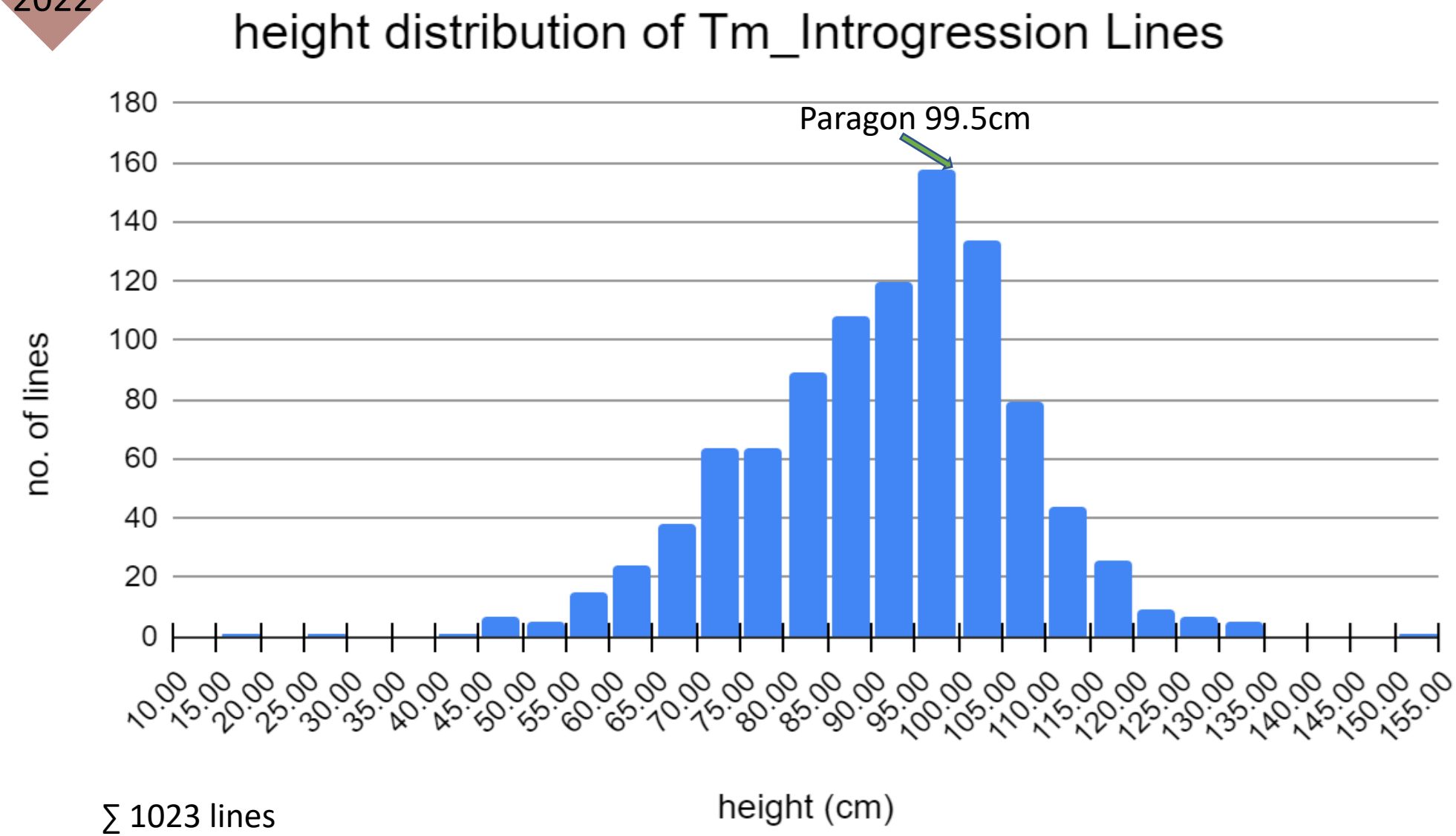
seed multipli-cation 2022



∑ 1023 lines

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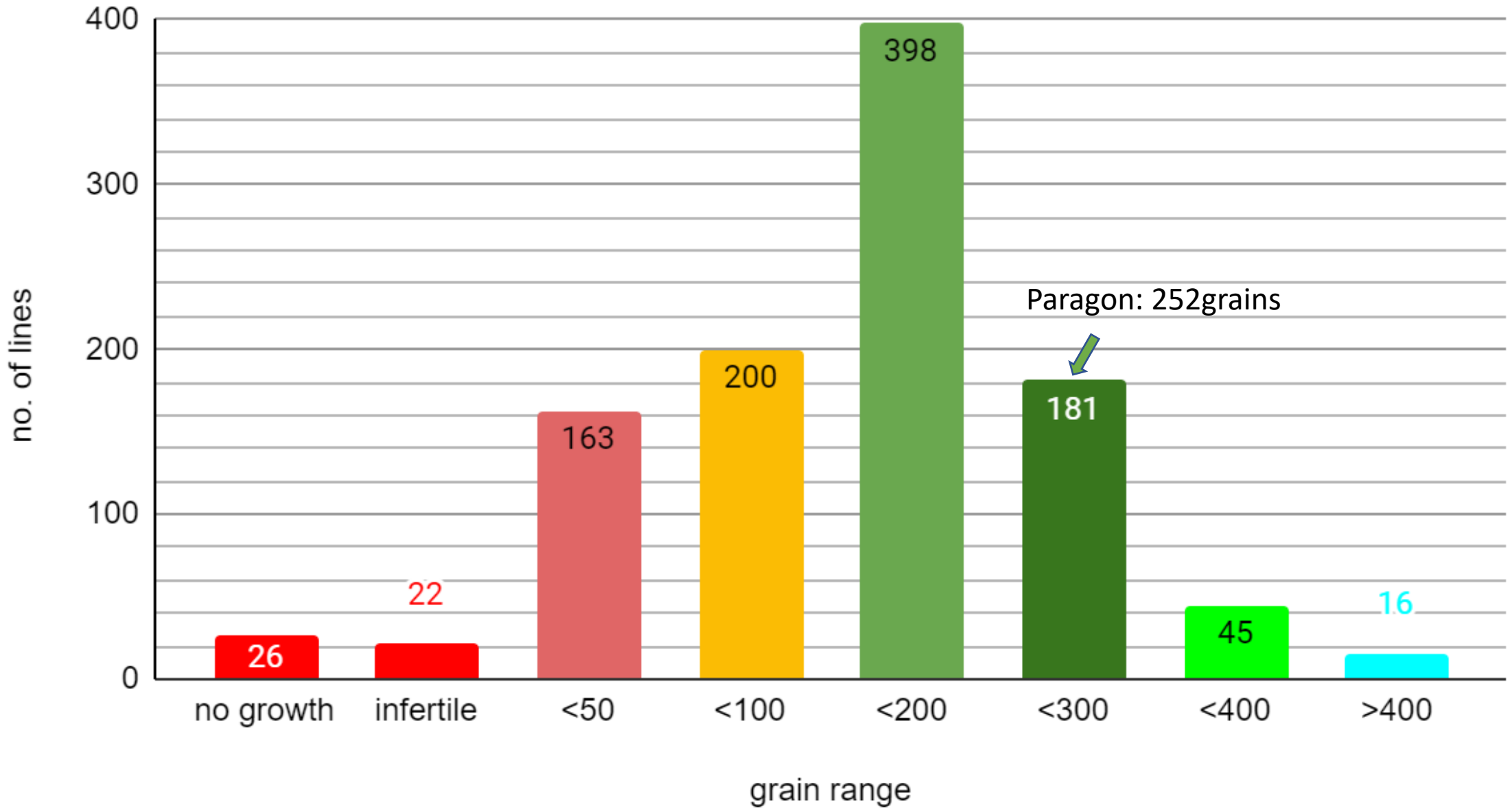
Grain Yields of Introgressed Lines



