



ROTHAMSTED
RESEARCH

Development of wheat with low-viscosity grain extracts using novel alleles of IRX9 orthologue TaGT43_2

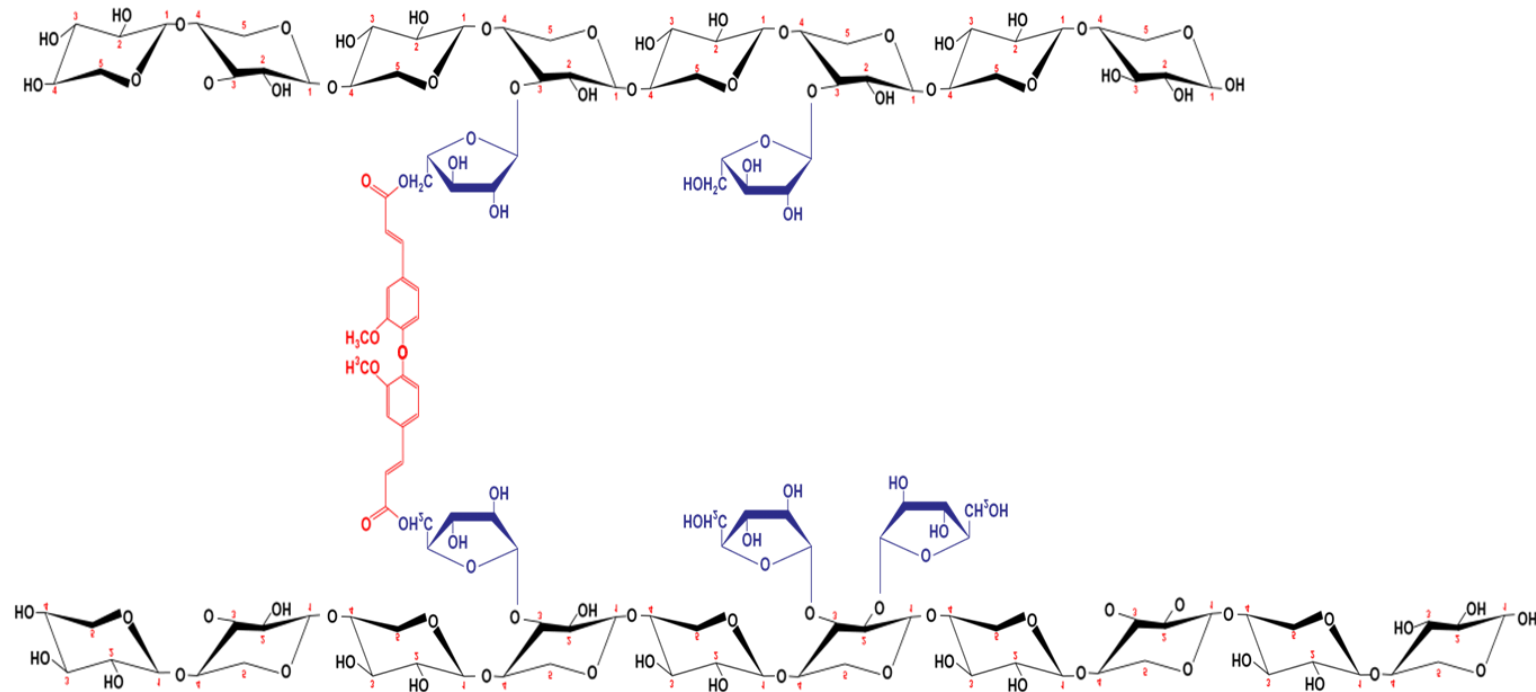
Till K Pellny
Rothamsted Research

Background



ROTHAMSTED
RESEARCH

- Arabinoxylan (AX) major polysaccharide in wheat grain (70%)



Background



ROTHAMSTED
RESEARCH

- Arabinoxylan (AX) major polysaccharide in wheat grain (70%)
- Key determinant of end use quality
- Plays important role in human health, distillery and animal feed

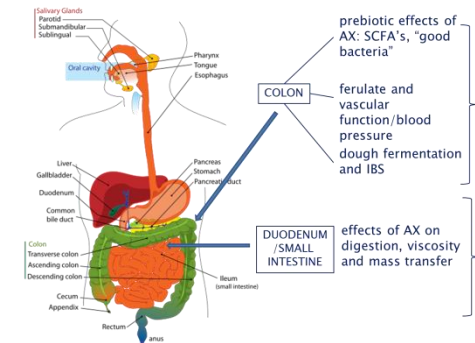


Decreased soluble AX would be desirable for alcohol production and use as animal feed.



Increased amounts of soluble AX would be beneficial for human consumption.

Role of AX fibre in the human GI tract



AX is a key determinant of end-use quality.



ROTHAMSTED
RESEARCH

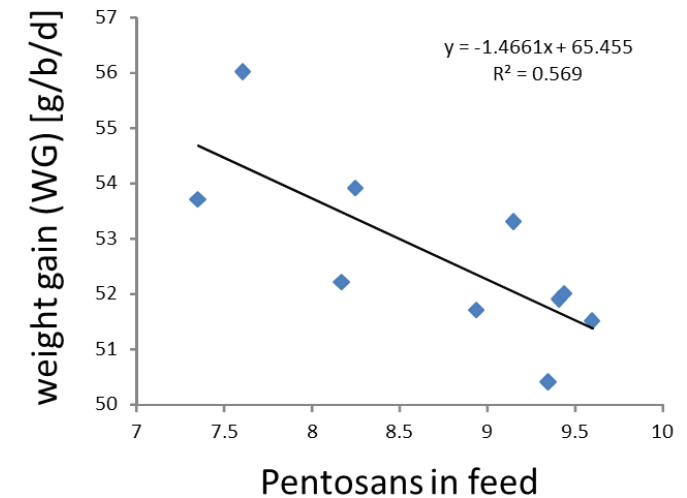
High extract viscosity is detrimental when used for animal feed e.g. leading to sticky faeces in poultry.

More than 50 billion chickens are raised annually world-wide as a source of food, for both their meat and their eggs.

Value of UK poultry production is greater than £2 billion.



Less pentosans lead to better food conversion rate.



DR VASIL PIRGOZLIEV HARPER ADAMS UNIVERSITY

Breeding Target Low Viscosity



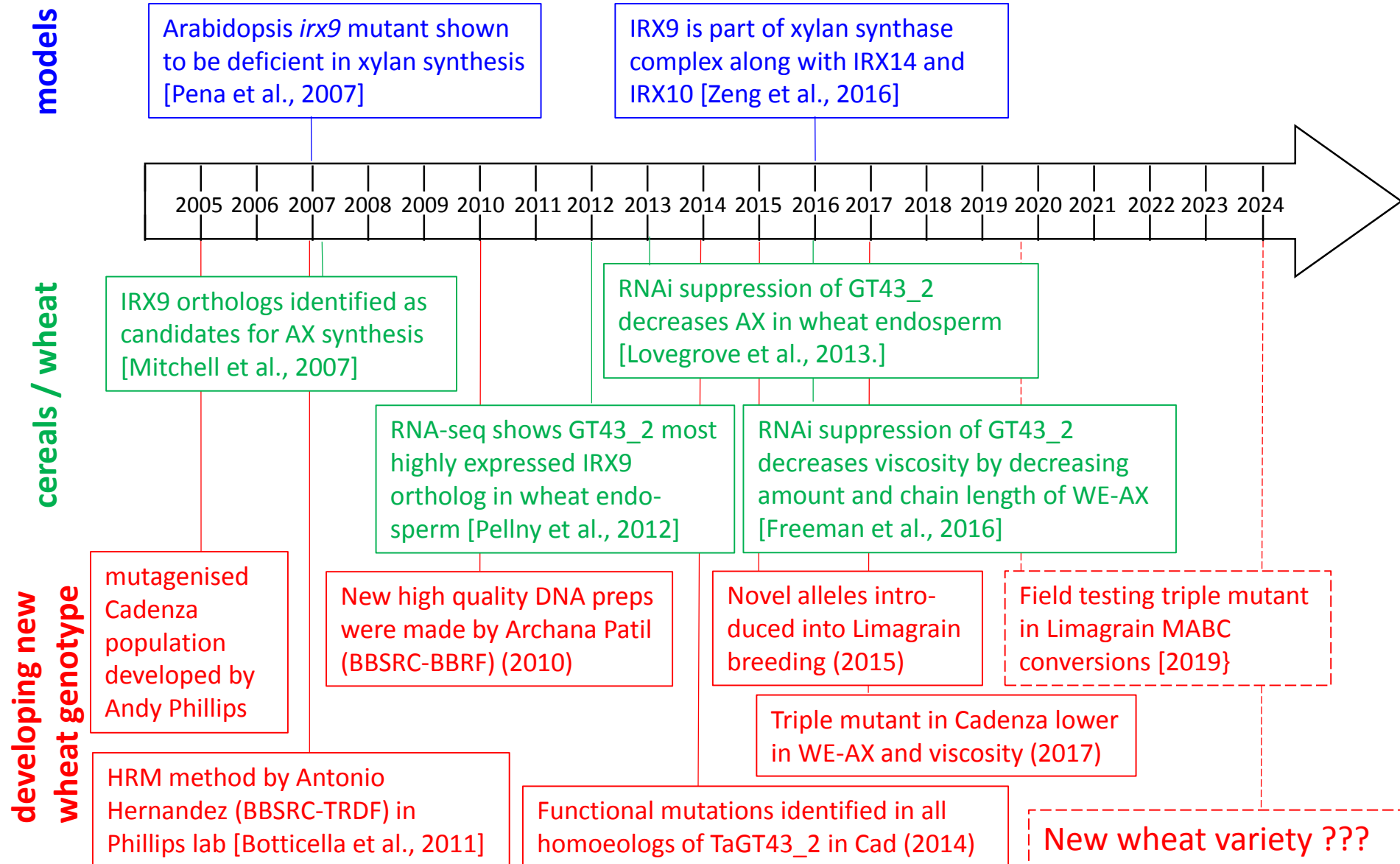
ROTHAMSTED
RESEARCH

- It is cheap to add enzymes to chicken feed to reduce viscosity.
- Scotch Whisky is a premium product.
- No additives are allowed.
- Breeding a low viscosity wheat variety.

