Genome editing for low acrylamide wheat

Sarah Raffan
PhD Student
Rothamsted Research
and University of Bristol

Acrylamide

ROTHAMSTED RESEARCH

- The processing contaminant, acrylamide, was unexpectedly found in cooked foods, mainly those derived from plants, in 2002.
- Acrylamide causes cancer in rodents and is regarded as a probable (Group 2a) carcinogen in humans. It also affects development, and at high doses the nervous system and fertility.
- Fried, baked and roasted potato and cereal products and coffee are the major contributors to dietary intake in Europe.
- Bread, breakfast cereals, biscuits, cereal snack products, cakes, pies, batter, chips, crisps, roast potatoes, and all types of coffee are all affected.

Wheat as a major source of dietary acrylamide

ROTHAMSTED RESEARCH

- Wheat represents one of the major sources of dietary acrylamide intake, with products such as biscuits, breakfast cereals and toast all showing relatively high acrylamide concentrations.

Contribution (%) of different food groups to dietary acrylamide intake for adults in the UK									
Potato Products				Cereal Products					
French Fries	Crisps	Oven Potatoes	Total	Biscuits	Crisp Bread	Bread	Breakfast Cereals	Muesli	Total
41.3	8.5	17.3	67.1	6.3	2.0	15.0	5.0	3.6	31.9

Journal of Cereal Science **59**, 382-392; data from EFSA, 2011

Regulations for acrylamide in food products as set by the European Commission

ROTHAMSTED RESEARCH

- The EFSA's Expert Panel on Contaminants in the Food Chain issued a report in 2015, stating that the margins of exposure for acrylamide indicate a concern for neoplastic (tumour-inducing) effects.
- New risk management measures were proposed in June 2017, including new, lowered Benchmark Levels and compulsory Codes of Practice.
- These came into force on 11th April 2018.

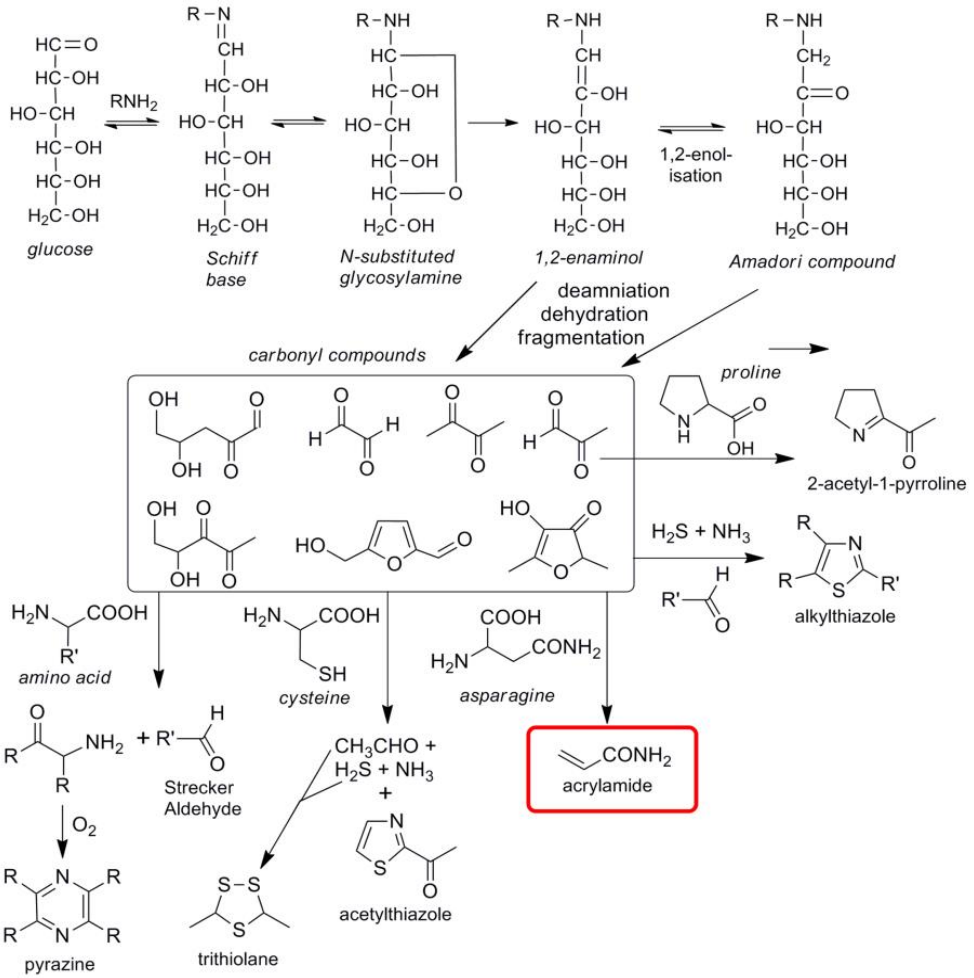
Food	Indicative Value 2013 (parts per billion)	Benchmark Level 2017 (parts per billion)
Soft bread (wheat)	80	50
Soft bread (other)	150	100
Breakfast cereals	400	300
Biscuits	500	350
Crackers	500	400
Crispbread	450	350
Gingerbread	1000	800
Cereal-based baby foods	50	40