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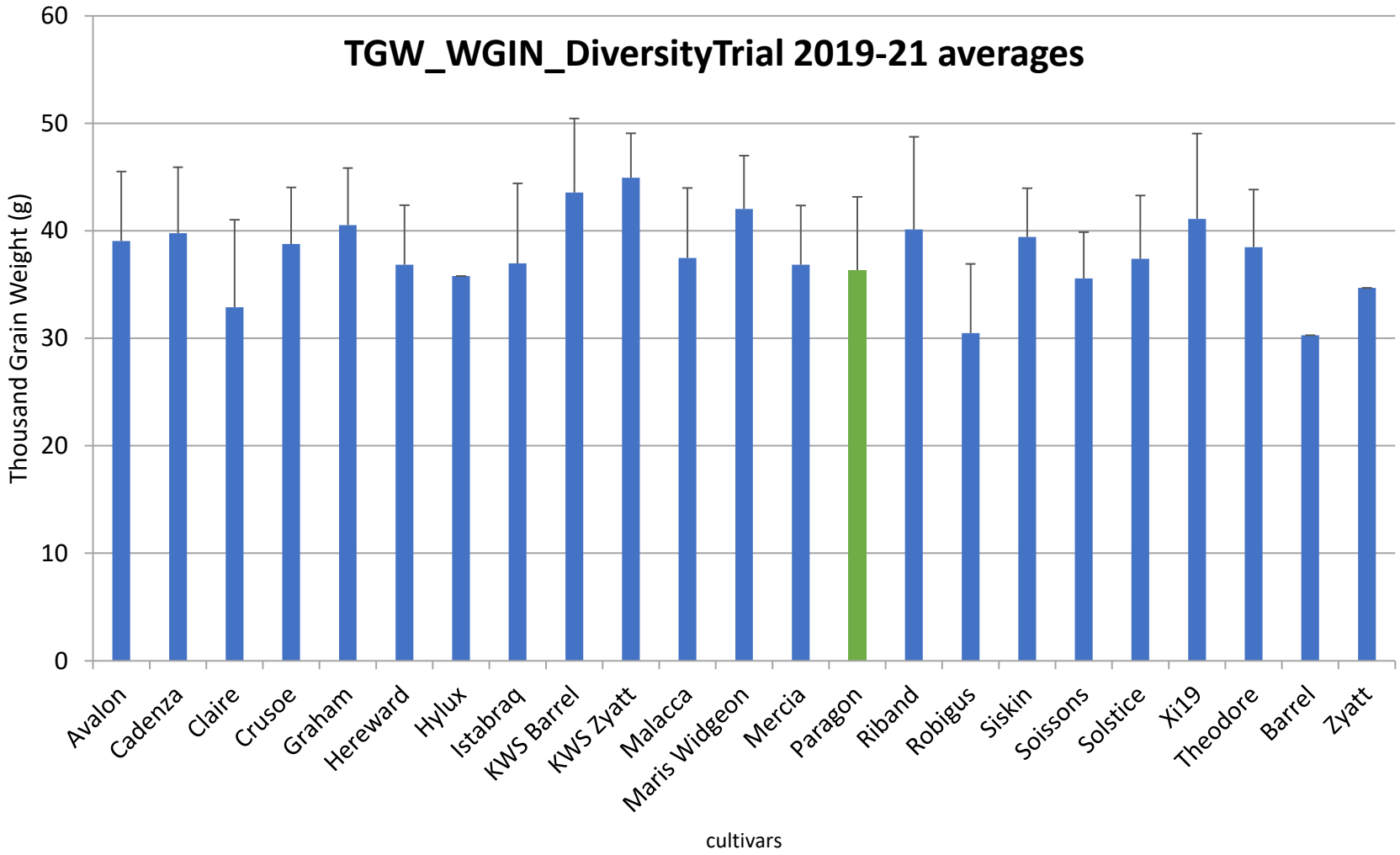
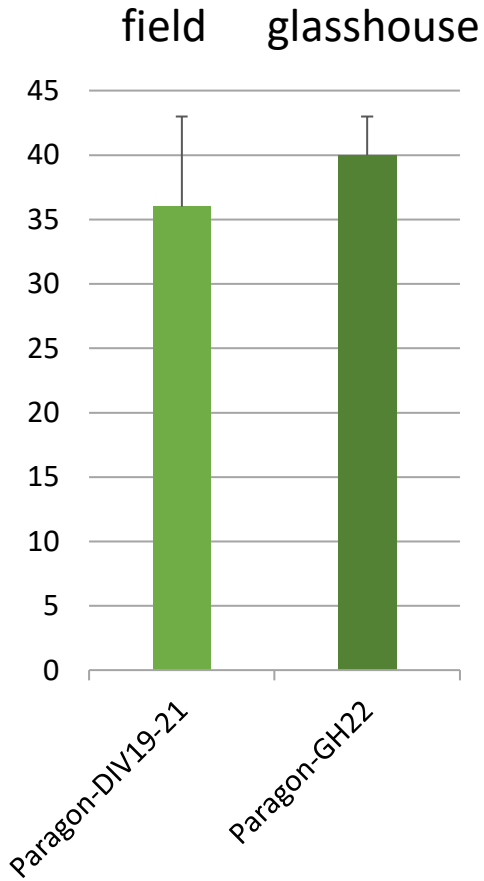
Thousand Grain Weights (TGW) of Wheat Cultivars used in UK Agriculture





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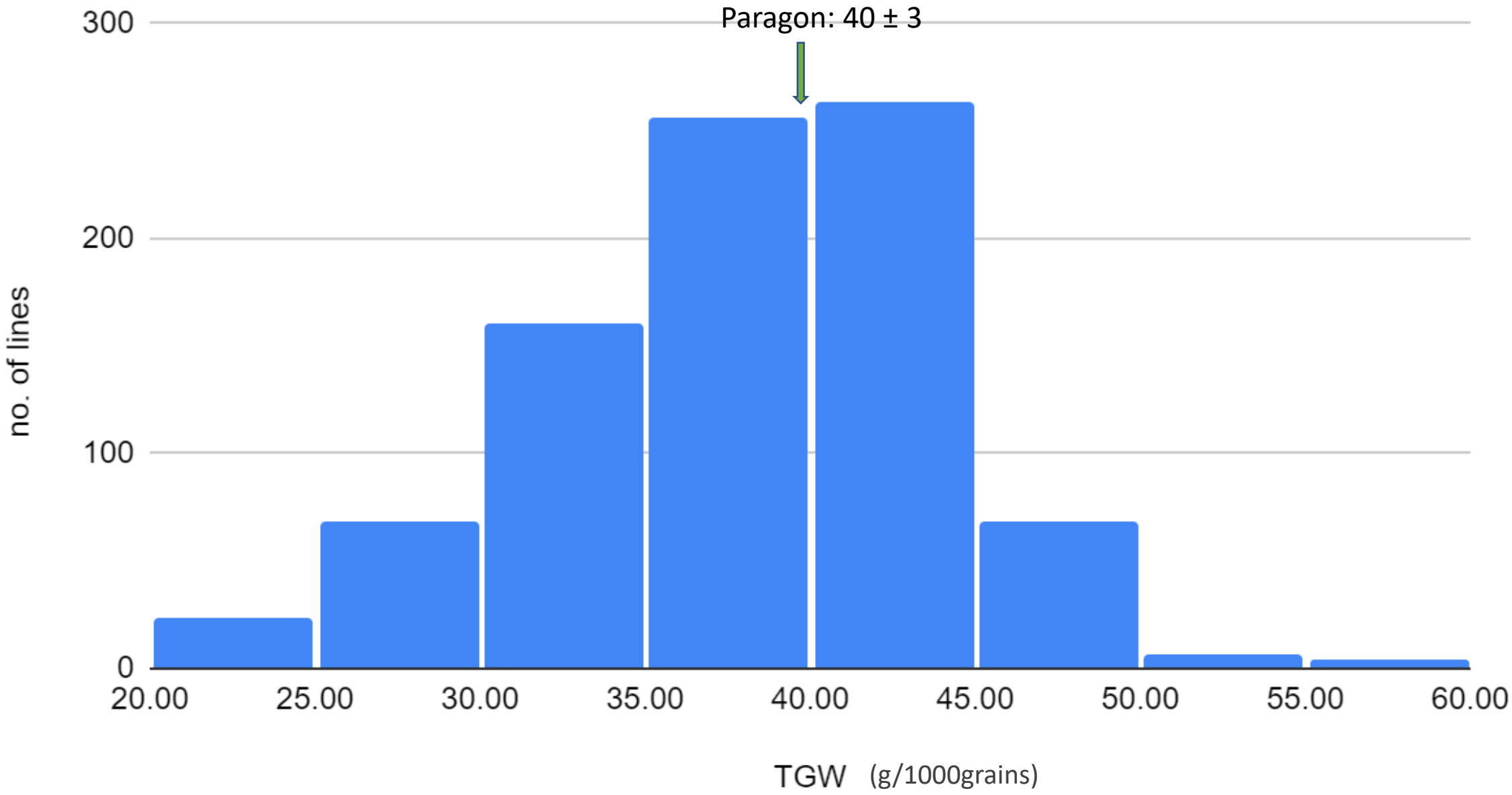
TGW distribution of T.mon Introgression Lines in Glasshouse