From Arabidopsis mutant to new wheat variety in 20 years?



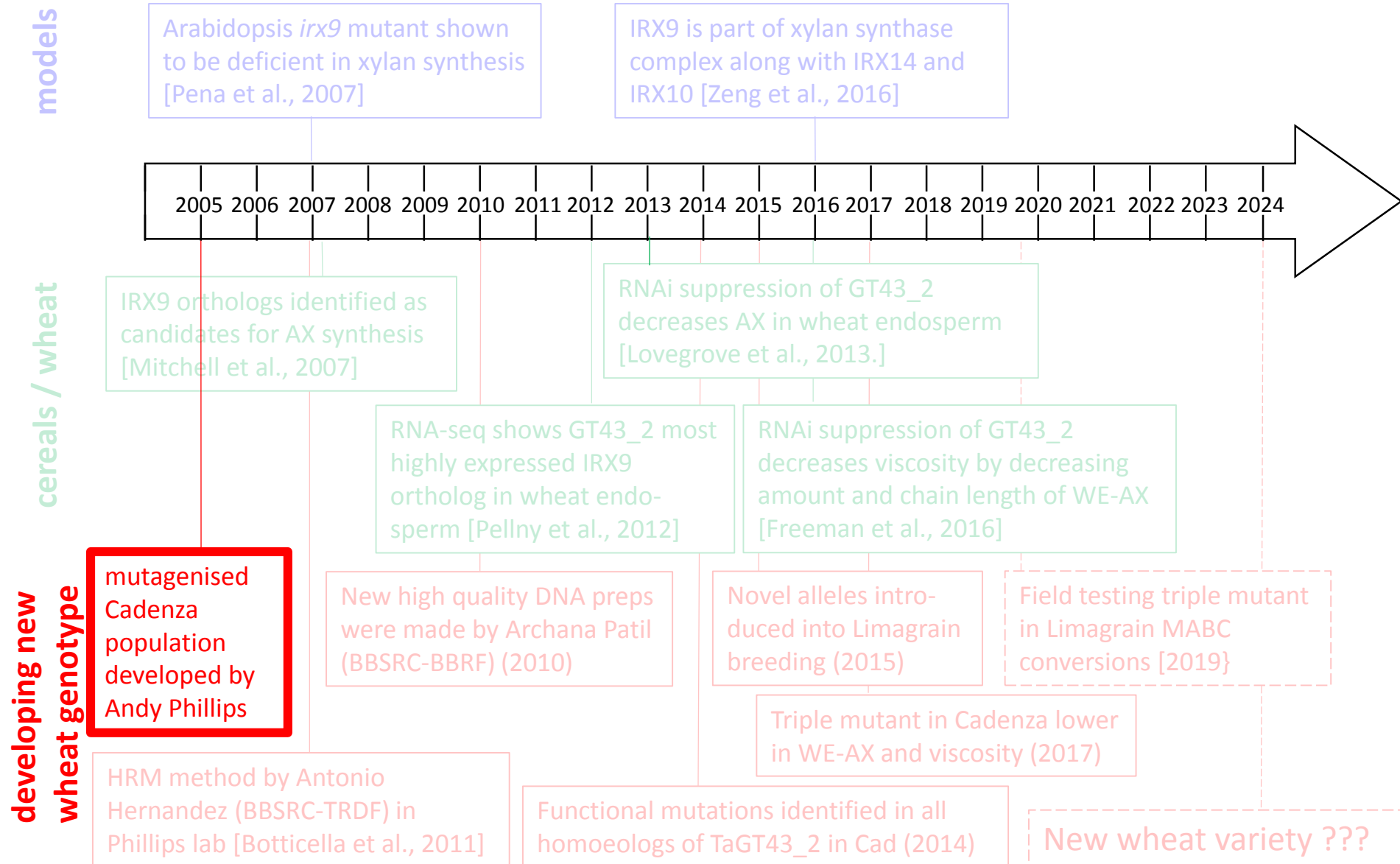
ROTHAMSTED
RESEARCH



From Arabidopsis mutant to new wheat variety in 20 years?



ROTHAMSTED
RESEARCH



Mutagenized Bread Wheat Population



ROTHAMSTED
RESEARCH

- UK Spring bread wheat cultivar – Cadenza
- Used maximum tolerable EMS conc: 0.9%
- Single seed descent to avoid duplication
- M_2 leaves harvested for DNA
- M_3 seeds bulked to M_4/M_5
- Mutation rate ≈ 35 mutations/Mb
- Despite hexaploid genome, observe a wide range of phenotypes



Andy Phillips
Mariann Rakszegi, Martonvásár

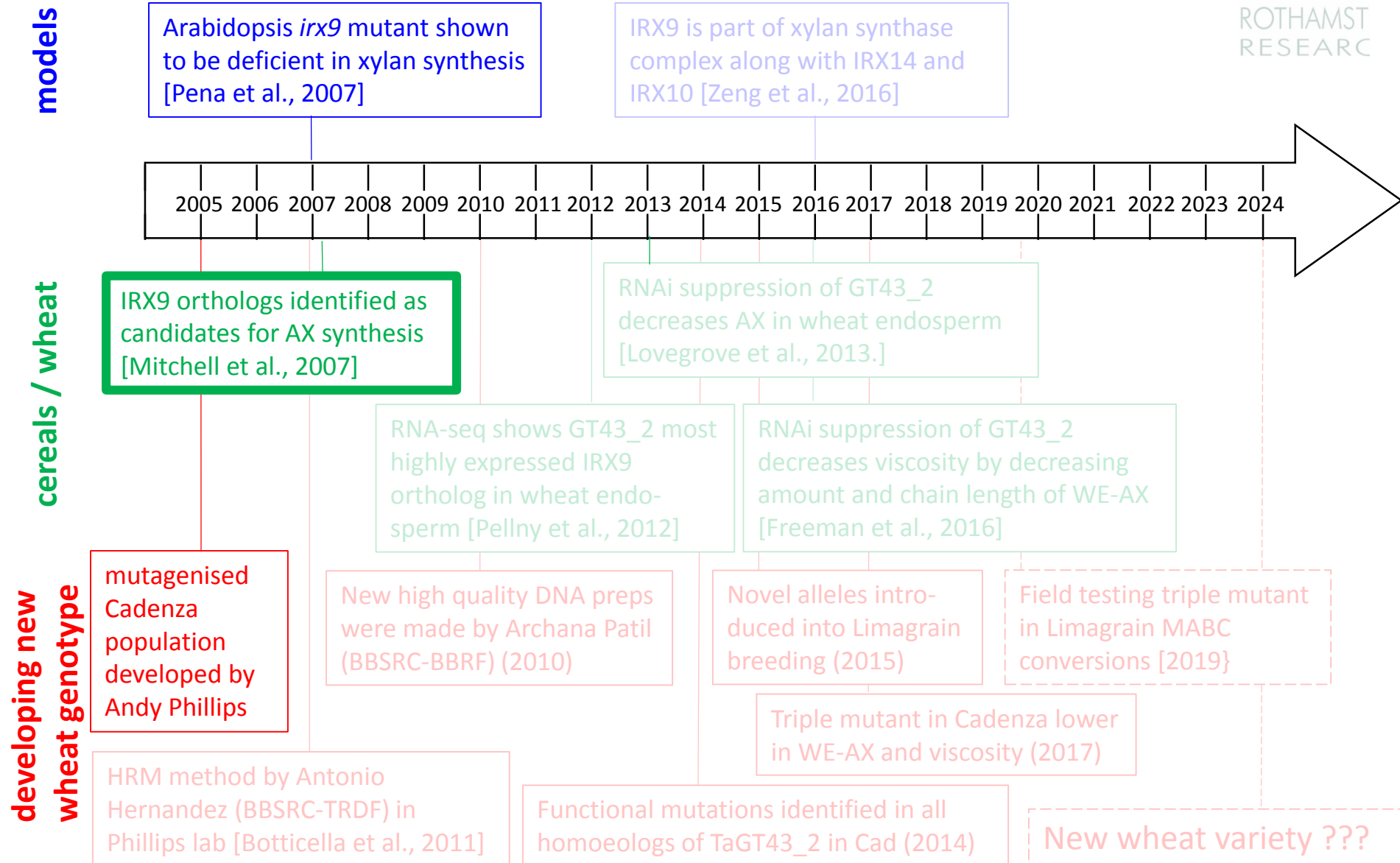
From Arabidopsis mutant to new wheat variety in 20 years?



