

Agronomic and genetic approaches to reduce the risk of acrylamide formation in wheat products



Nigel G. Halford
Rothamsted Research

ACRYRED Conference. Acrylamide in Cereal Products:
Progress and Prospects
June 3rd- 4th 2026



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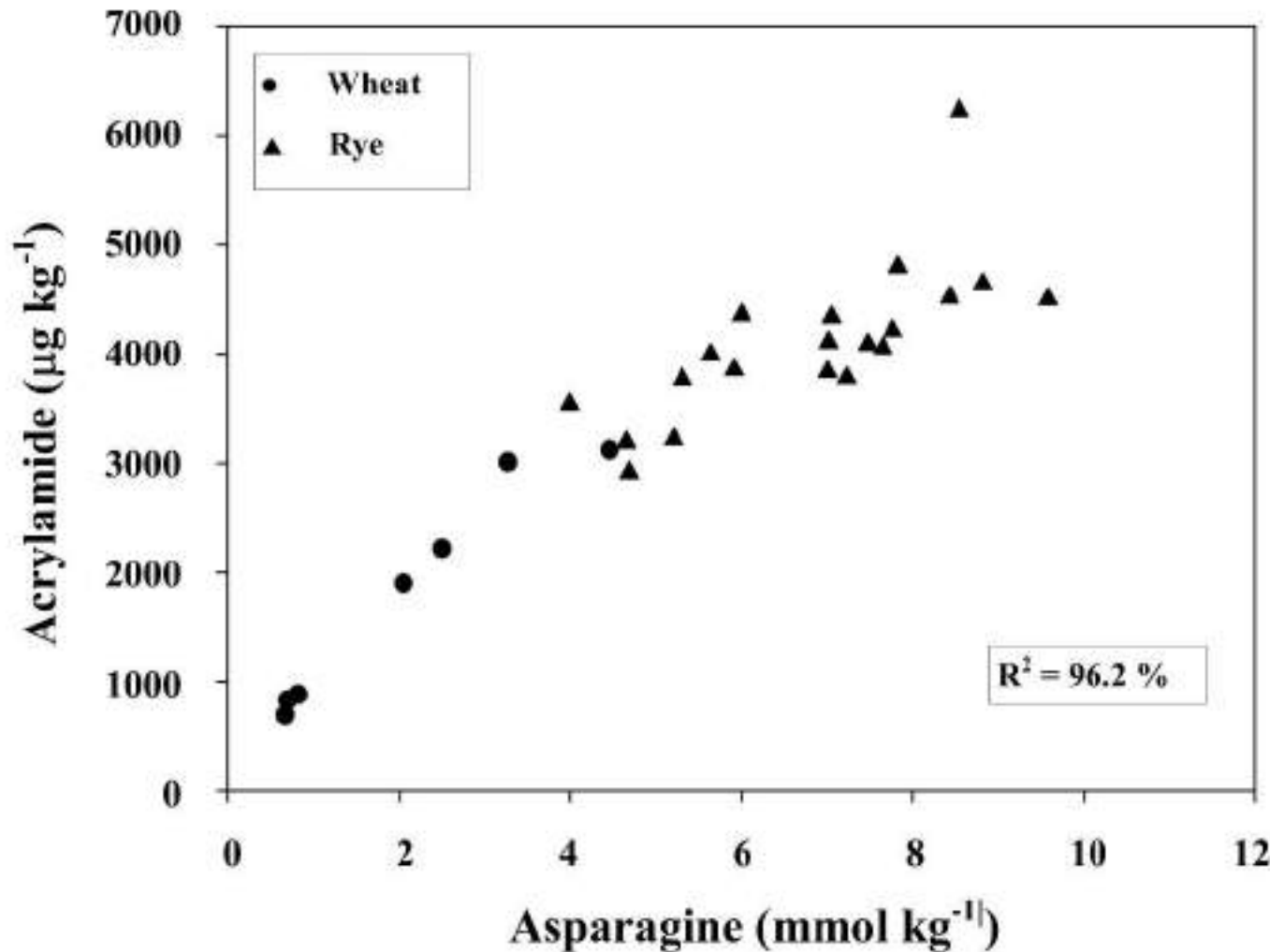


Biotechnology and
Biological Sciences
Research Council

Free (non-protein) asparagine concentration determines acrylamide formation in heated flour