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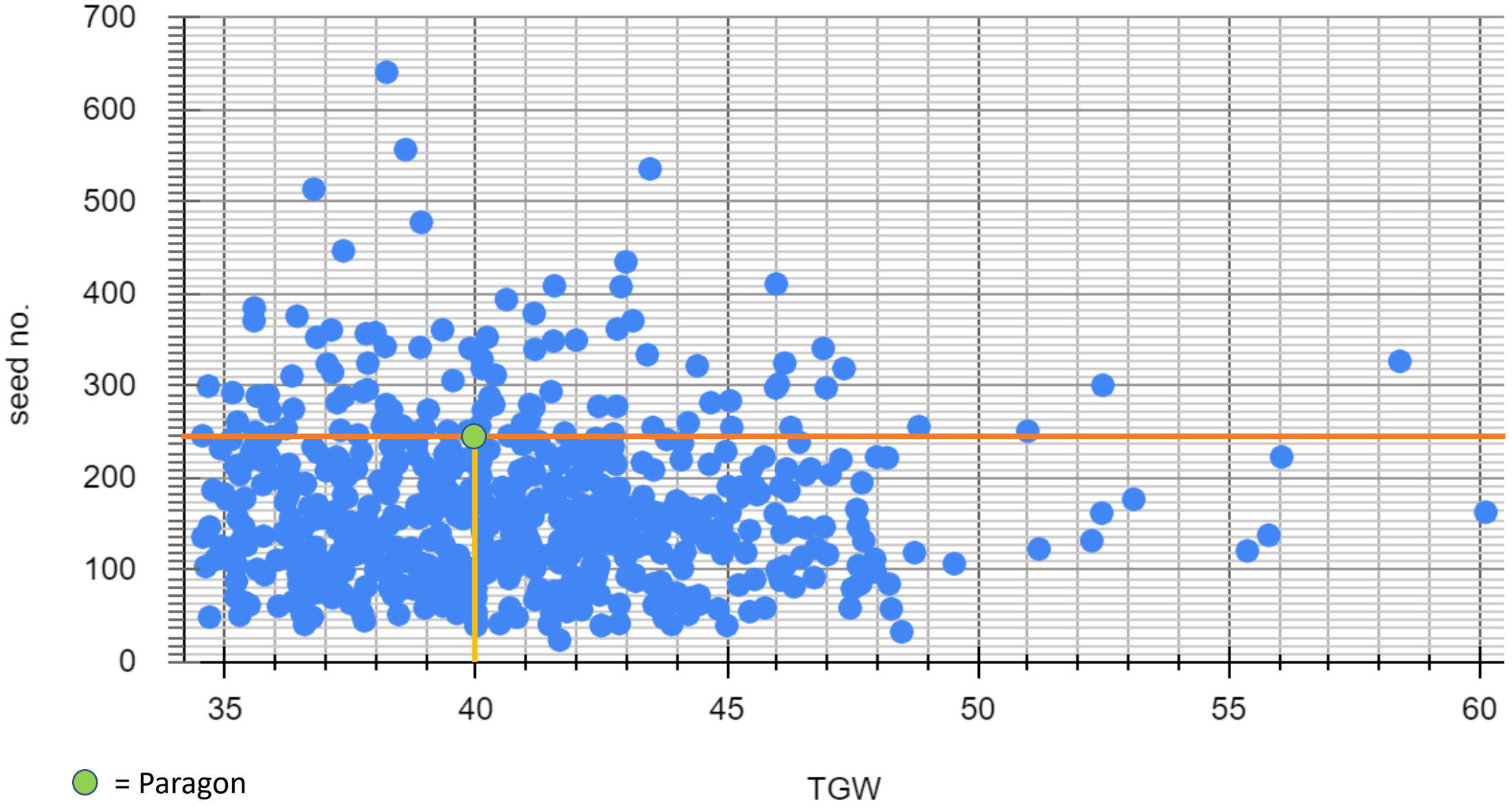
Scatterplot of TGW vs Seed Numbers for all Introgression Lines



= Paragon

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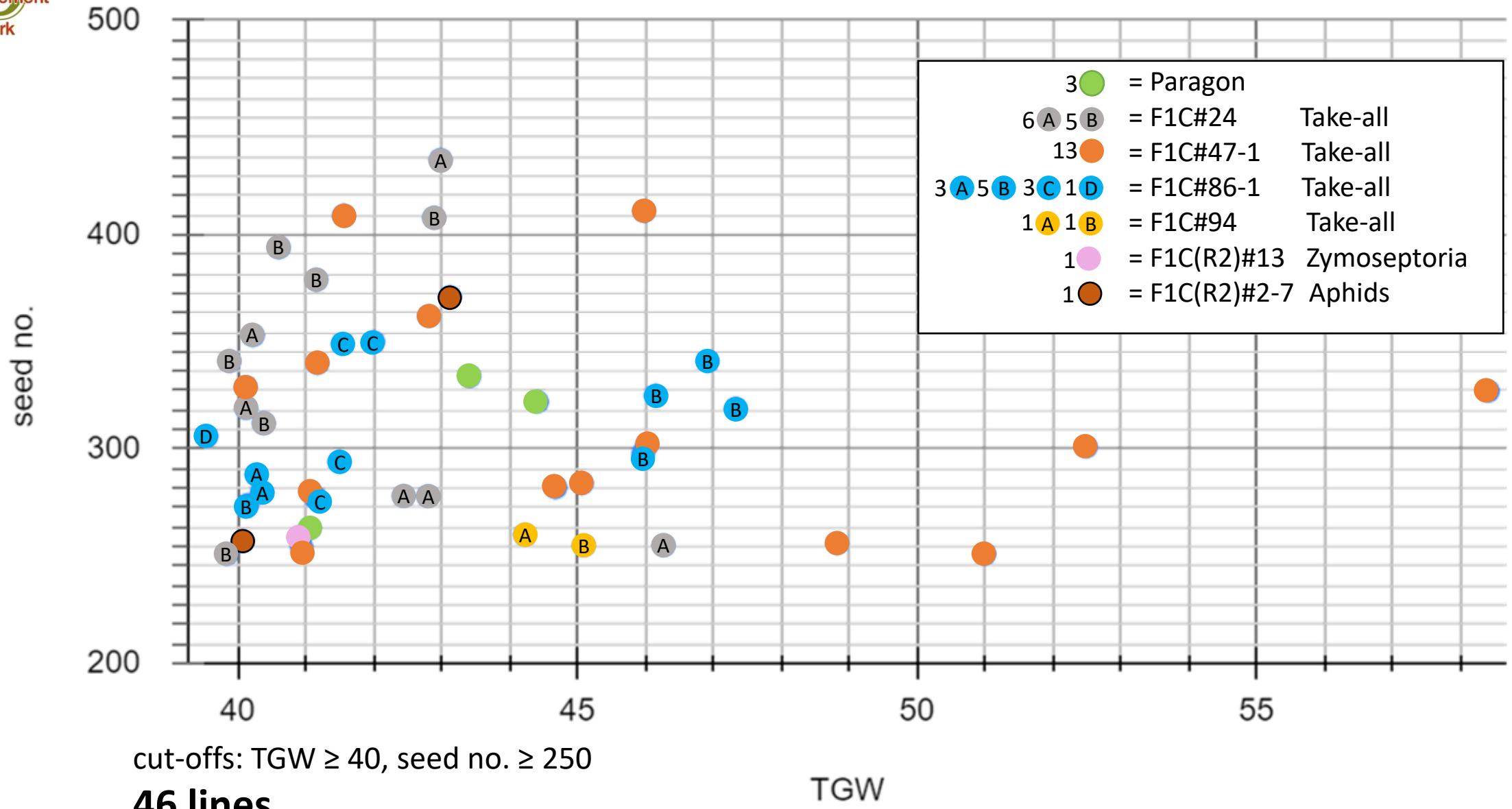
Scatterplot of TGW vs Seed Numbers for Paragon exceeding Introgression Lines



cut-offs: TGW ≥ 40, seed no. ≥ 250

46 lines

7 grains in 1 Spikelet – the way forward or just an aberration?

pot #791: SSD3 385-1 | F1C#94 | BC1-X186-1

Summary

Grain Yield

- 35% of Tmon introgression lines have a TGW equal or larger than Paragon, but most have lower grain count
- BUT – **43 individual lines (4%)** have larger grain count **and** TGW, promising larger yields in the field straight away, ie no need to backcross to high yielding cultivars

Height

- considerable height variation from 15cm to 154cm (Paragon 99.5cm) makes these lines useful for novel genes influencing height

Disease Control

- novel **Disease Lesion Mimics** for control of leaf pathogens
- Yellow Rust resistance (6 lines <5% disease in field trial 2020, field trial confirmation needed)

other traits

- some lines segregating for dark grain (nutritional benefits?)
- ear morphology
- awn types
- possible high(er) fibre content and other nutritional benefits (TBC)

if you wanted to be involved, please come and look at our field trial this summer