ROTHAMST
RESEARCH



ROTHAMSTED
RESEARCH

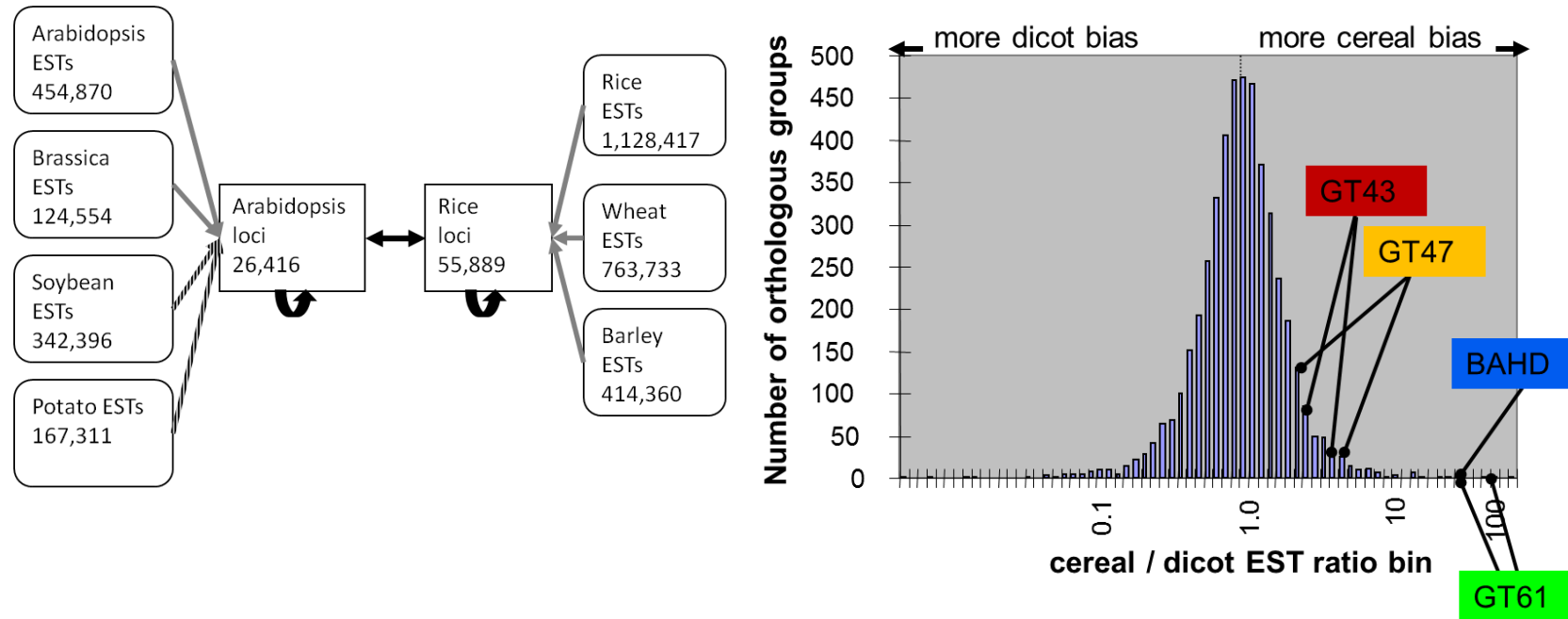


IRX9 orthologs identified as candidates for AX synthesis



ROTHAMSTED
RESEARCH

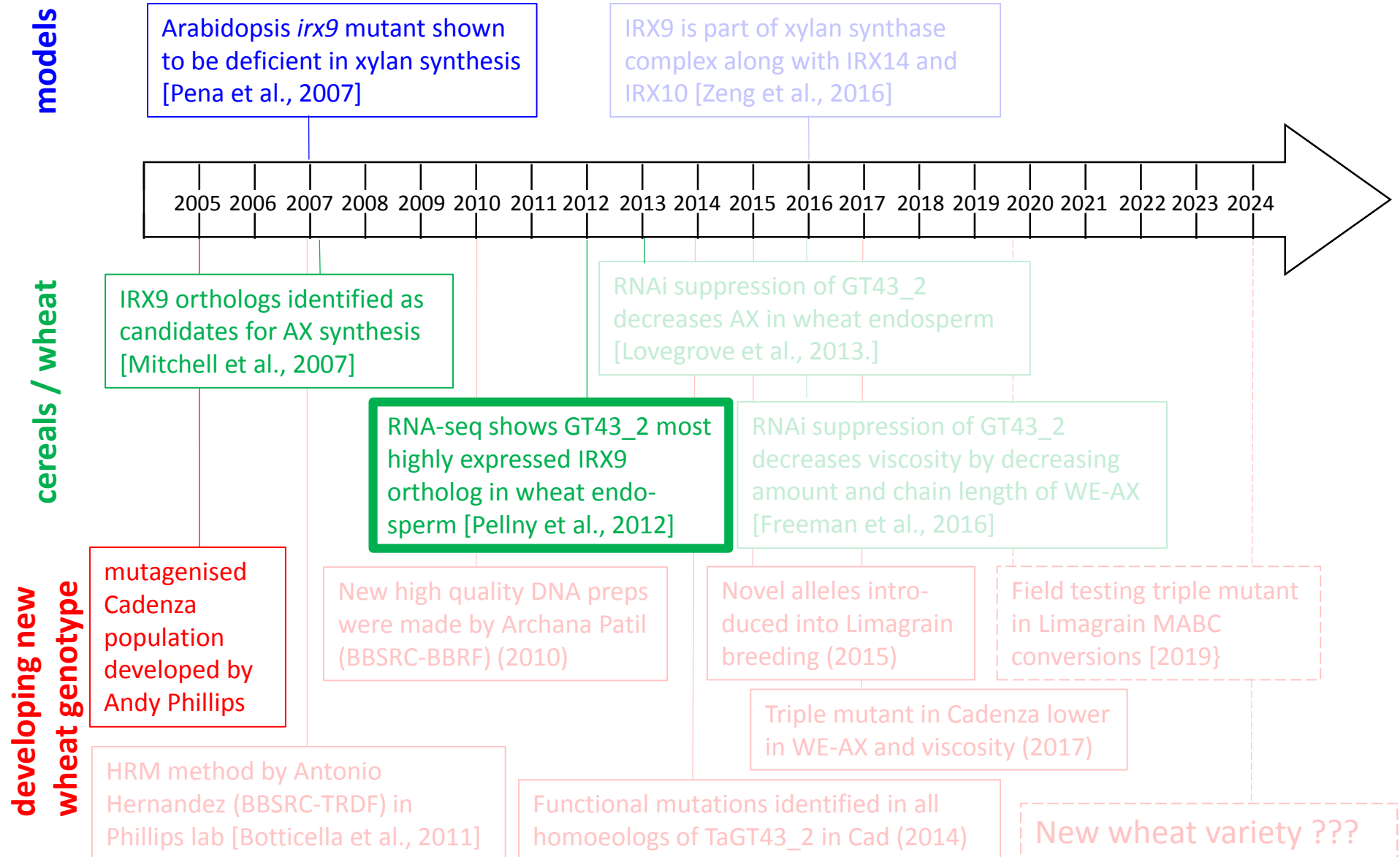
- Difference in expression in monocots vs eudicots



From Arabidopsis mutant to new wheat variety in 20 years?