Note that the tolerance level set for drinking water is 1 ppb.

Acrylamide formation

ROTHAMSTED RESEARCH

Acrylamide is formed from the reaction of free (soluble, non-protein) asparagine with reducing sugars during the later stages of the Maillard reaction, which is also responsible for giving cooked foods their characteristic flavours, aromas and colours.

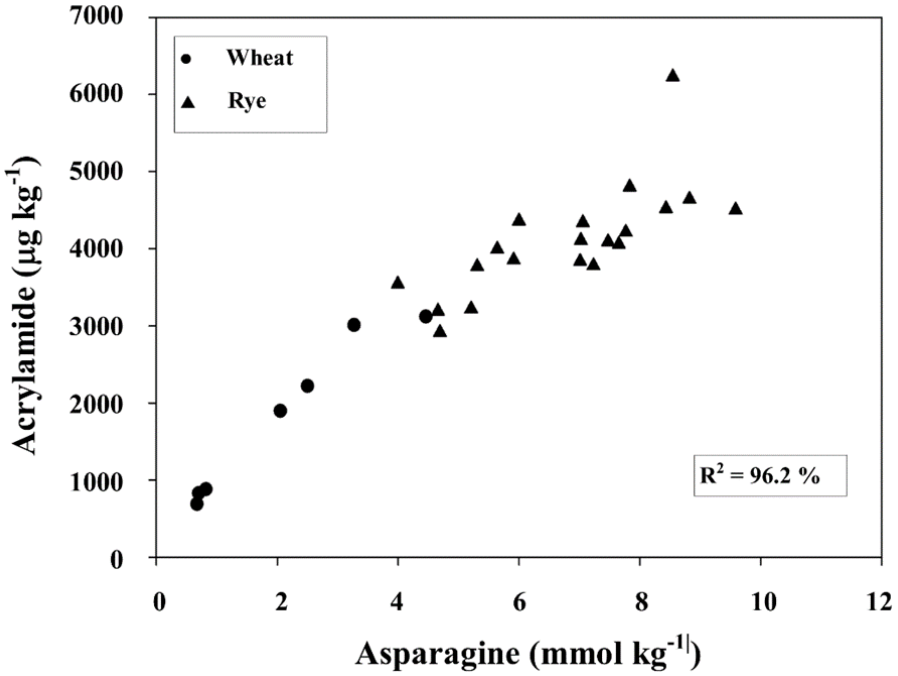


Annals of Applied Biology **158,** 1-25
Journal of Experimental Botany **63**, 2841–2851

Acrylamide formation

ROTHAMSTED RESEARCH

- Free asparagine and reducing sugars can therefore be regarded as precursors of acrylamide
- Asparagine concentration is the determining factor for acrylamide formation in cereal products.