Acknowledgments

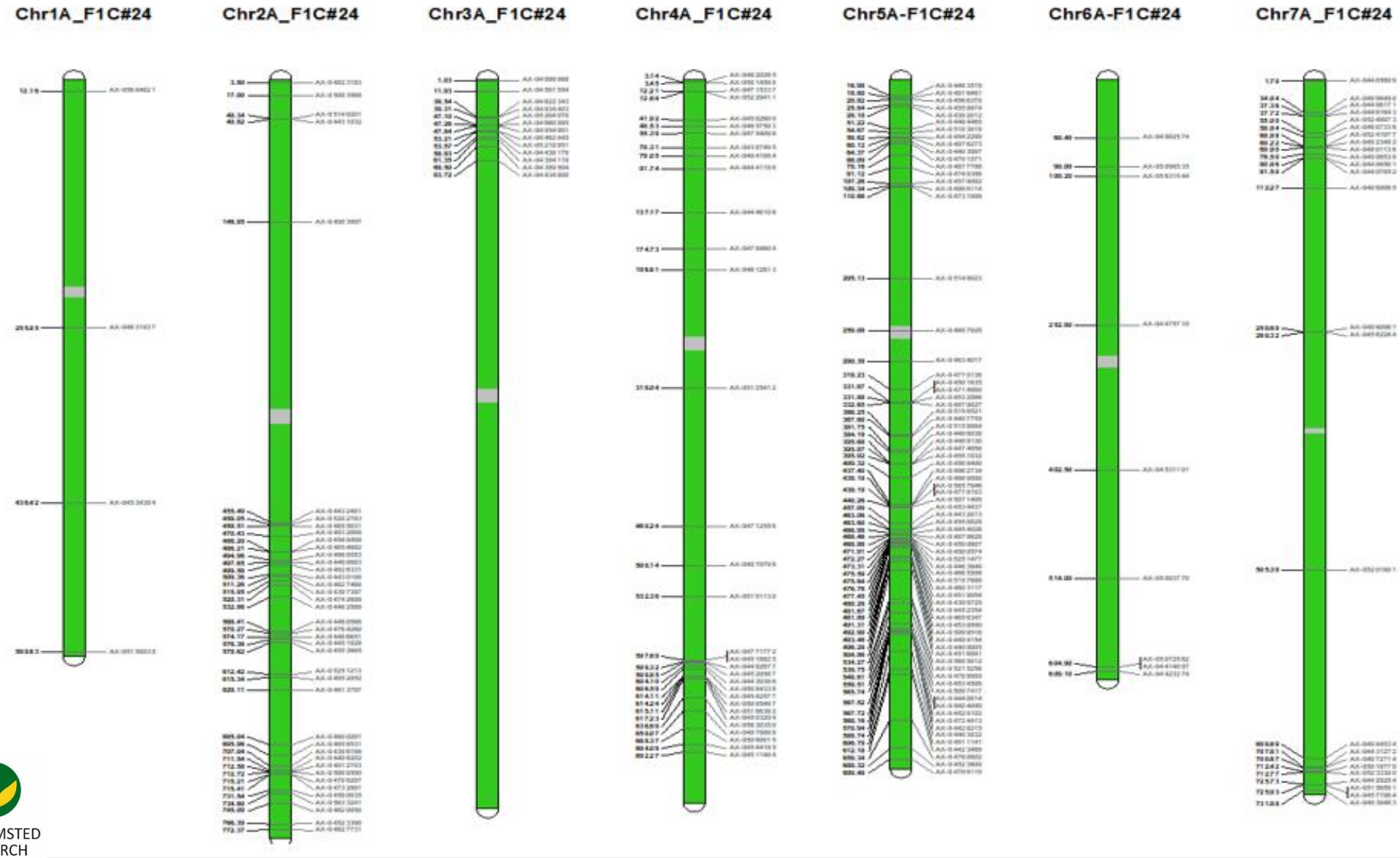
- **Defra** – only the longterm funding of WGIN made this project and successful outcome possible
- **Kim Hammond-Kosack** – helping out a lot (Field Trial 2020, SSD and multiplication), planning, moral support and being a great wife
- The **WGIN Management Team** for useful discussions, particularly Nick Bird (KWS) for many useful comments and suggestions
- Rothamsted Glasshouse staff
- Rothamsted field staff
- **Leo Barr** and **Nida Ghori** (the new Take-all leader at Rothamsted) for help with ear harvest, measurements and imaging of at the multiplication stage
- **Kirstie Halsey** for imaging the aleurone layers
- **Kostya Kanyuka** (NIAB, formerly RRes) for help with Zymoseptoria assays, genotyping and wheat genetic discussions
- **Bristol Genomics Facility** for genotyping (35k Breeders' Array)

PS: ...and now there are 20 balloons as well ☺

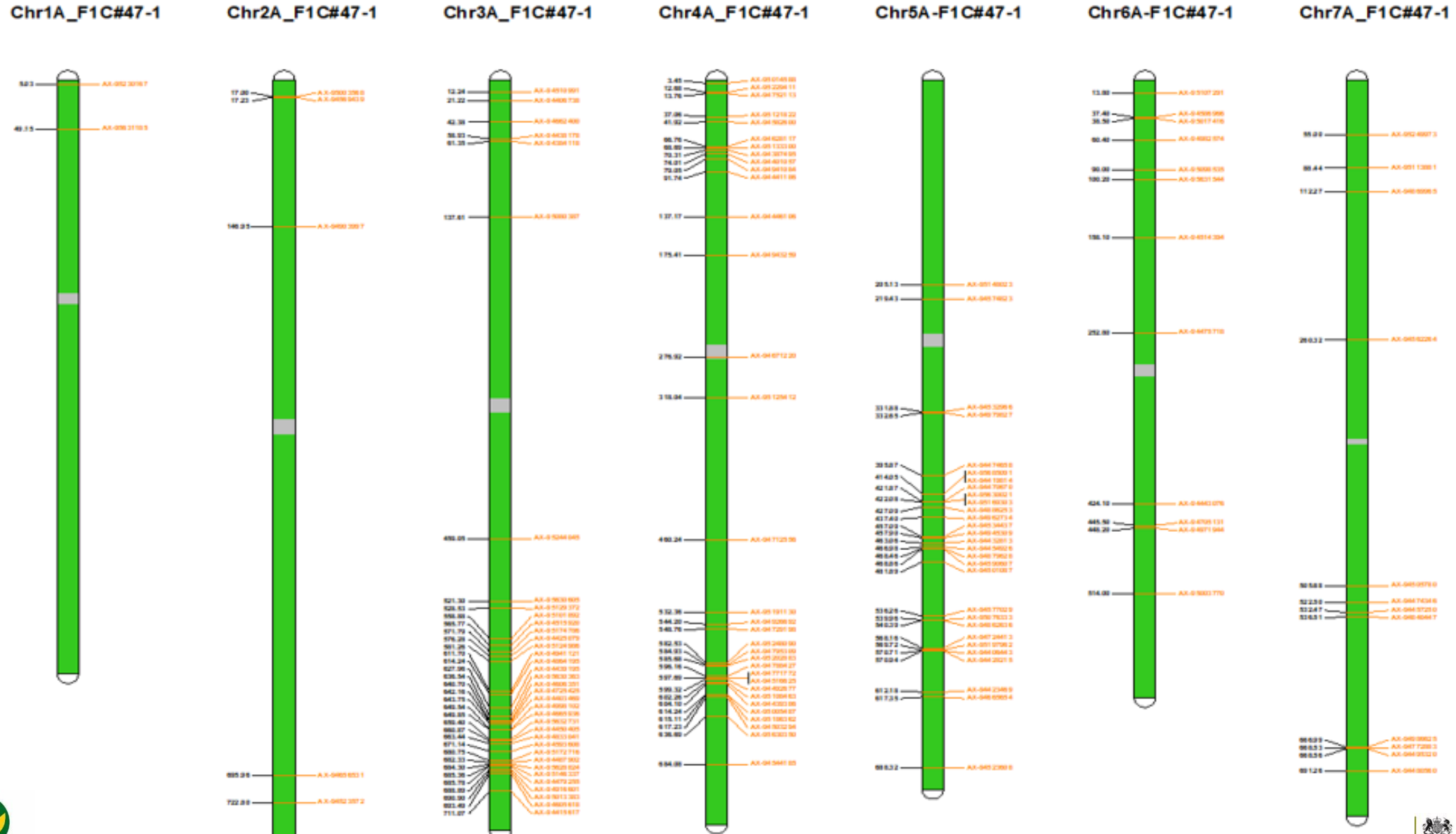
contact Mike at wgin.defra@rothamsted.ac.uk