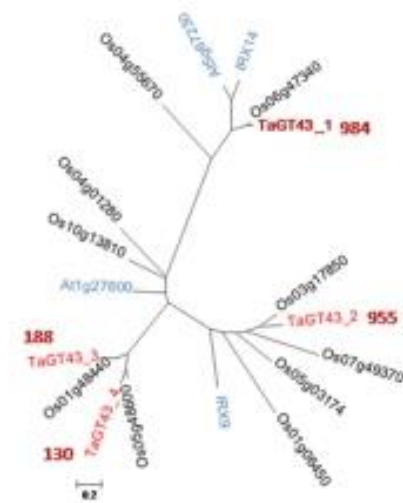
ROTHAMSTED
RESEARCH



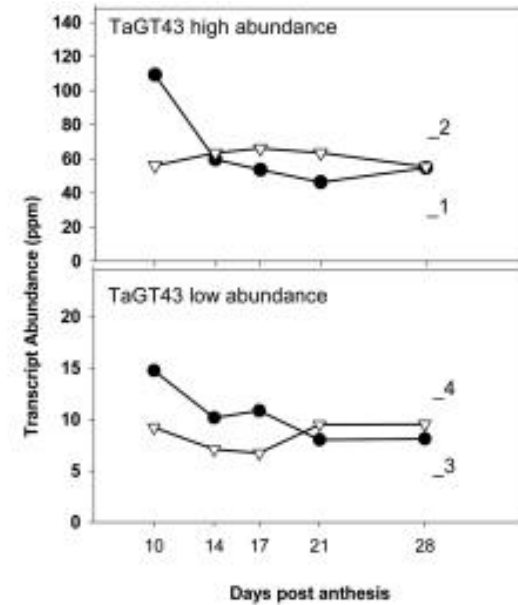
GT43_2 most highly expressed IRX9



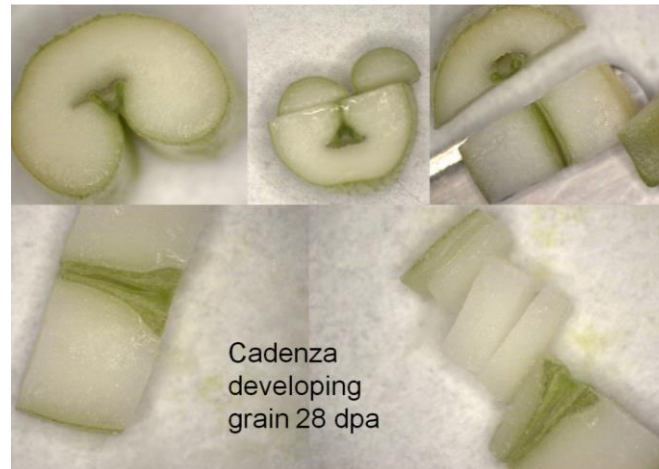
ROTHAMSTED
RESEARCH



GT43



- Next Gen transcript sequencing
- Pure endosperm dissection

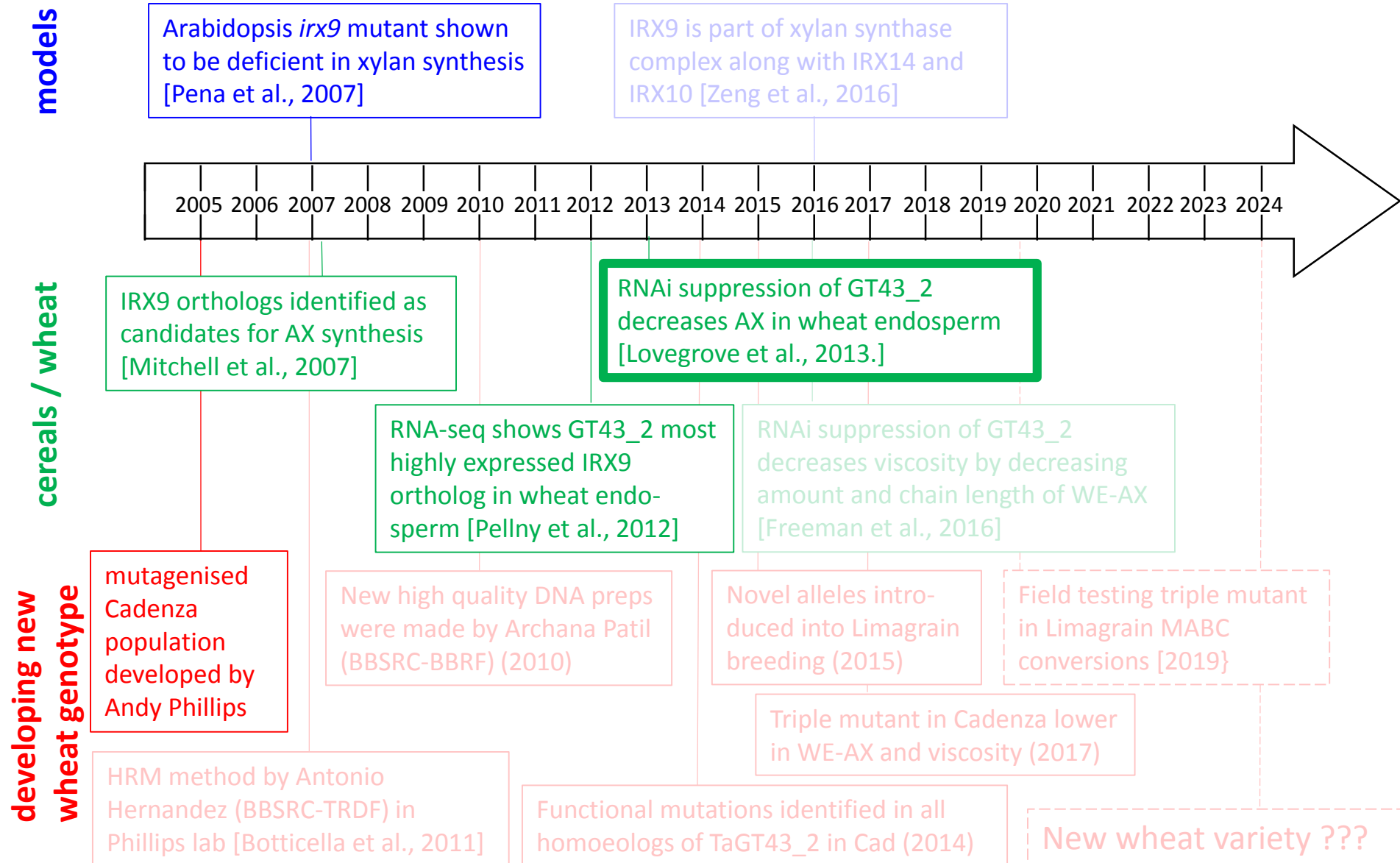


Pellny et al. 2012,
Plant Physiol

From Arabidopsis mutant to new wheat variety in 20 years?



ROTHAMSTED
RESEARCH

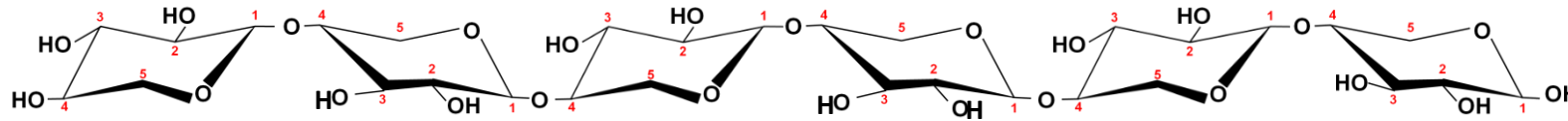


RNAi suppression of GT43_2 decreases AX

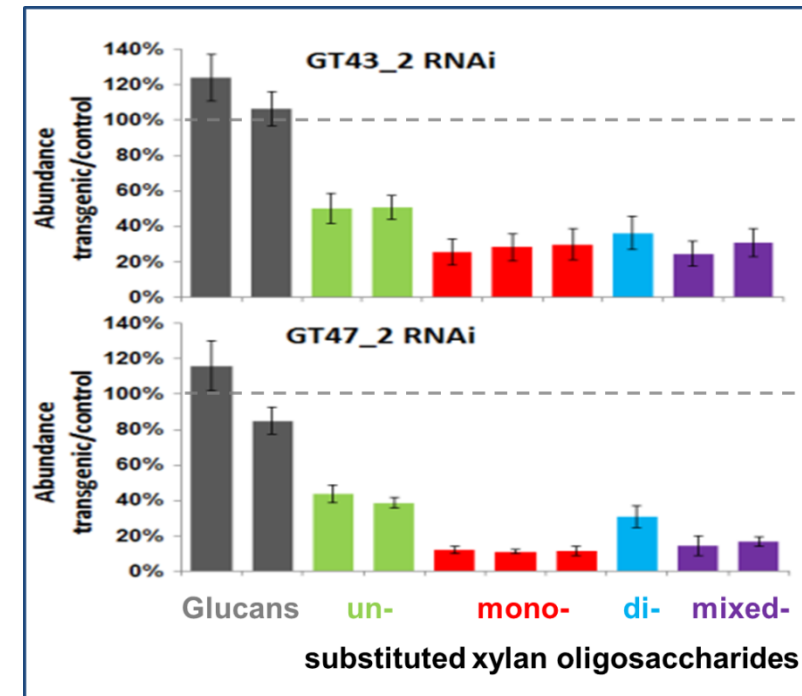


ROTHAMSTED
RESEARCH

Xylan Backbone



- TaGT43_2 ortholog to AtIRX9
- TaGT47_2 ortholog to AtIRX10
- Involved in xylan backbone synthesis
 - RNAi TaGT43_2 and TaGT47_2 suppression give very similar phenotypes
 - All AX oligosaccharide peaks are decreased with a maximum decrease of 80%



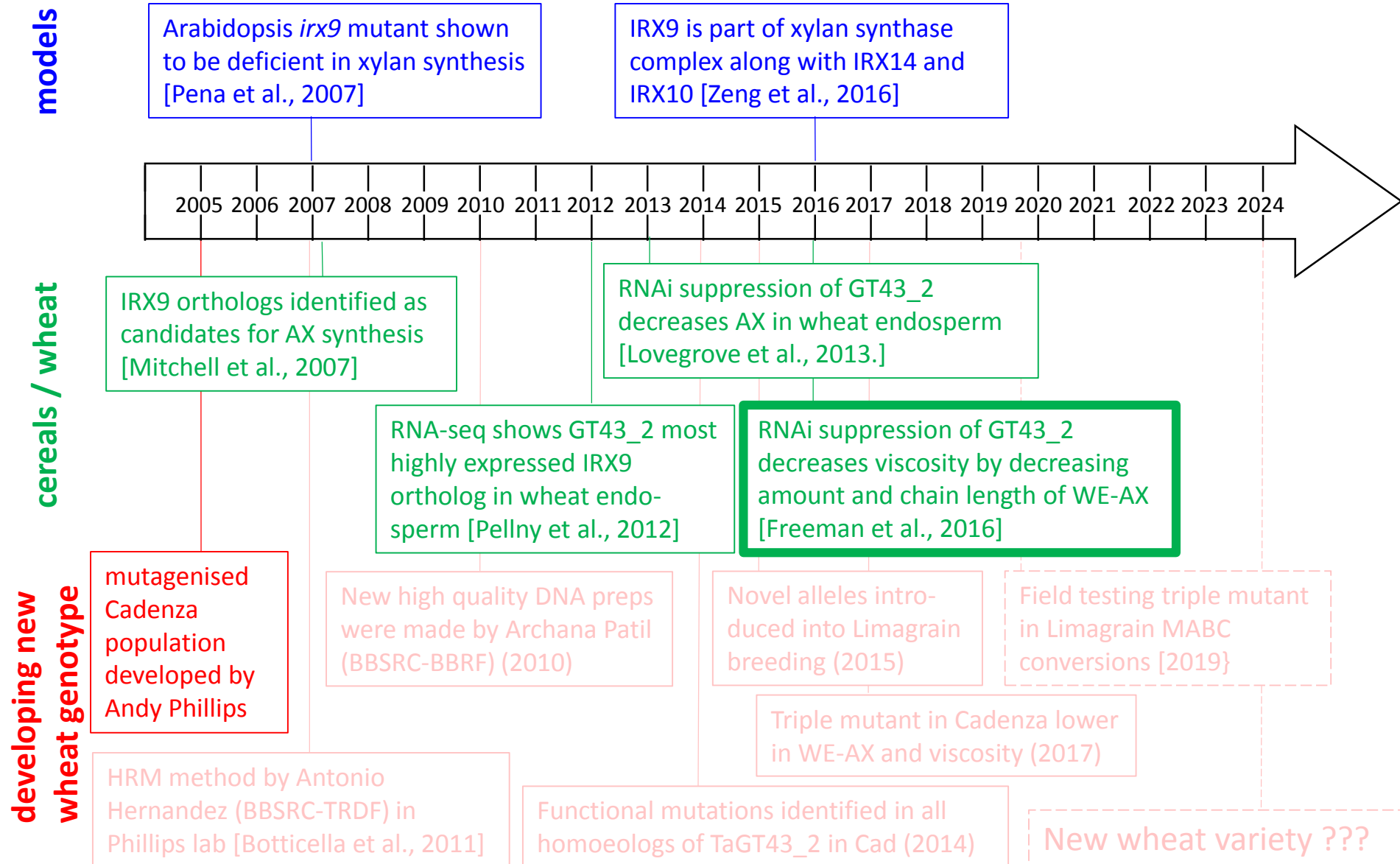
Enzyme fingerprinting

Lovegrove et al. 2013,
Plant Physiol

From Arabidopsis mutant to new wheat variety in 20 years?