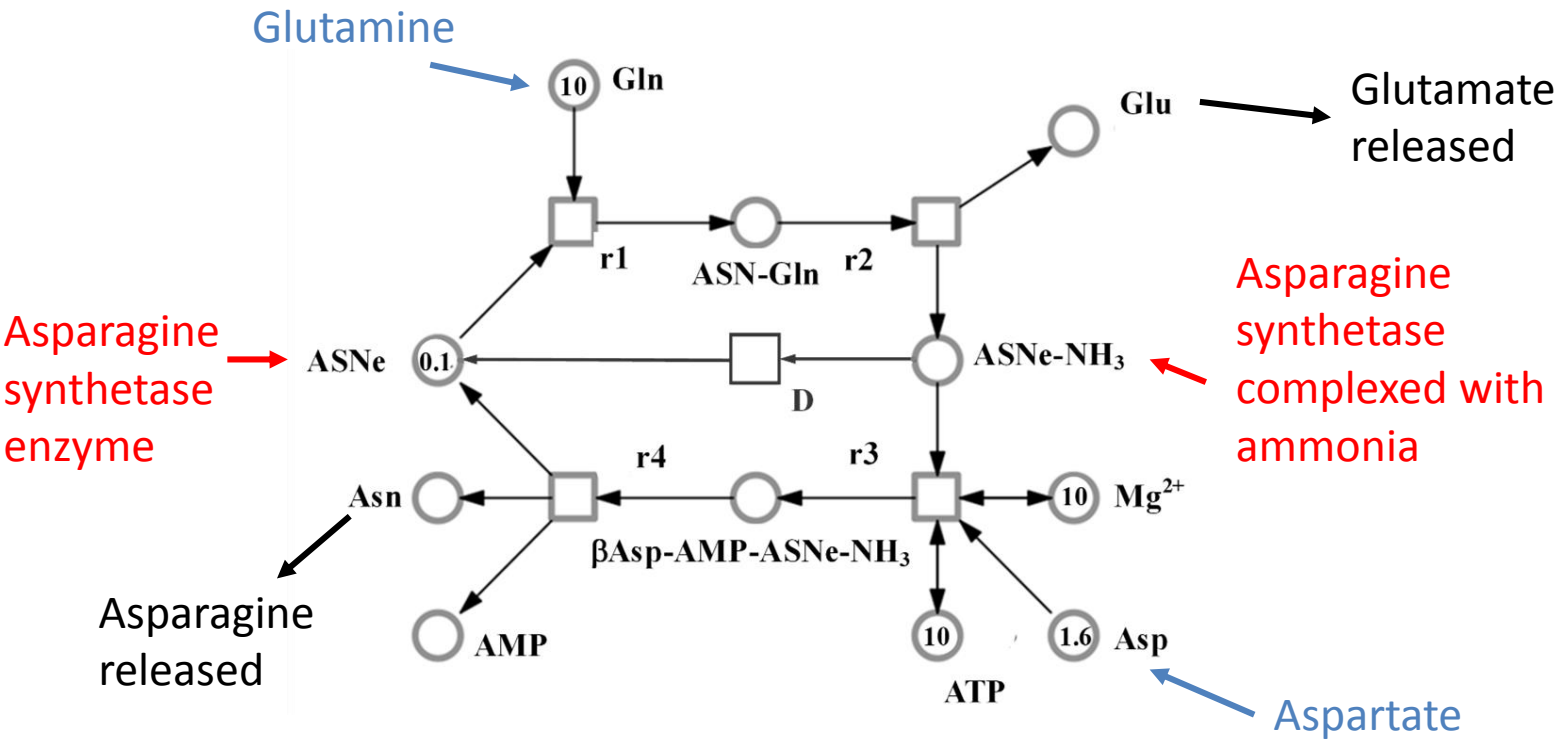
Acrylamide formation (µg/kg) (ppb) plotted against free asparagine concentration (mmol/kg) in wheat and rye flour heated at 180 ˚C

Journal of Cereal Science **59**, 382-392.