Introgression in the A Genome of the five F1 Complex Plants

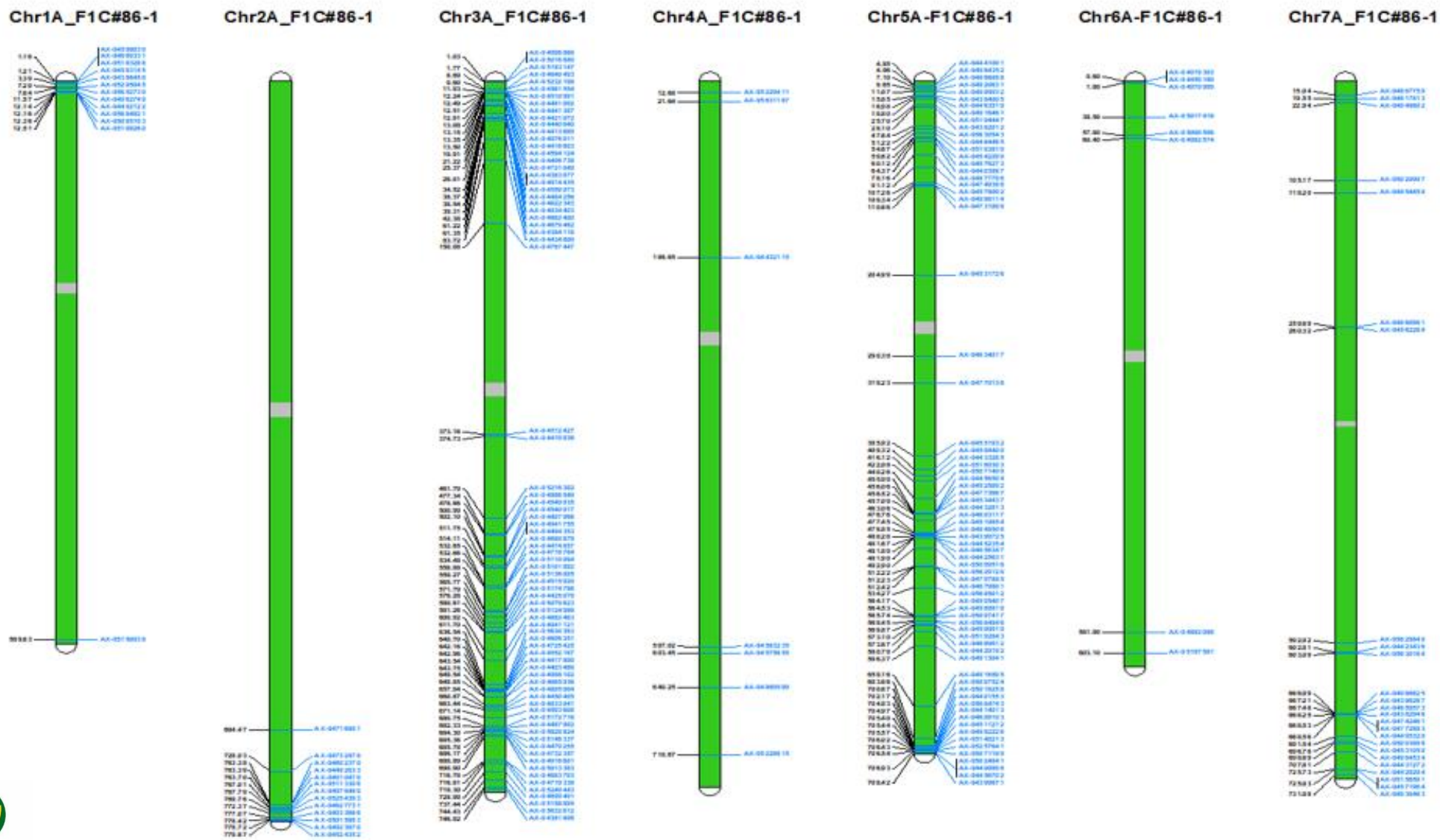
1. **F1C#24** – [Kronos x MDR031] x Paragon



2. **F1C#47-1** – [Kronos x MDR031] x Paragon



3. **F1C#86-1** – [Hoh501 x MDR031] x Paragon



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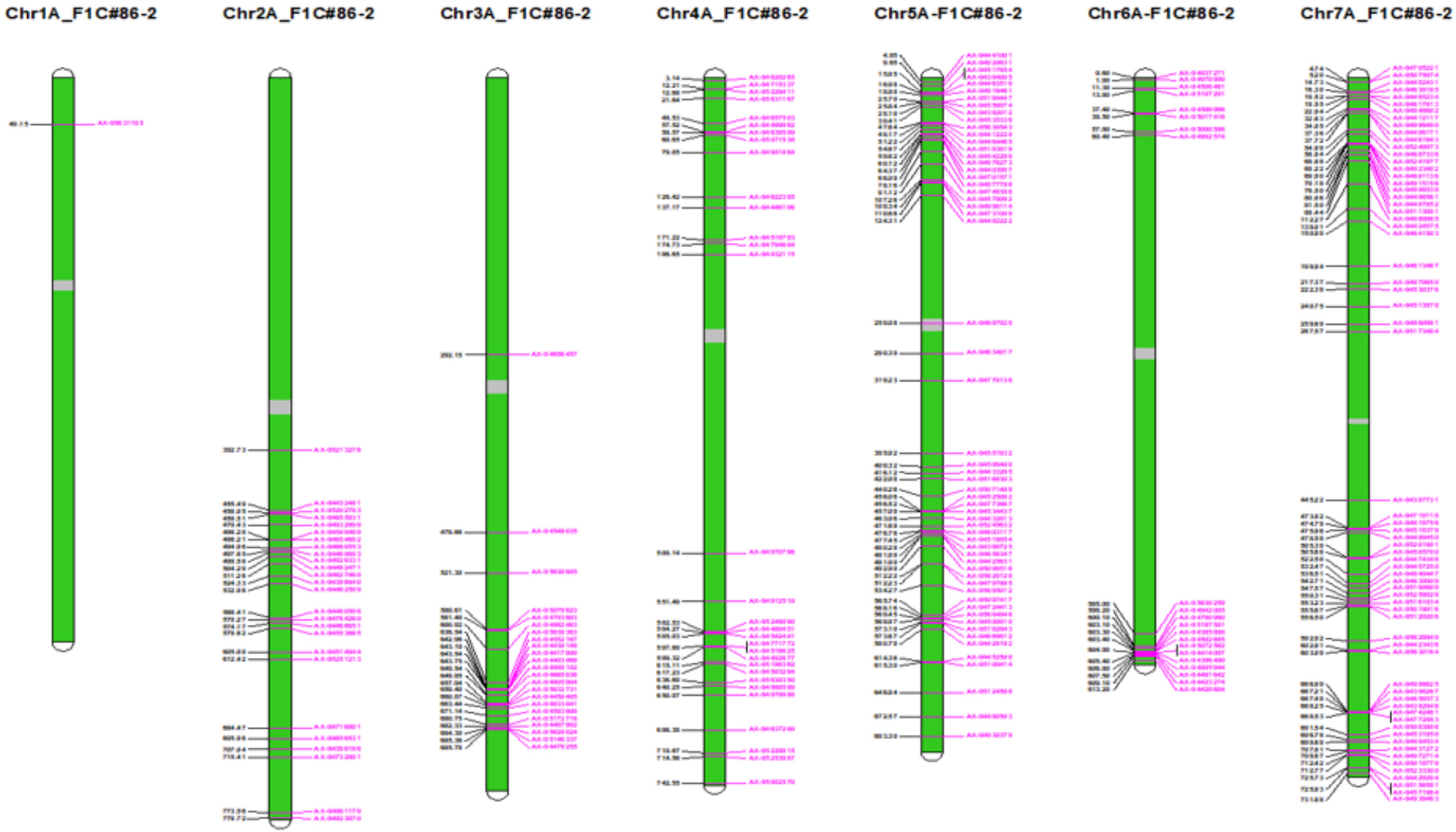
4. **F1C#86-2** – [Hoh501 x MDR031] x Paragon