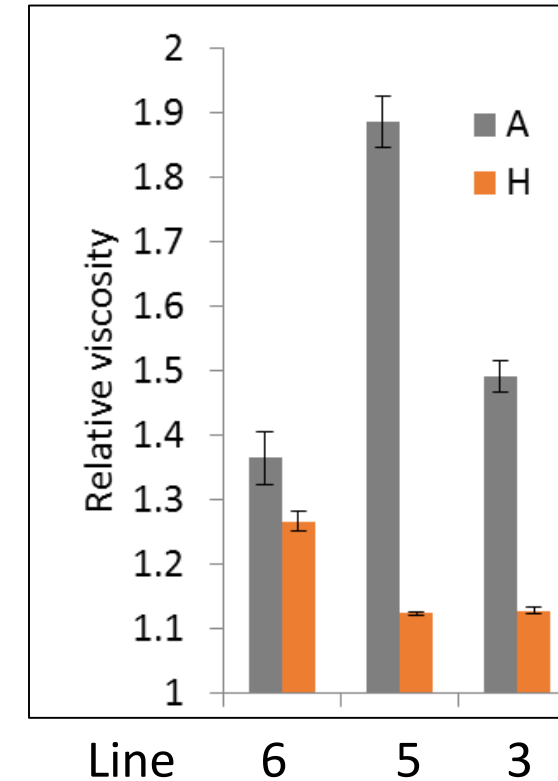
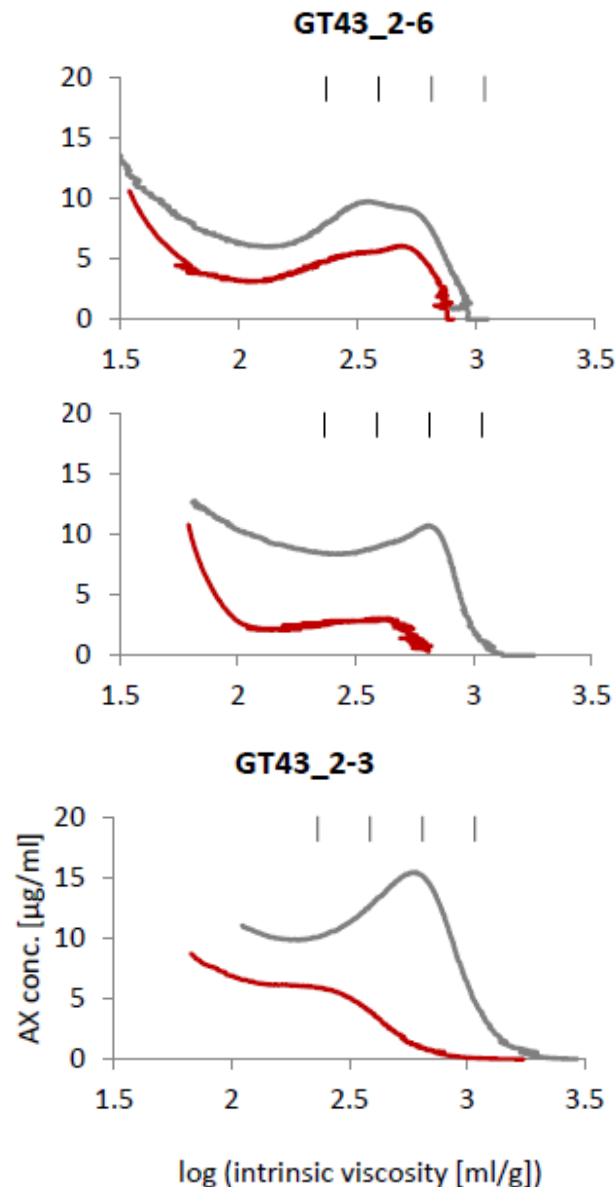
ROTHAMSTED
RESEARCH



GT43_2 RNAi decreases viscosity by decreasing amount and chain length of WE-AX



ROTHAMSTED
RESEARCH

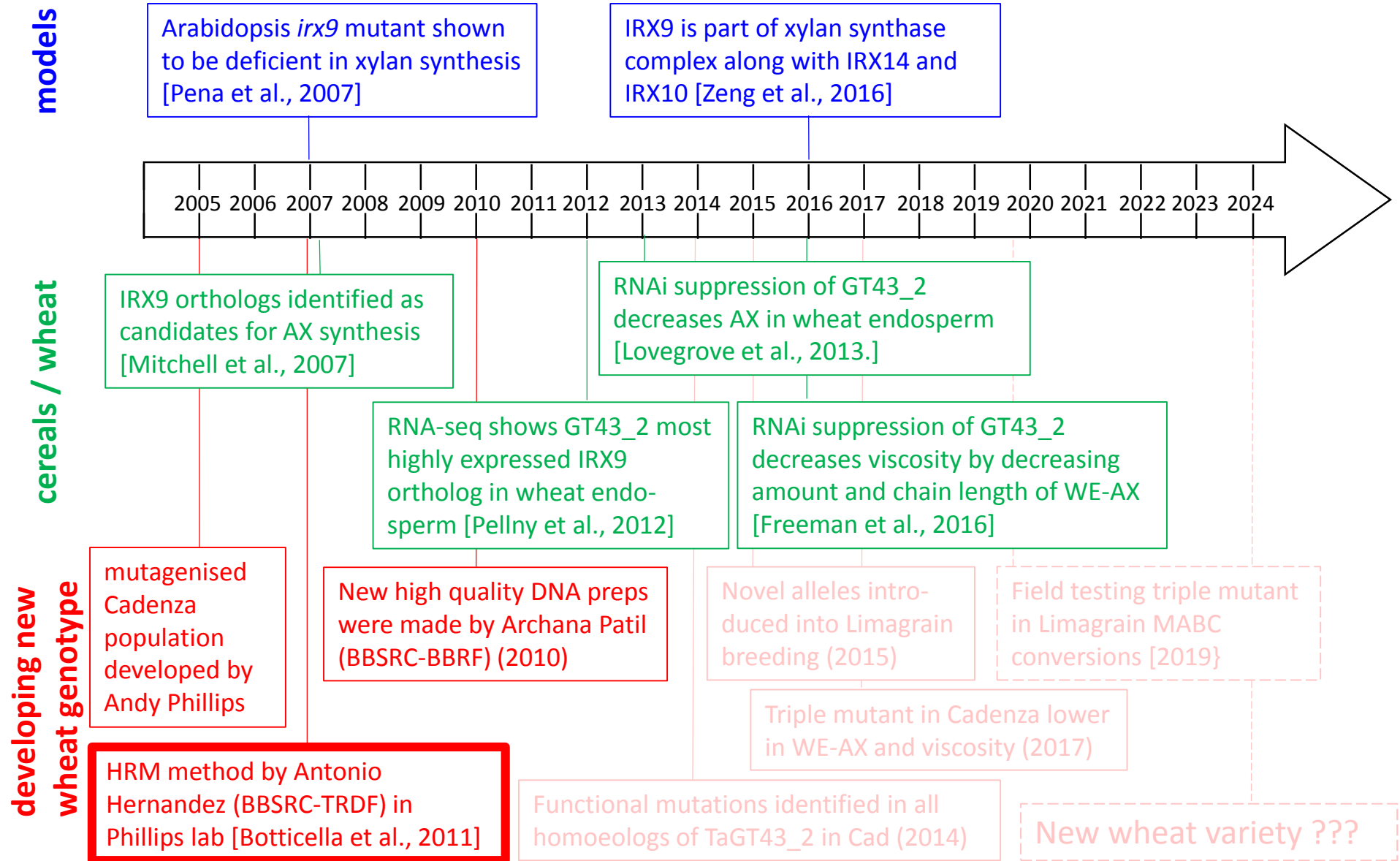


Freeman et al. 2016,
Plant Biotech J

From Arabidopsis mutant to new wheat variety in 20 years?



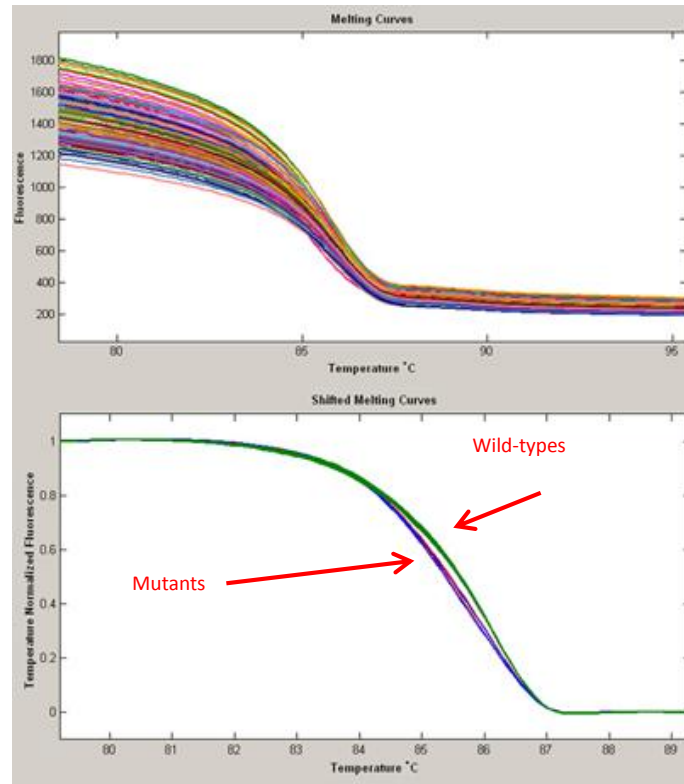
ROTHAMSTED
RESEARCH



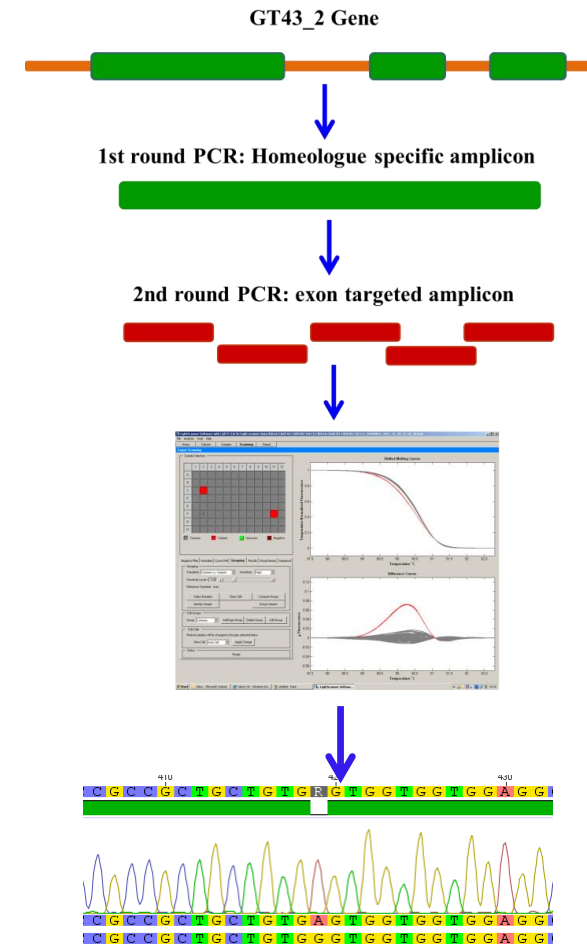
High-Resolution Melt Analysis



ROTHAMSTED
RESEARCH



High resolution melt curve analysis



- HRM very successful but slow!!
- In silico identification of deletions

In silico Identification of Mutations



ROTHAMSTED
RESEARCH

The screenshot shows a web browser window with multiple tabs. The active tab is 'www.wheat-tilling.com'. The website header includes logos for Wheat TILLING, John Innes Centre, Earham Institute, and Rothamsted Research. The main heading is 'Welcome to the *in silico* wheat Target Induced Local Lesions In Genome (TILLING) website'. Below this, a paragraph describes the resource: 'This resource consists of TILLING populations developed in tetraploid durum wheat cv 'Kronos' and hexaploid bread wheat cv 'Cadenza' as part of a joint project between the University of California Davis, Rothamsted Research, The Earham Institute, and John Innes Centre. We have re-sequenced the exome of 1,535 Kronos and 1,200 Cadenza mutants using Illumina next-generation sequencing, aligned this raw data to the IWGSC Chinese Spring chromosome arm survey sequence, identified mutations, and predicted their effects based on the protein annotation available at Ensembl Plants.'