Asparagine Biosynthesis

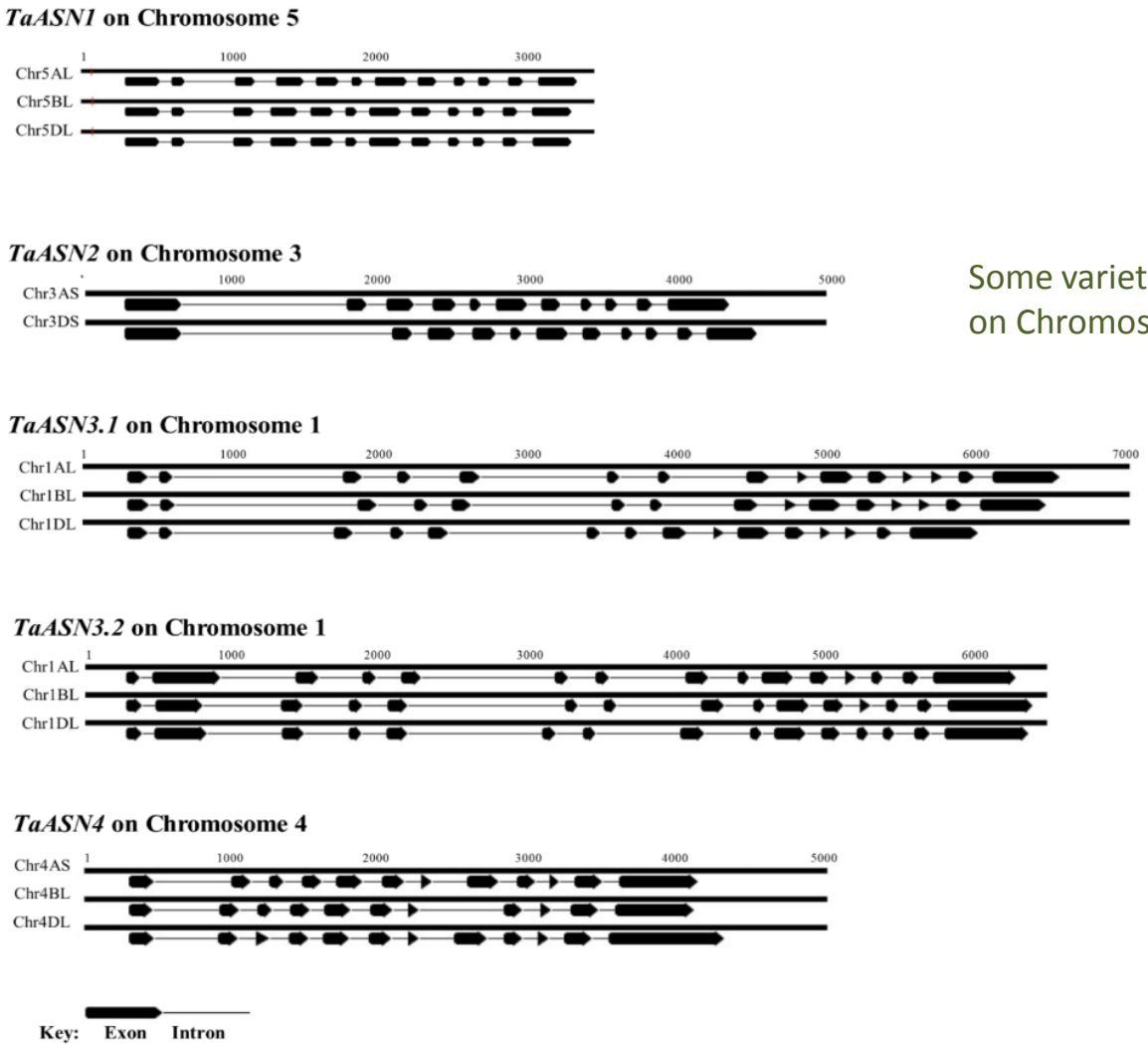
ROTHAMSTED RESEARCH

- Asparagine is synthesised via the ATP-dependent transfer of an amino group from glutamine to aspartate, and glutamate is released as a by-product.
- This reaction is catalysed by a family of enzymes called the asparagine synthetases (ASNs).