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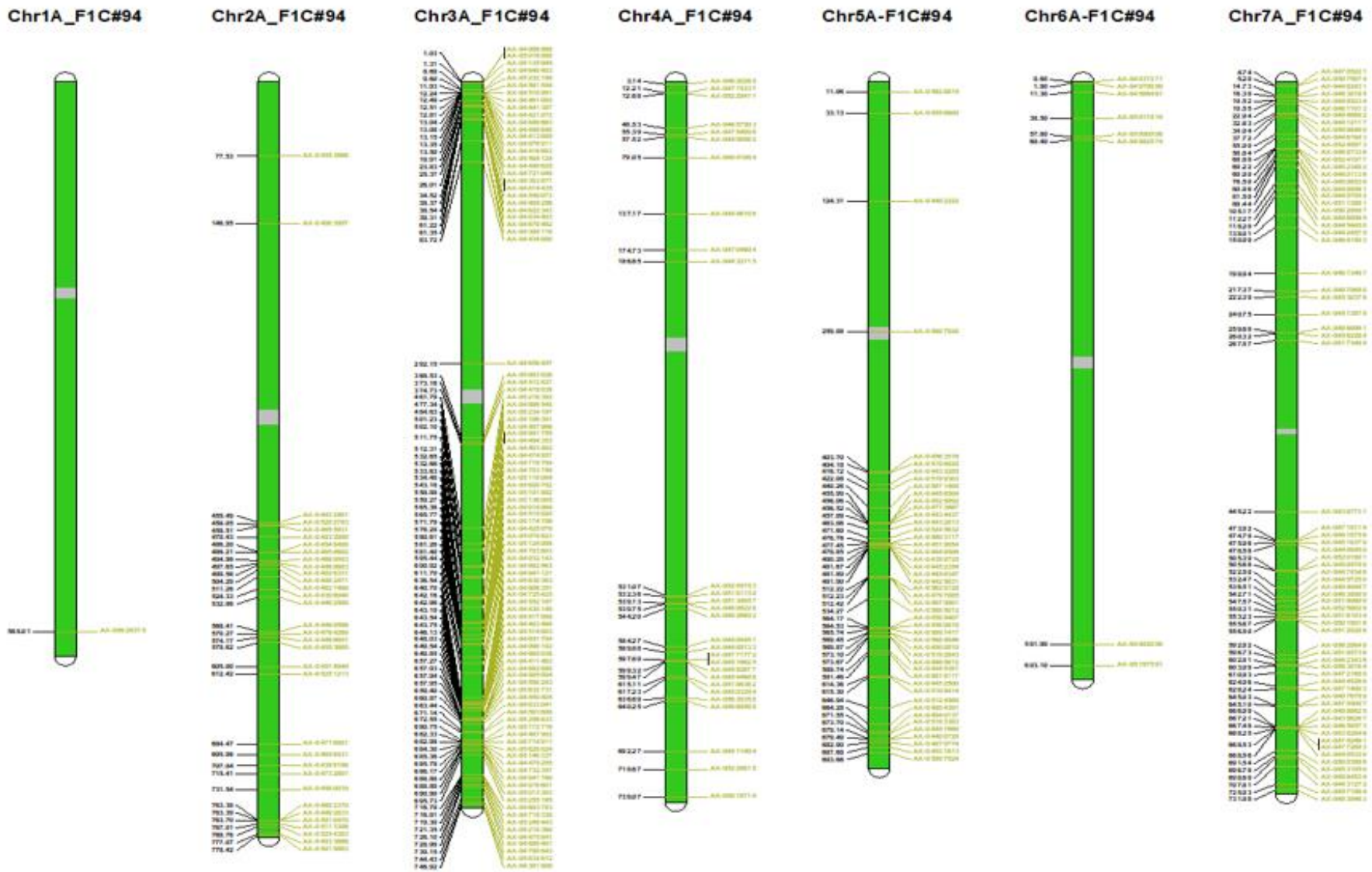
5. **F1C#94** – [Hoh501 x MDR031] x Paragon



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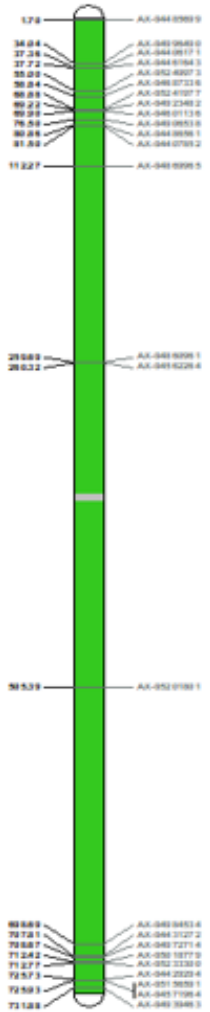
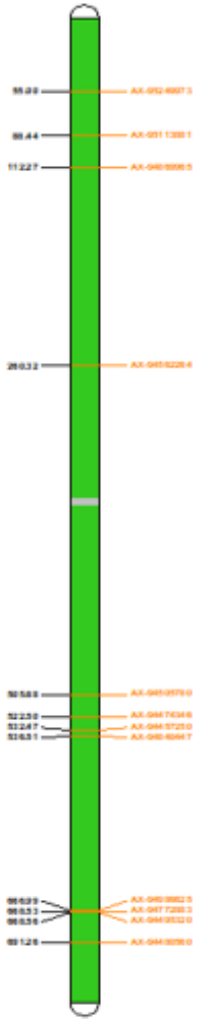
Chromosome 7A differences

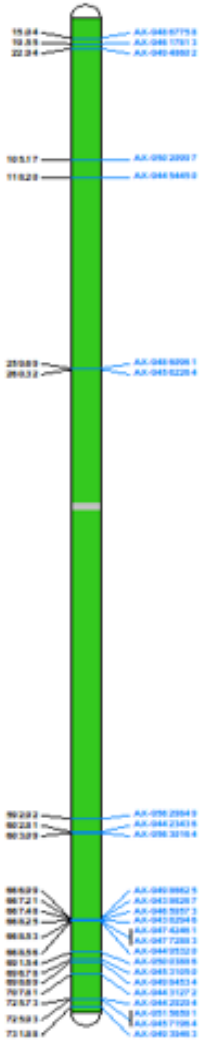
Chr7A_F1C#24



Chr7A_F1C#47-1



Chr7A_F1C#86-1



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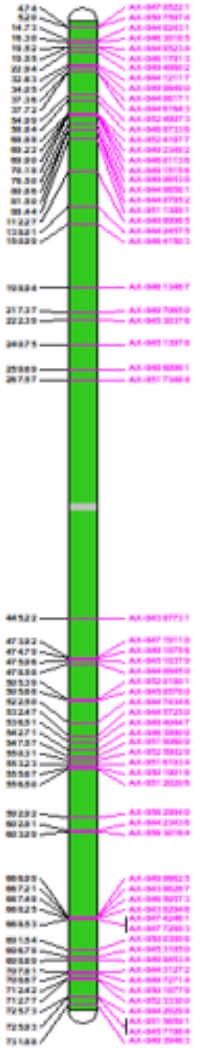
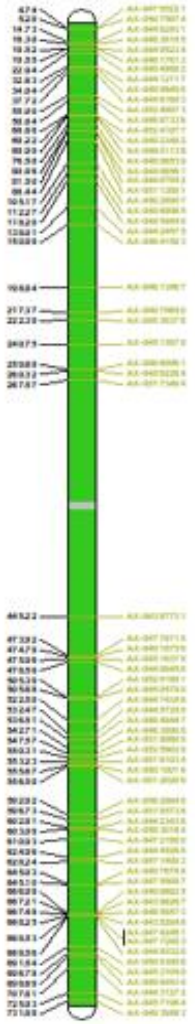


Chr7A_F1C#94



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Field Trial Summary

1. **Seven plants** from 4 introgressed lines have **high yellow rust resistance**