The interface is divided into two main sections: 'Search TILLING data' and 'BLAST Scaffold'.

Search TILLING data

Population ☐ Cadenza ☐ Kronos ☒ Both
Type in list of search terms (scaffold, line or gene)

Search the database by:
gene (eg. Traes_1AL_9EC1E6F0C; Traes_1AL_9EC1E6F0C.1),
scaffold (eg. IWGSC_CSS_2AL_scaff_6343779; 2AL_6343779) or,
mutant line (eg. Cadenza0250)

Examples: Traes_1AL_9EC1E6F0C, Traes_1AL_9EC1E6F0C.1,
IWGSC_CSS_2AL_scaff_6343779, 2AL_6343779, Cadenza0250

Or choose an input file containing terms:

BLAST Scaffold

Paste query sequence(s) or drag file containing query sequence(s) in FASTA format here ...

Nucleotide databases

☒ IWGSC CadenzaU KronosU v1

Advanced Parameters:

SeedStor

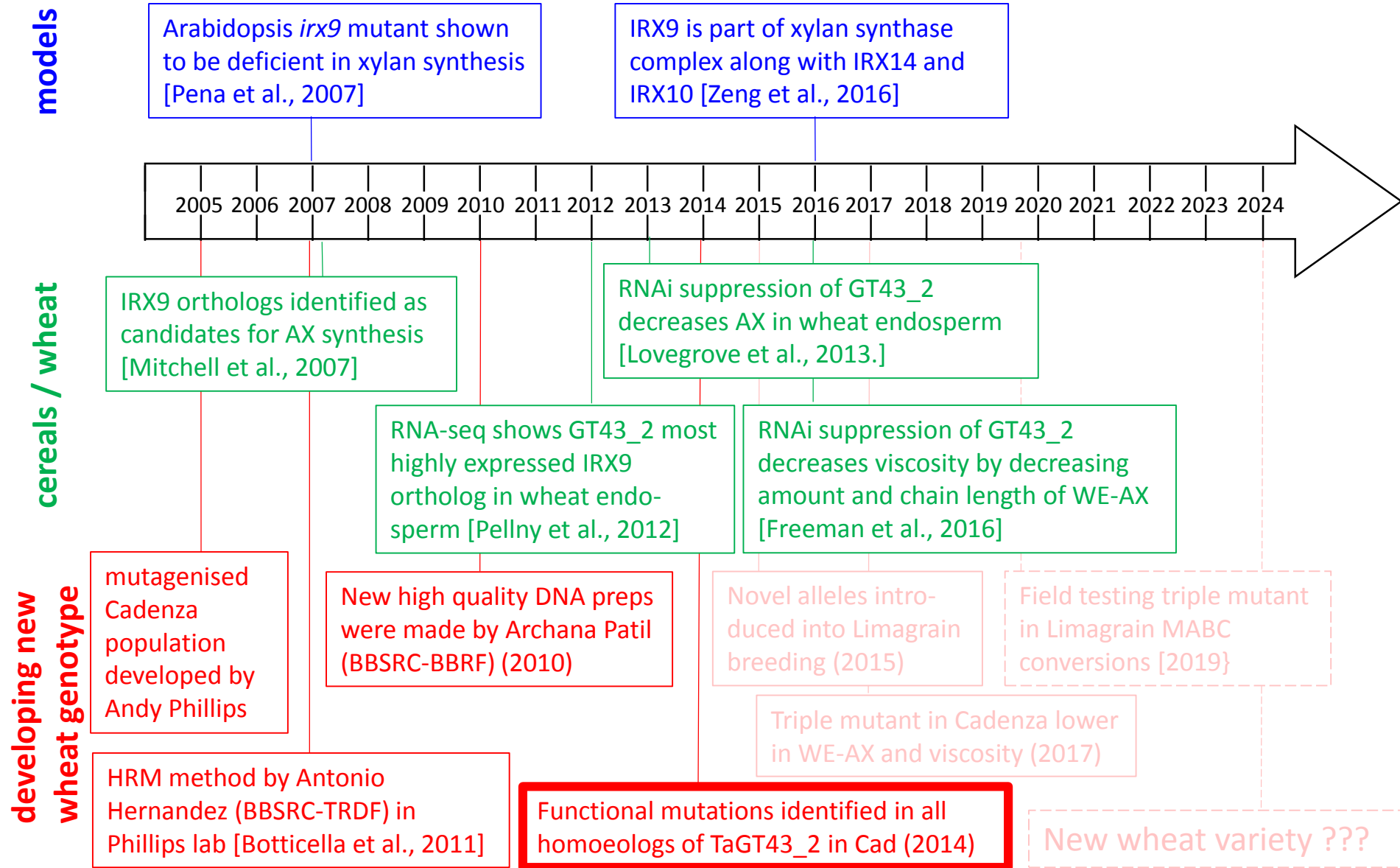
ional Resources
ional resources and documentation of the website and populations
s: To order seeds please visit the SeedStor site at www.seedstor.ac.uk You will need the mutant line identifier to request seed (e.g. nza1111 or Kronos1111) For help, comments and bug reports, please contact Ricardo Ramirez-Gonzalez

<http://www.wheat-tilling.com/>

From Arabidopsis mutant to new wheat variety in 20 years?



ROTHAMSTED
RESEARCH

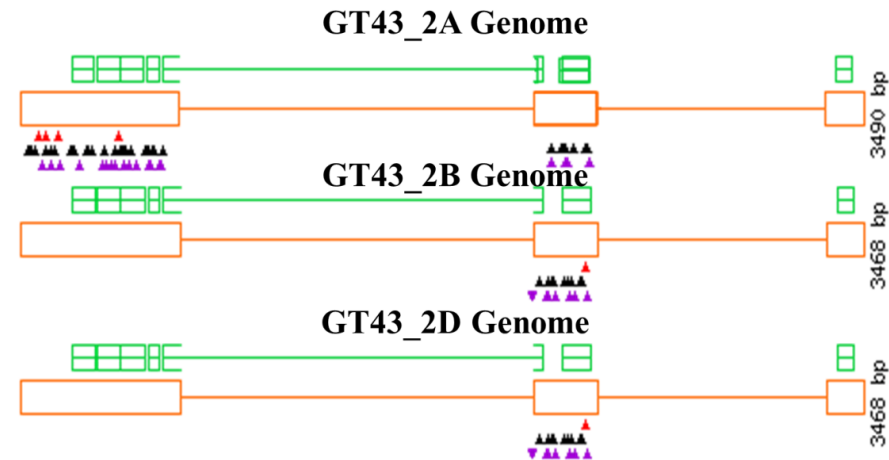
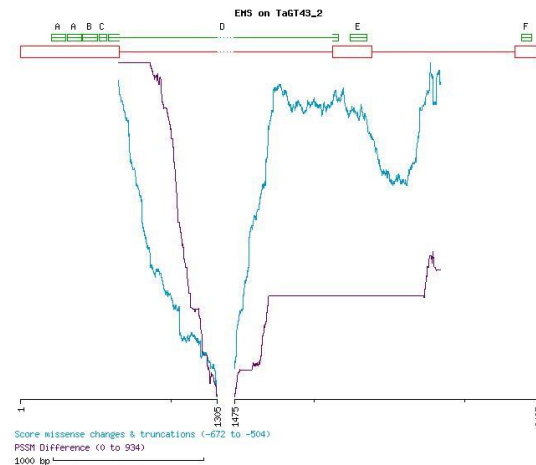


Functional mutations identified in all homoeologs of TaGT43_2



ROTHAMSTED
RESEARCH

CODDLe (Choose codons to Optimize the Detection of Deleterious Lesions) analysis



Genome	Size (bp)	M ₂ plants screened	Mutation
A	912	2150	77
B	259	2150	31
D	259	2150	34