Wheat asparagine synthetase genes

ROTHAMSTED RESEARCH

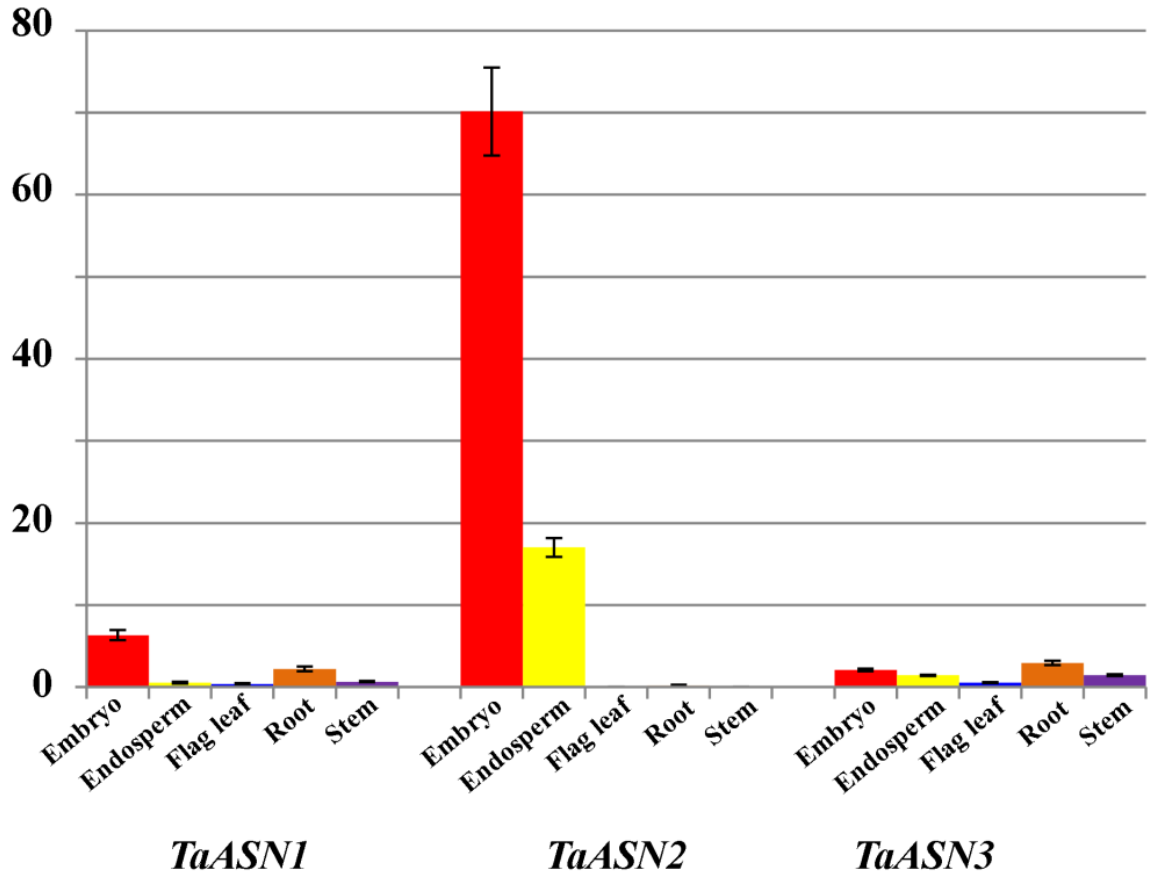


Some varieties lack a *TaASN2* gene on Chromosome 3B

Frontiers in Plant Science **8**, 2237

Differential expression of asparagine synthetase genes in different tissues of wheat

ROTHAMSTED RESEARCH



The embryo is in the bran fraction while the endosperm is the white flour fraction.

TaASN2 is an obvious target for genetic intervention.

The graphs show the NRQ means and standard errors.

Journal of Cereal Science **68**, 122-131

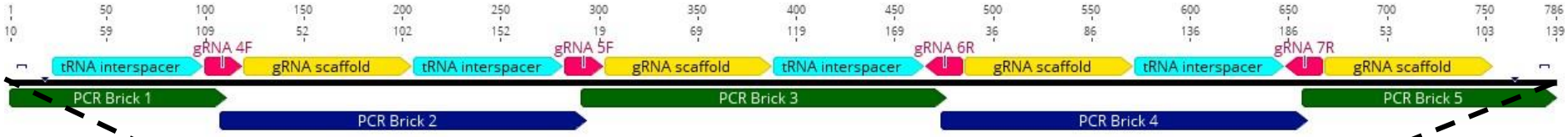
Knocking out *TaASN2*: Genome editing with CRISPR-Cas9

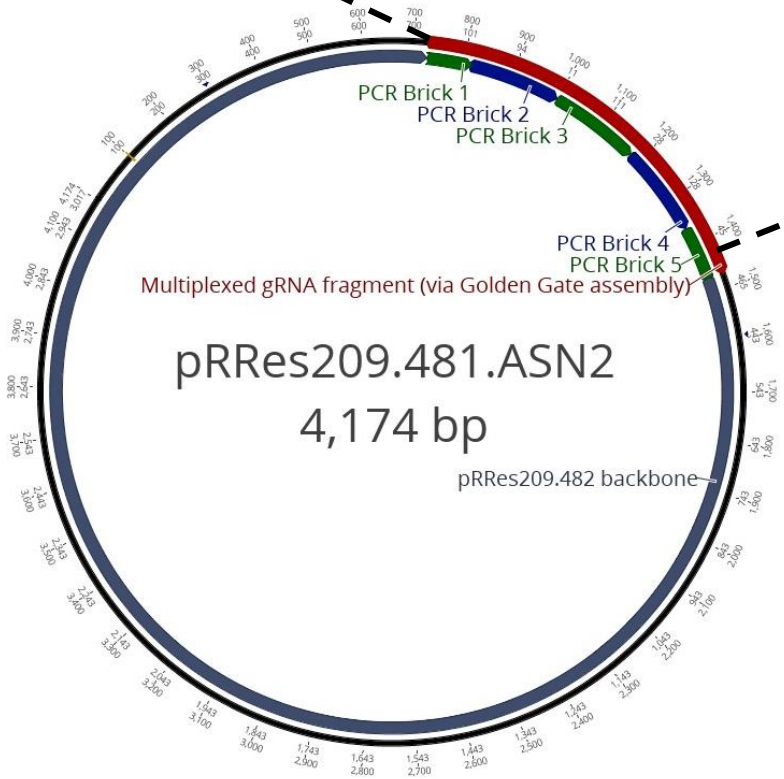
ROTHAMSTED RESEARCH

- Four gRNAs were designed to target the first exon of *TaASN2*.
- A plasmid construct was created containing the 4 gRNAs and used to transform wheat embryos by particle bombardment.