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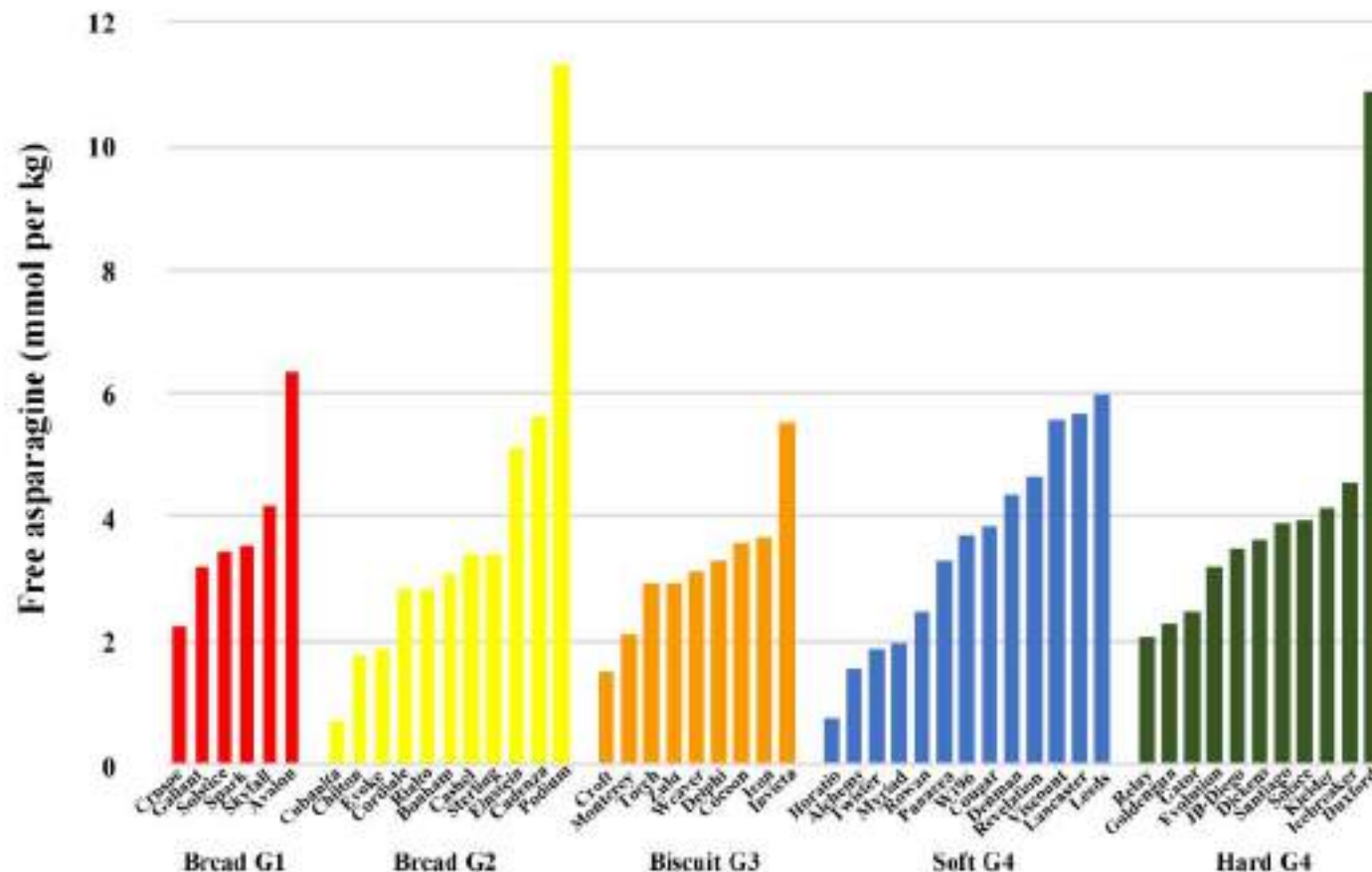
Acrylamide formation in heated flour produced from wheat and rye grain, plotted against free asparagine concentration.

Acrylamide concentrations in commercial cereal products depend on the concentration of free asparagine in the raw material and the processing and quality control methods used by manufacturers.

Varietal differences in wheat



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Free asparagine concentration in grain of winter wheat varieties from the UK's AHDB Recommended List grown in a field trial in the UK.