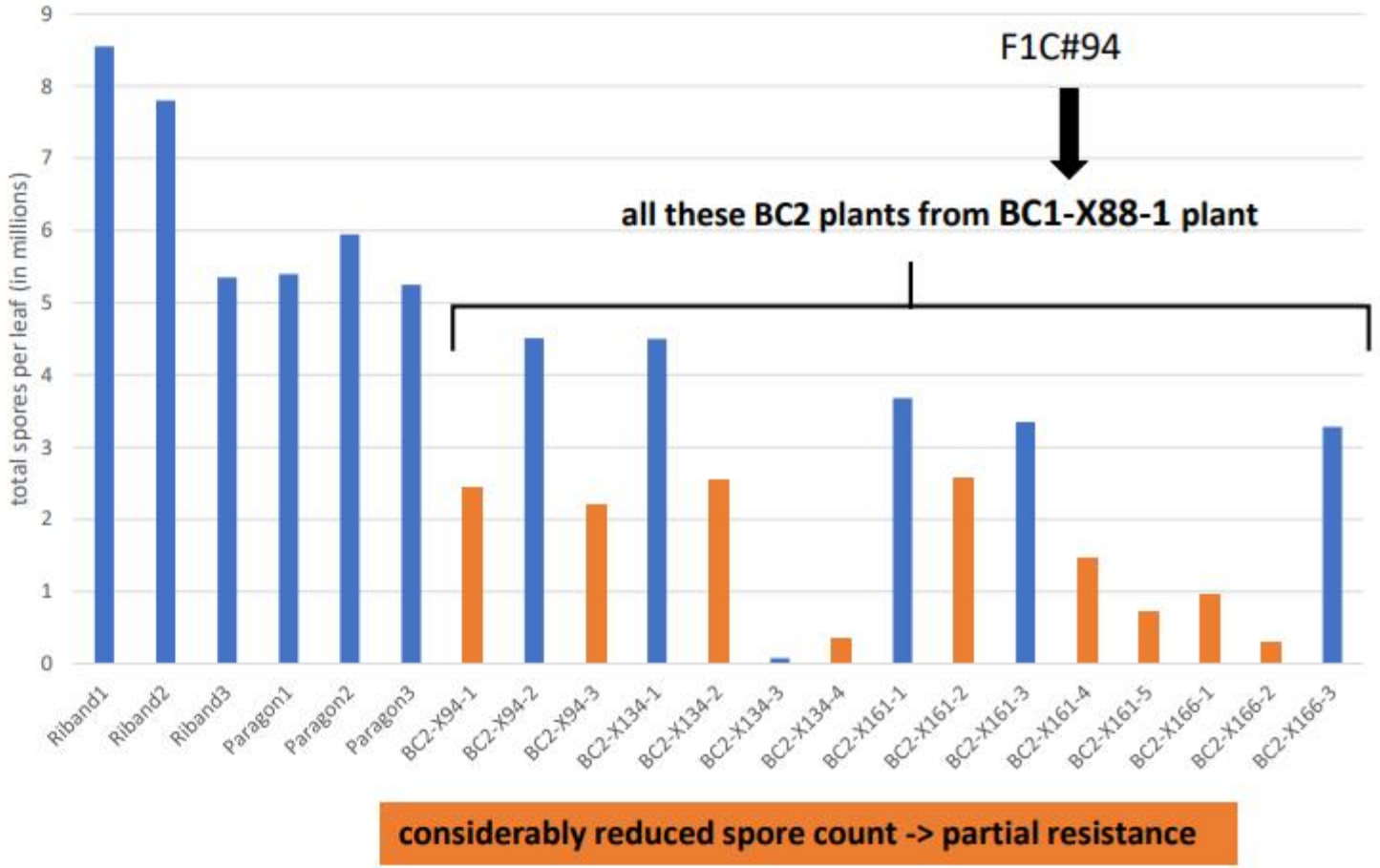
2. **Individual plants** in each row clearly **segregating** ==> they are genetically different

3. Many phenotypic differences (Awns, ear shape, leaf shape, waxiness, height...) show that **introgression has taken place.**

4. 41 individual plants are **Disease Lesion Mimics** (DLM) - they mimic disease lesions **without fungal spores** present on leaf:

"Lesion mimics (LMs) are disease-like symptoms that occur randomly on plant green leaves in the absence of pathogens. A previous study showed that LMs are related to enhanced resistance to a broad spectrum of diverse pathogen races and programmed cell death (PCD)." (Liu et al, 2021)

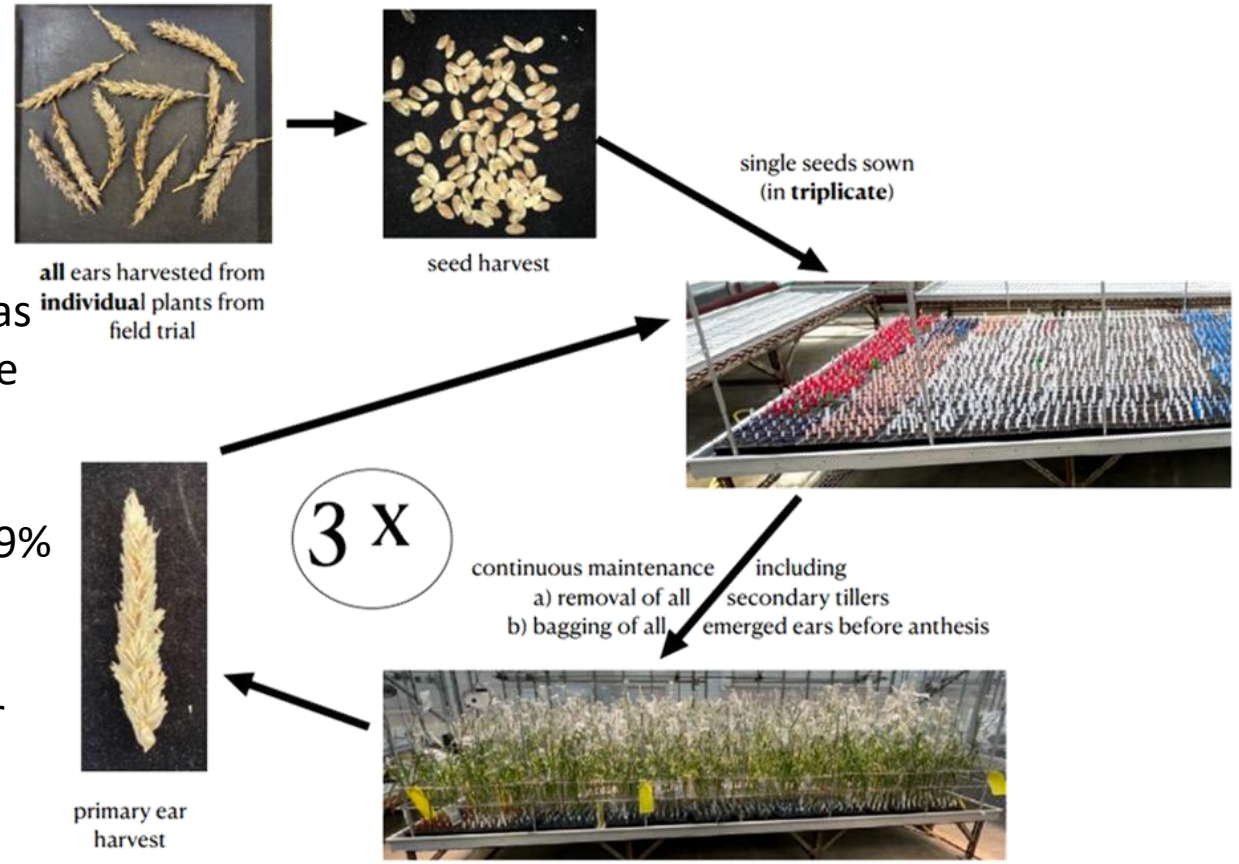
Kostya Kanyuka



selfing 2021

Triticum monococcum Introgressed Plants - Single Seed Descent (SSD)

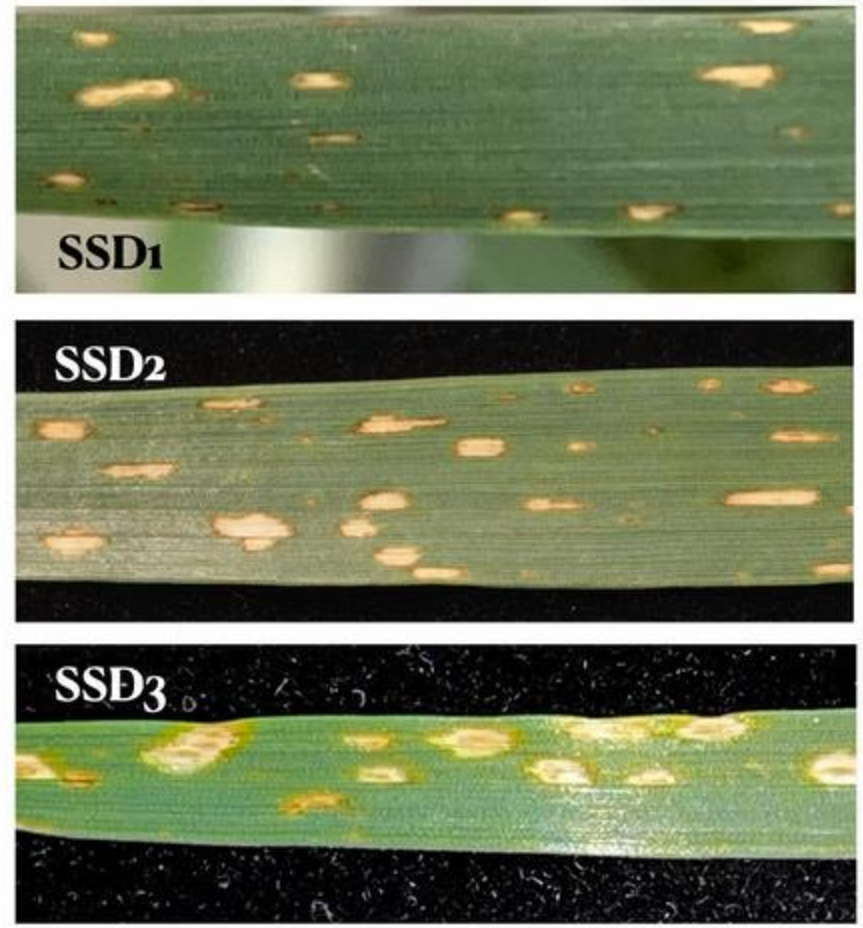
- **why?** Most (all?) introgressed plants are **heterozygous**
- several rounds (≥3) of SSD used to **make plants homozygous** - from 87.5% in field trial to **99.21% homozygosity after SSD3**
- **how?**