The gRNA plasmid construct – pRRes209.481.ASN2

ROTHAMSTED RESEARCH





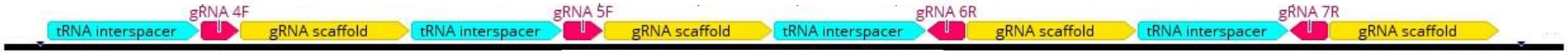
A gRNA construct was generated via a Golden Gate assembly, **pRRes209.481.ASN2**, based on the multiplex system proposed by Xie *et al.* (2015), containing a 4-gRNA polycistronic gene interspaced with tRNAs.

Proceedings of the National Academy of Sciences **112(11)**, 347–355

The gRNA plasmid construct – pRRes209.481.ASN2

ROTHAMSTED RESEARCH

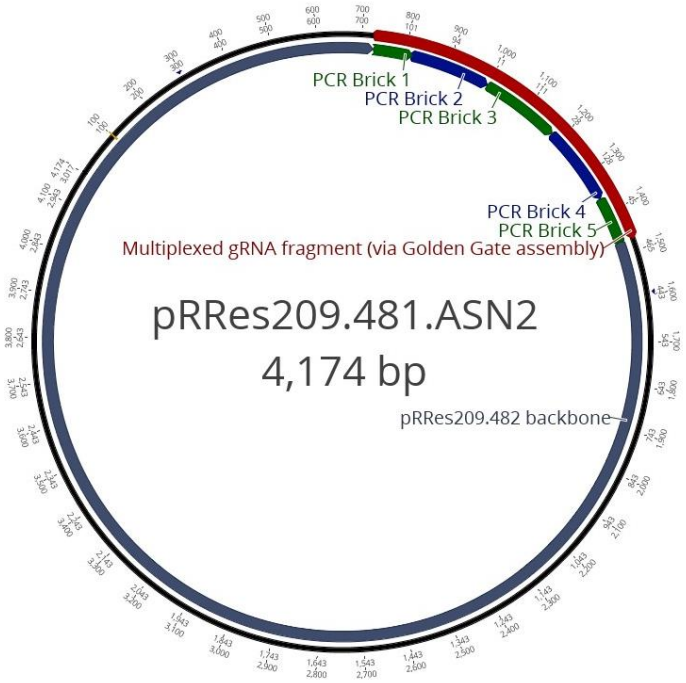
The polycistronic 4-gRNA structure consisted of gRNAs (20bp guide sequences + gRNA scaffold) interspaced with tRNAs. The tRNAs are cleaved out by the plant's endogenous tRNA processing enzymes to release the 4 mature gRNAs



Proceedings of the National Academy of Sciences **112(11)**, 347–355

The gRNA plasmid construct – pRRes209.481.ASN2

ROTHAMSTED RESEARCH



The construct was used alongside a *Cas9* vector, pRRes217.486, and a plasmid which confers BASTA resistance, to transform *T. aestivum* cv. Cadenza embryos via particle bombardment.