GT43_2

A genome: 5 Stop codons

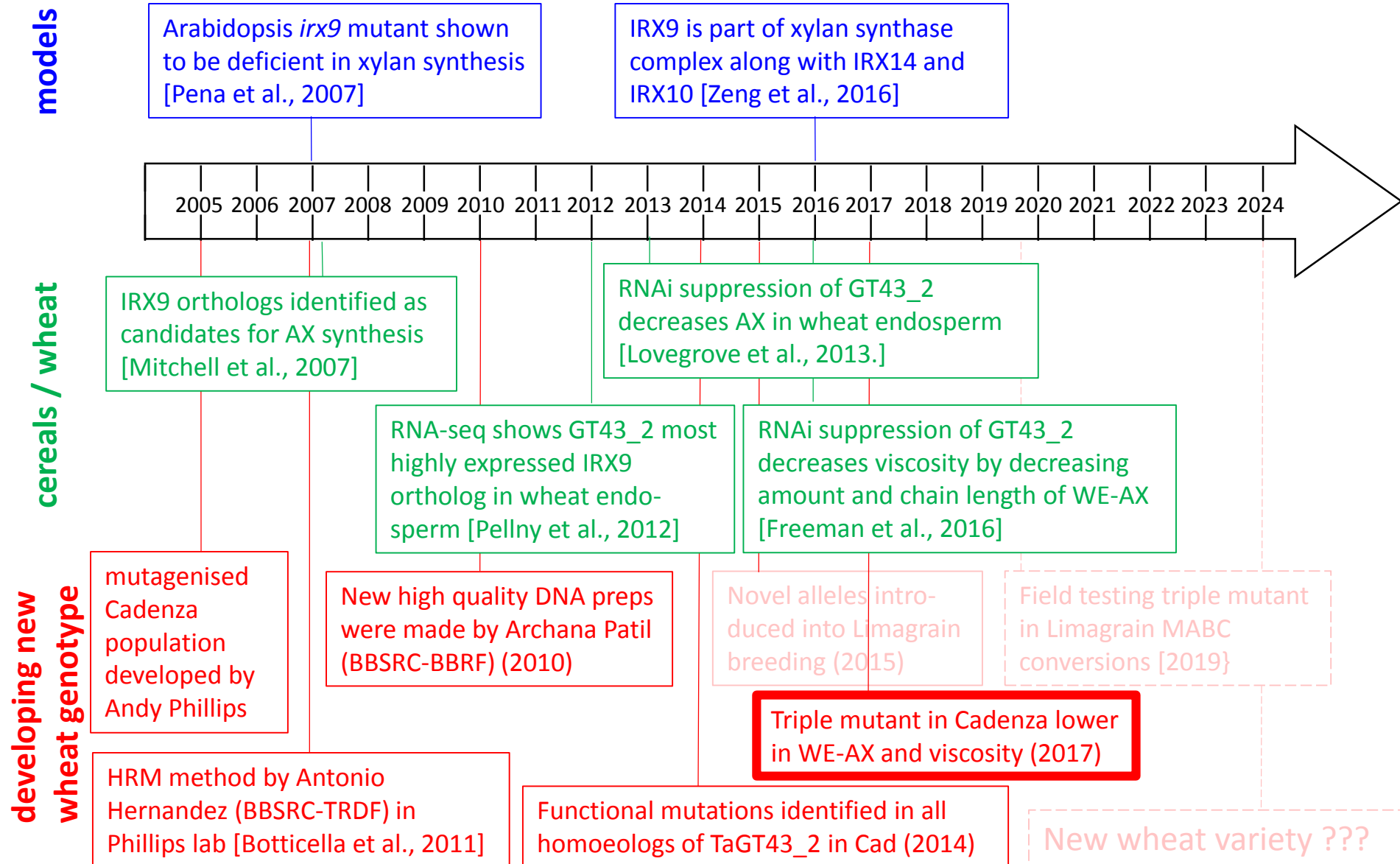
B genome: 1 Stop codon

D genome: 1 Stop codon

From Arabidopsis mutant to new wheat variety in 20 years?



ROTHAMSTED
RESEARCH

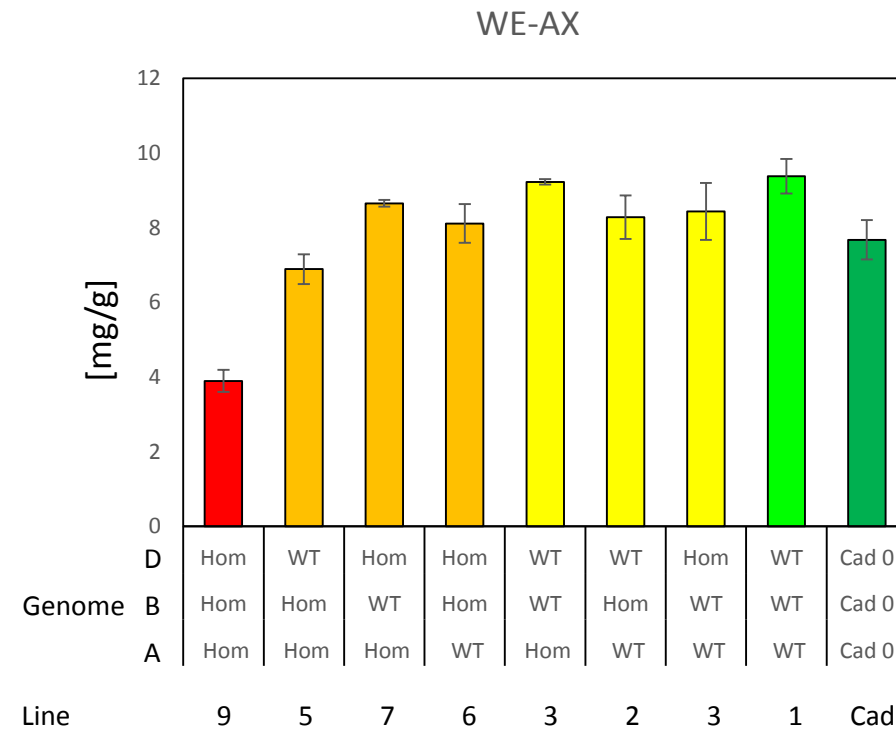


Triple mutant in Cadenza lower in WE-AX



ROTHAMSTED
RESEARCH

- 2016 Field trial

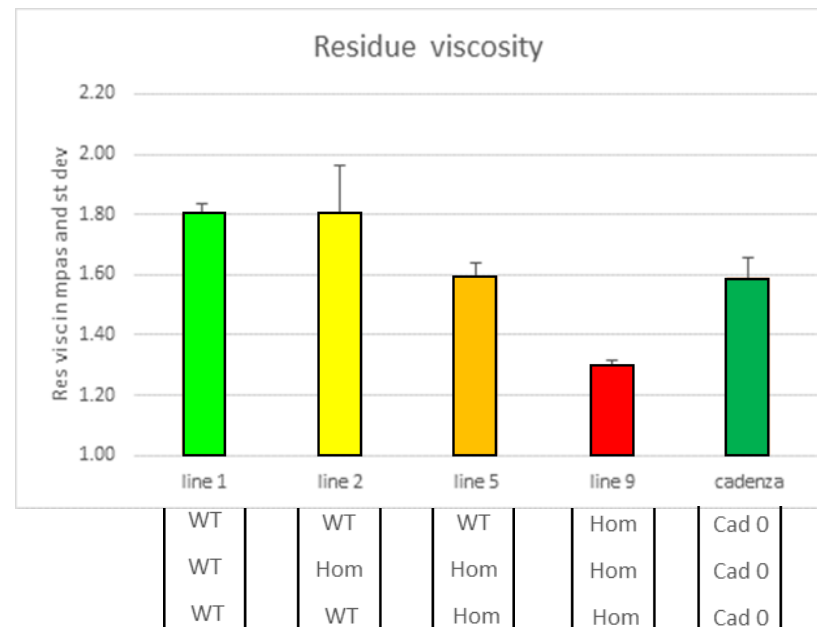


Triple mutant in Cadenza Lower in Viscosity



ROTHAMSTED
RESEARCH

- Reduction in WE-AX leads to reduction of Viscosity



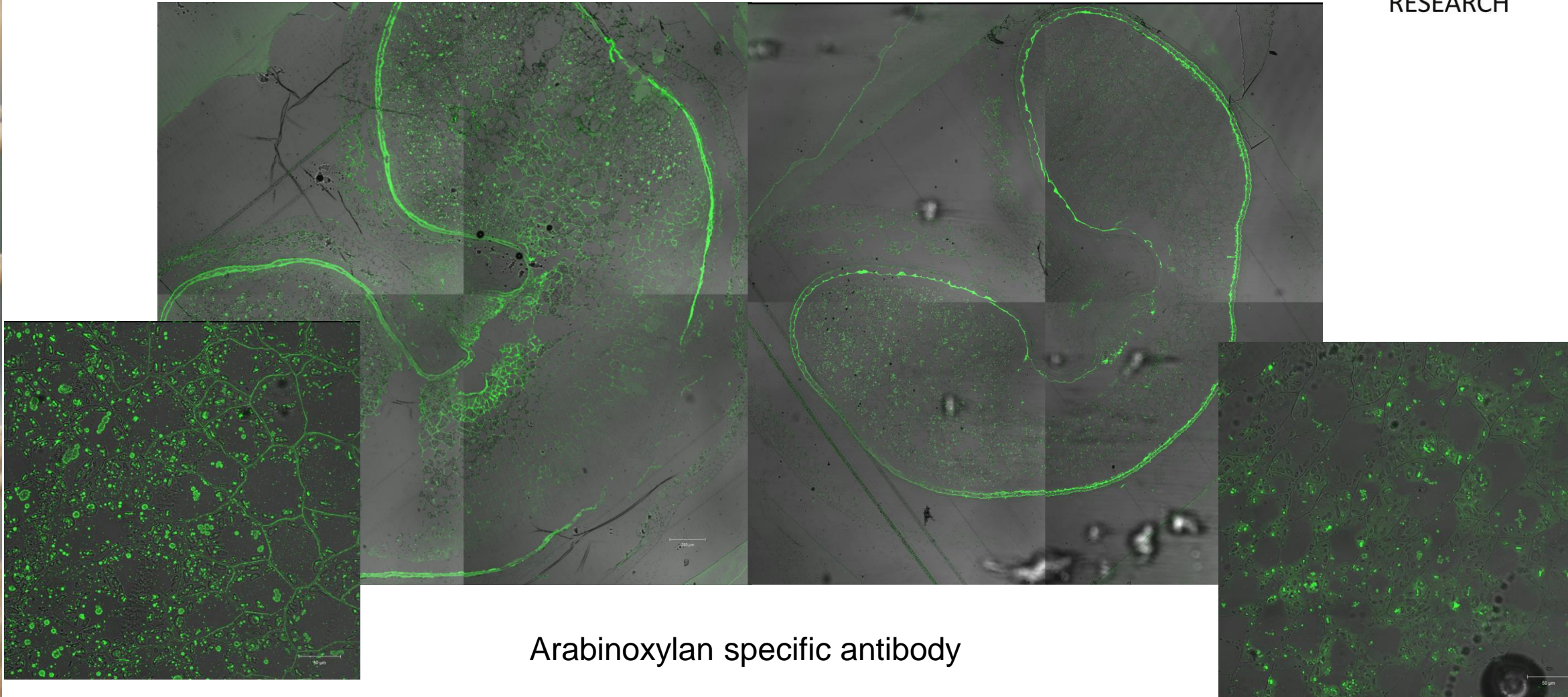
Triple Mutant in Cadenza Have Normal Grains