- grain from **437** Field Trial plants used for SSD: 3 grains per plants in individual cells
- additionally, grain from 142 BC3 plants was also used for SSD – all BC3 plants originate from the field BC1 trial lines
- 3 rounds of SSD would make all plants >99% homozygous
- sowing 3 grains for each plant allowed for further scoring of awn segregation
- all ears photographed and catalogued at each stage (at harvest) FT, SSD1 SSD2 SSD3

SSD	FT plants	BC3 plants	DLM plants (lines)
FT	509	0	41 (16)
SSD1	1326	426	4?
SSD2	843	373	43 (13)
SSD3	797	354	87 (23)

16 of FT DLMs replicated in SSDs



Wheat Genetic Improvement Network

Field Trial 2020			FT	SSD1	SSD2	SSD3
#	Line	Plant				
170	BC1-X12-1	#118-P2				170-1
171		#118-P4			171-3	171-3
172		#118-P5				172-3
177		#123-P4			177-1	
91		#22-P3				91-1
163		#22-P6				163-1
168		#57-P5				168-3
183	BC1-X12-3	#38-P6				183-3
22		#64-P6	22			
83	BC1-X87-1	#108-P4				83-2
344		#135-P5				344-1
96		#31-P1				96-1
82		#46-P6				82-2
236	BC1-X173-1	#157-P2				236-1
48		#157-P3	48			
433	BC3-X6-1					433-3
438	BC3-X10-2				438-2	438-1,3
441	BC3-X20-1				441-3	
446	BC3-X31-1				446-1,2,3	
490	BC3-X172-1					490-1
16	BC1-X147-1	#36-P5	16			
23		#52-P4	23			
221		#124-P3				221-1
325	BC1-X75-1	#39-P6				325-1
30		#94-P1(P2)	30			
2		#94-P3(P4)	2			
308	BC1-X74-1	#79-P1				308-1
313		#103-P1				313-1
49	BC1-X115-1	#101-P2	49			
279	BC1-X189-2	#120-P1				279-3
28		#129-P1	28		28-3	
74		#129-P2	74			74-2
29		#129-P4	29			
75		#129-P5	75			
24		#30-P1	24		24-1,2,3	24-3
68		#30-P2	68			
69		#30-P3	69			
70		#30-P4	70	70-3	70-3	70-1,3
25		#30-P5	25	25-1	25-1,3	
71		#30-P6	71		71-1,2,3	71-1,2,3
72		#58-P2			72-1,2,3	72-1,3
26		#58-P4	26			
27		#58-P6	27		27-2	27-2
12	BC1-X224-1	#78-P3	12			
113	BC2-X94-1					113-1
488	BC3-X171-1					488-3

397	BC2-X65-1	#134-P3				397-1
390		#21-P2				390-1
391		#21-P3				391-2
394		#76-P4				394-3
396		#83-P4				396-1
131	BC1-X88-1	#119-P1				131-1
134		#119-P4				134-1,3
140		#141-P4			140-1	140-3
142		#141-P6				142-2
128		#48-P2				128-1
129		#48-P3				129-3
85		#48-P6				85-3
144	BC1-X113-1	#65-P2				144-2
31	BC1-X113-3	#75-P4	31			
209	BC1-X122-1	#155-P1				209-1,2
36		#155-P3	36			36-1,3
32		#24-P2	32			32-1,3
33		#24-P3	33		33-1,2,3	
34		#24-P4	34			34-1,2
35		#45-P2	35		35-3	35-1
202		#45-P6				202-1
207		#97-P5				207-2
115	BC2-X134-2				115-2	
358	BC2-X150-1	#100-P2				358-1
362		#128-P4				362-2
347		#29-P3				347-3
348		#29-P4				348-1
350		#29-P6				350-1
352		#71-P2				352-2
353		#71-P3				353-2
354		#71-P4				354-2
355		#71-P5				355-1,2,3
45	BC2-X150-2	#133-P3	45			
46		#133-P5	46			
389	BC2-X150-3	#156-P5			389-3	
383		#87-P1				383-2
37		#87-P4	37			
519	BC3-X99-3					519-2
47	BC1-X161-3	#147-P2	47			
38	BC1-X186-2	#35-P2	38			
7		#53-P2	7			
39		#53-P5	39			39-2
40		#90-P3	40		40-1	
248		#90-P4				248-1
11	BC1-X187-1	#142-P3	11			11-2
41		#32-P4	41			
4		#47-P4	4			
9		#47-P5	9			
268	BC1-X187-2	#149-P5			268-2,3	
67		#40-P2				67-2
262		#55-P3				262-3
42		#85-P2	42		42-1,2,3	
103		#85-P3			103-3	
273	BC1-X187-3	#28-P6				273-2
43		#73-P3	43			
275		#93-P2				275-3
44		#93-P4	44			

408	R2#13-1-1 (MDR308)	#104-P3			408-2,3	408-3
408		#104-P3				408-3
412		#130-P5				412-1
413		#130-P6			413-3	413-3
413		#130-P6				413-3
425	R2#14-1-1 (MDR308)	#154-P5				425-3
417		#56-P3			417-2	
101		#56-P5		101-1	101-1	101-1
102		#56-P6				102-1
422		#86-P5	422-2		422-1,2,3	
559	R2#2-7 (MDR049)	BC2(R2)-X104-2				559-2
574		BC2(R2)-X120-2				574-3
575		BC2(R2)-X121-1				575-3

- **1 plant** (#70 row30-plant4) has DLM in FT and all SSDs ==>

  DLM stably inherited already

- 5 plants have DLM in FT and ≥ 2 SSDs
- 1 plant (#101 row56-plant5)has

  DLM in all SSDs ==>

  DLM stably inherited already

- 7 plants have DLM in SSD2 &3 ==>

  DLM stably inherited as well?

- thought of the day: all plants with SSD3 DLM could also have stable inheritance.

Department for Environment Food & Rural Affairs

ROTHAMSTED RESEARCH

Dark Wheat?!

SSD3 harvest+grain cleaning:

- 25 (out of 1018) ears harvested have blackish or dark-coloured grain
- all dark grain originate from only 1 F1C (F1C#94) and 4 FT lines created in only 2 BC1 crosses (82 % X186 and 18% X187)
- this trait is stable in 3 SSD3 plants - ie SSD3-P1, SSD3-P2 & SSD3-P3 have coloured grain
- some also segregate for this trait: eg for SSD3#359, SSD3-P1 and SSD3-P2 are coloured whereas P3 has 'normal' completely white(red?) grain

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Rising Demand for Healthy Foods-Anthocyanin Biofortified Colored Wheat Is a New Research Trend

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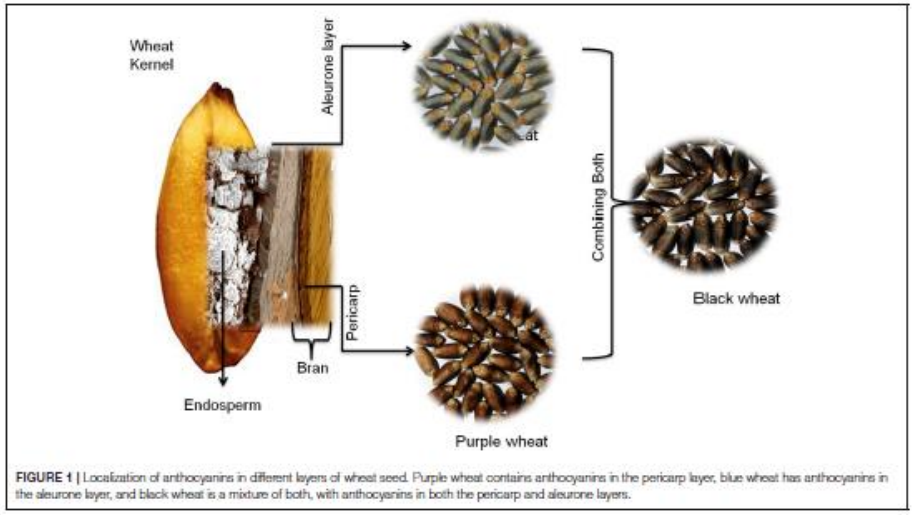

FIGURE 1 | Localization of anthocyanins in different layers of wheat seed. Purple wheat contains anthocyanins in the pericarp layer, blue wheat has anthocyanins in the aleurone layer, and black wheat is a mixture of both, with anthocyanins in both the pericarp and aleurone layers.