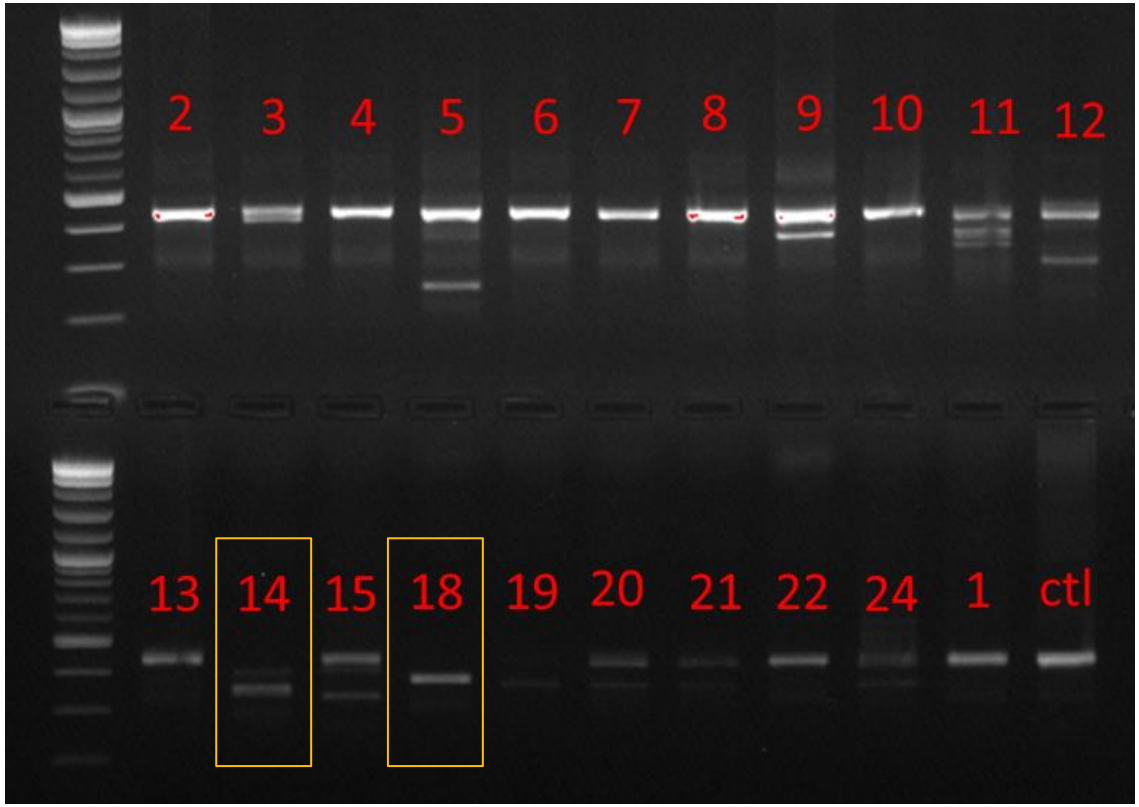
Bombardment	Plant Number	+ve Cas9	-ve Cas9
B3654	24	20	4
B3656	11	7	4
B3662	15	14	1
B3675	29	26	3
B3677	8	8	0
B3678	5	2	3
Total	92	77	15

Detection of editing events through PCR amplification of target region

ROTHAMSTED RESEARCH

PCR amplification of target region for ‘bandshift’ identification.