ROTHAMSTED
RESEARCH

Control

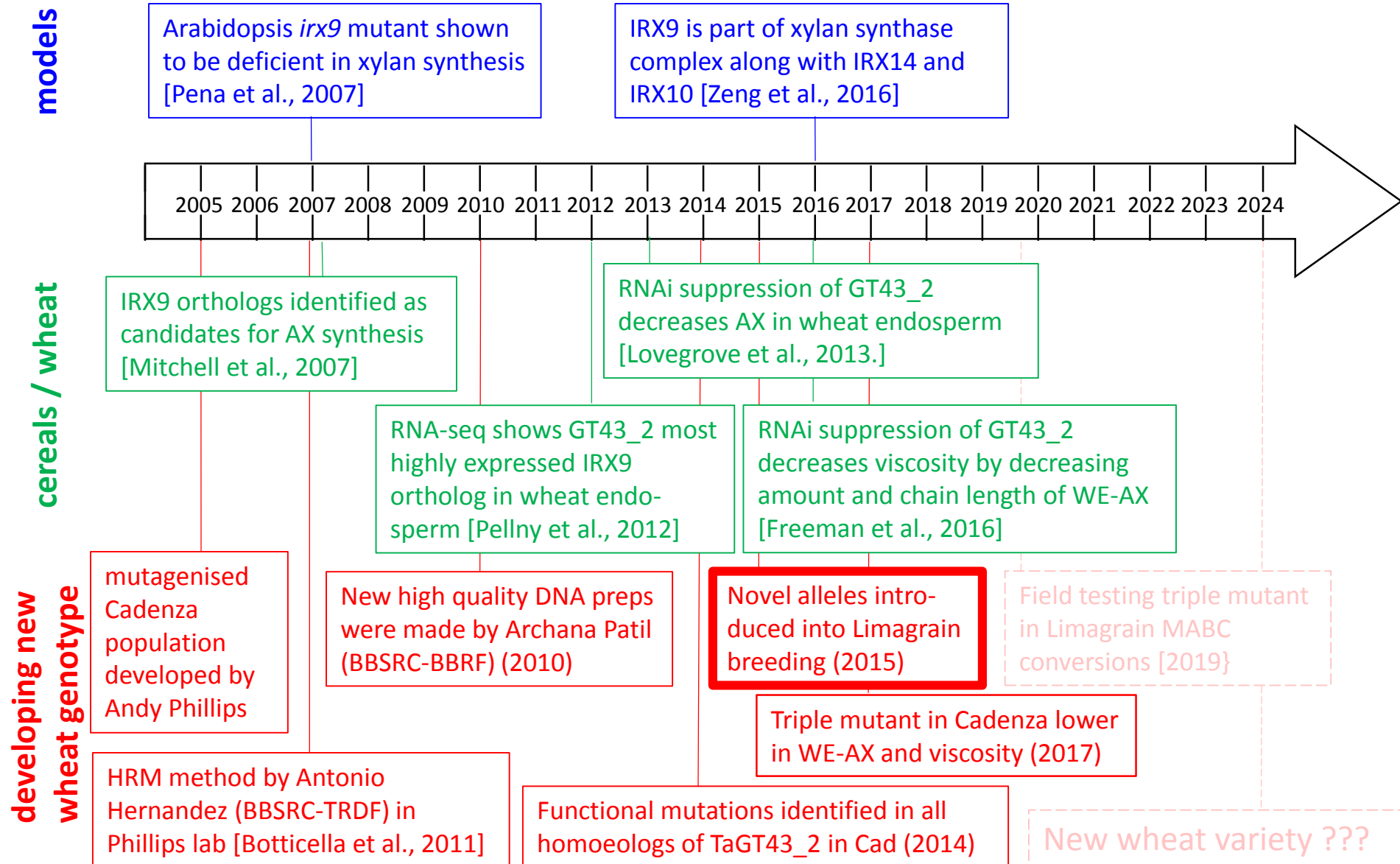
Triple Mutant



From Arabidopsis mutant to new wheat variety in 20 years?



ROTHAMSTED
RESEARCH



Marker Assisted Back Crossing

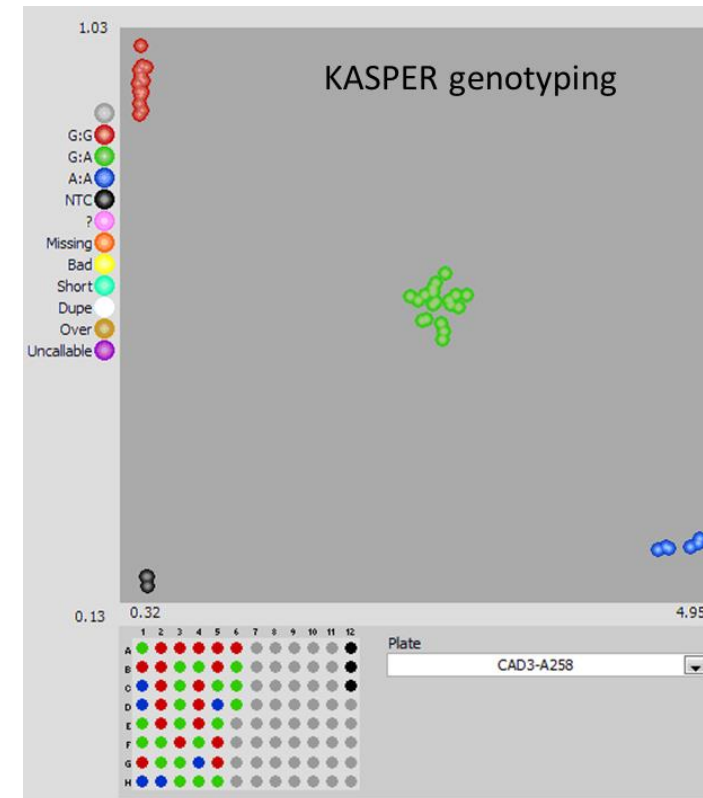


ROTHAMSTED
RESEARCH

Limagrain are introgressing the mutation into two recent listed Group 3 varieties.

Problems to address during the backcrossing:

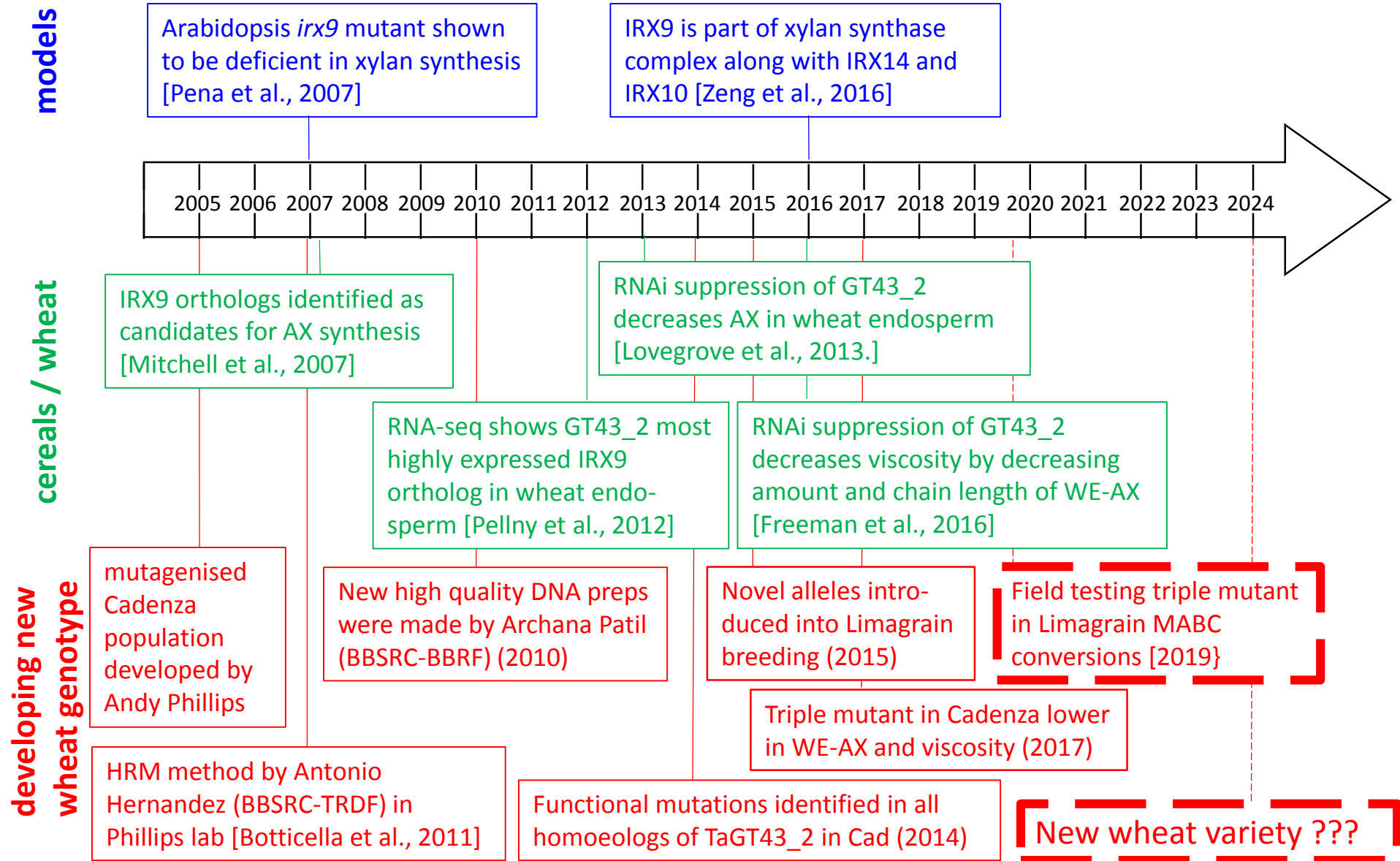
- Background mutation load
- Cadenza is a hard milling, bread variety with no Rht dwarfing gene and a low vernalisation requirement



From Arabidopsis mutant to new wheat variety in 20 years?



ROTHAMSTED
RESEARCH



The Future



ROTHAMSTED
RESEARCH

- Field Testing Near Isogenic Lines of Elite Material with and without mutations
- Breeding new varieties will only start from there
- Pilot distilling study
- Marketing
 - How to achieve a premium?

Acknowledgements

RRes Cell Wall Group:

Archana Patil

Amy Plummer

Alison Lovegrove

Kirstie Halsey

Jackie Freeman

Mark Wilkinson

Ondrej Kosik

Peter Shewry

Rowan Mitchell

Andy Phillips

*Plant Hormone signalling
group*

Glass house staff



Funding:

Designing Seeds ISP

BBF0142951

BBSRC Follow-on and

InnovateUK



ROTHAMSTED
RESEARCH