This screens for large deletions or insertions only

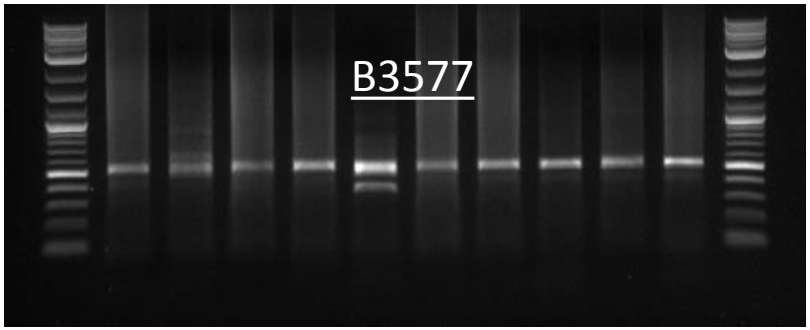


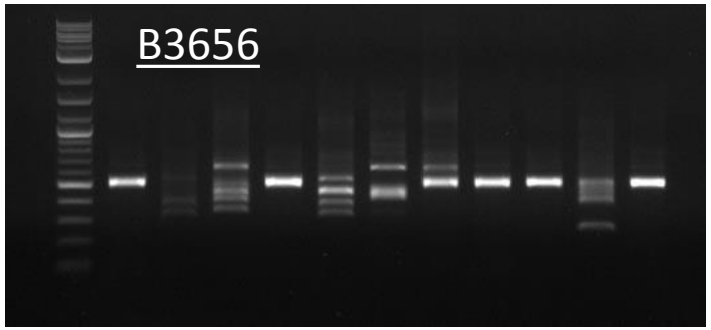
‘Wild-type’ band

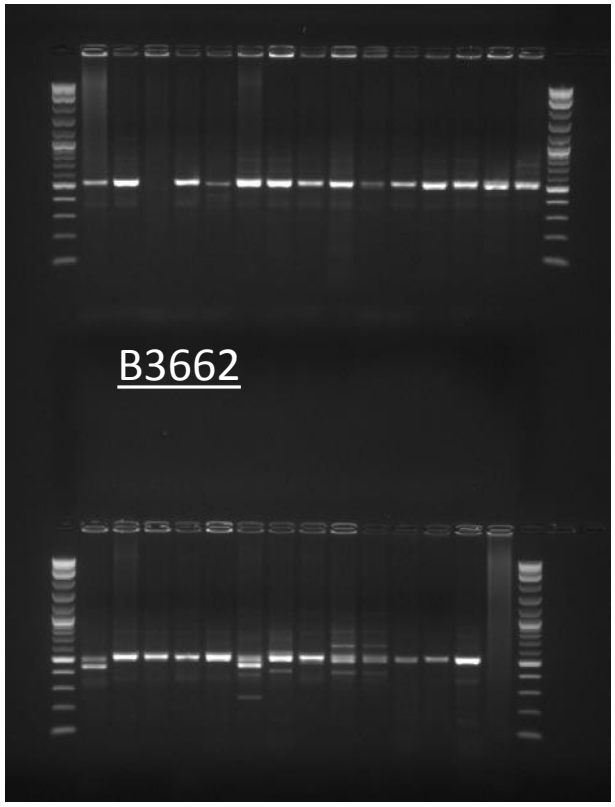
‘Wild-type’ band

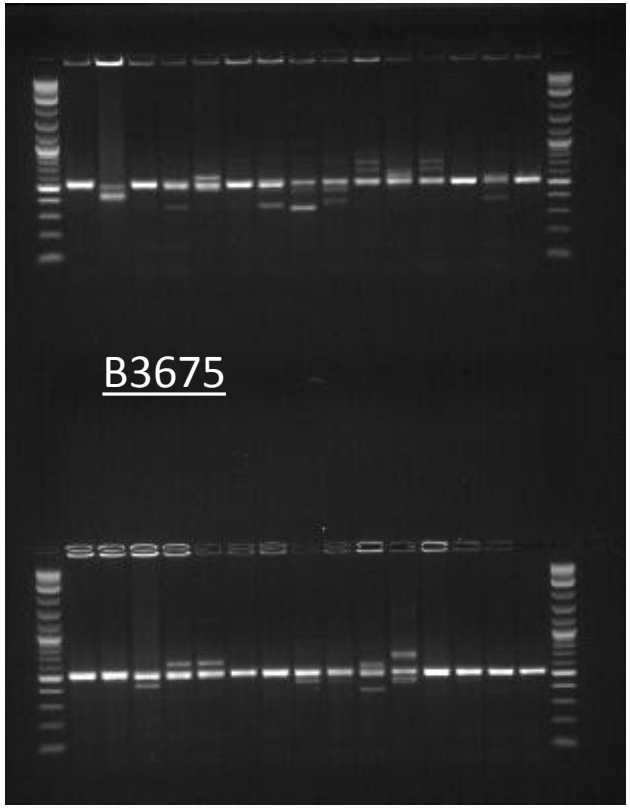
Detection of editing events through PCR amplification of target region using conserved primers

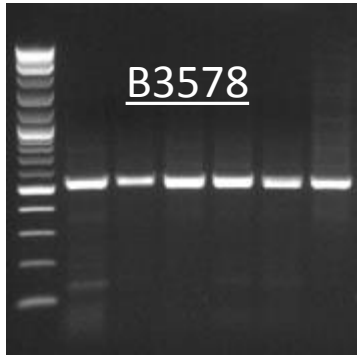
ROTHAMSTED RESEARCH











Knocking out *TaASN2*: TILLING lines

ROTHAMSTED RESEARCH

- We have mutant lines carrying mutations in each of the *TaASN2* genes
- These are being crossed to generate a null *TaASN2* line

Thanks to Andy Phillips, Rothamsted

Knocking out *TaASN2*

ROTHAMSTED RESEARCH

- We believe that the TILLING and genome editing approaches have the potential to solve the acrylamide problem, at least in the context of current regulations, but this depends on how the plants respond to the intervention

- It is possible that other genes will have to be targeted as well as or instead of *TaASN2*

Concluding remarks

- Step reductions in the acrylamide-forming potential of wheat may be possible using modern techniques, such as genome editing
- Genetic and agronomic approaches to solving the acrylamide problem could eventually lead to massive savings for the food industry
- Our impression is that while awareness of the acrylamide issue is high amongst large food producers, it is almost zero amongst SMEs and the catering sector. They need to wake up, because the Codes of Practice that now apply across the EU are ***compulsory***

Acknowledgements

- BBSRC SWBio iCASE DTP Studentship
- Industrial Partners:
  - AHDB, KWS UK Ltd., Saaten Union UK Ltd, RAGT Seeds Ltd, Syngenta UK Ltd, and Limagrain UK Ltd
- Rothamsted Research:
  - Nigel Halford, Damiano Martignago, Caroline Sparks and Cereal Transformations, Alison Huttly, Tanya Curtis
- University of Bristol:
  - Keith Edwards