- Free asparagine concentration is very sensitive to environmental factors and $G \times E$, and rankings can change between sites and from year to year.
- QTL have been difficult to identify and inconsistent between studies. [See Poster 19.](#)



Tanya Curtis

Varietal differences



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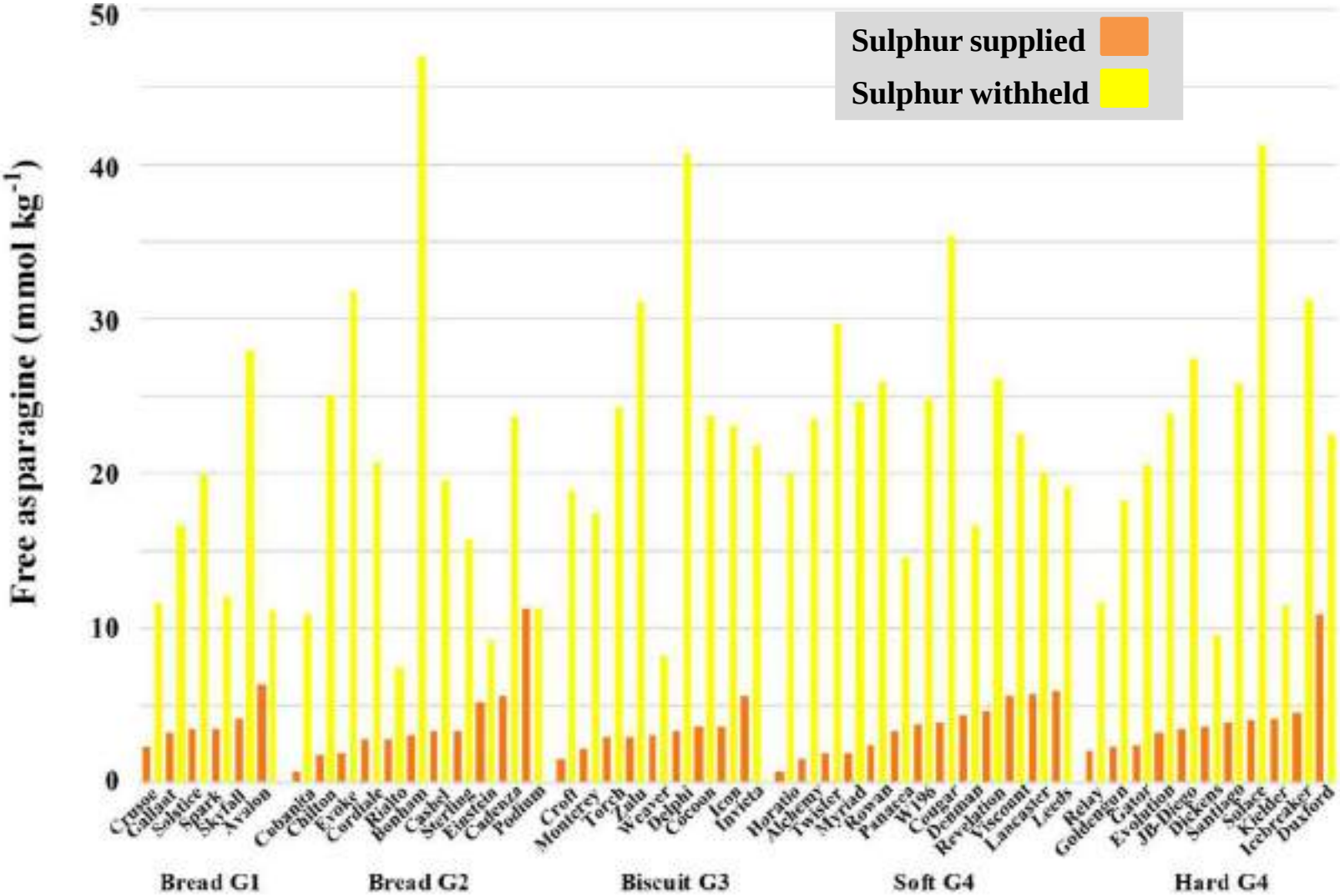


- Some wheat varieties were in the bottom half of the rankings on asparagine concentration over several field trials, and some were consistently in the top half, so there is scope for selecting low asparagine varieties.
- Most of the 'low' varieties were soft wheats, but selecting varieties for low asparagine simply on the basis that they are soft would be simplistic and potentially counter-productive (some soft varieties are high in asparagine).
- No-one is measuring free asparagine concentration in new varieties in the UK, and variety turnover is so rapid that a variety is likely to be obsolete by the time free asparagine concentration has been assessed over multiple seasons.
- We would support the inclusion of information on grain asparagine concentration in the UK's Recommended List variety descriptions.

Crop management: the importance of supplying wheat with sufficient sulphur



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Mean free asparagine concentration in the grain of 50 varieties of winter wheat grown in a field trial in 2012-2013. Data are shown from split-plots in which wheat in half the plot was supplied with nitrogen and sulphur (brown bars), while the wheat in the other half was supplied with nitrogen but not sulphur (yellow bars).

Sulphur was applied at a rate of 40 kg sulphur per hectare (100 kg per hectare SO₃ equivalent).

Ensuring sulphur sufficiency is included in the European Commission's compulsory Code of Practice for cereals.



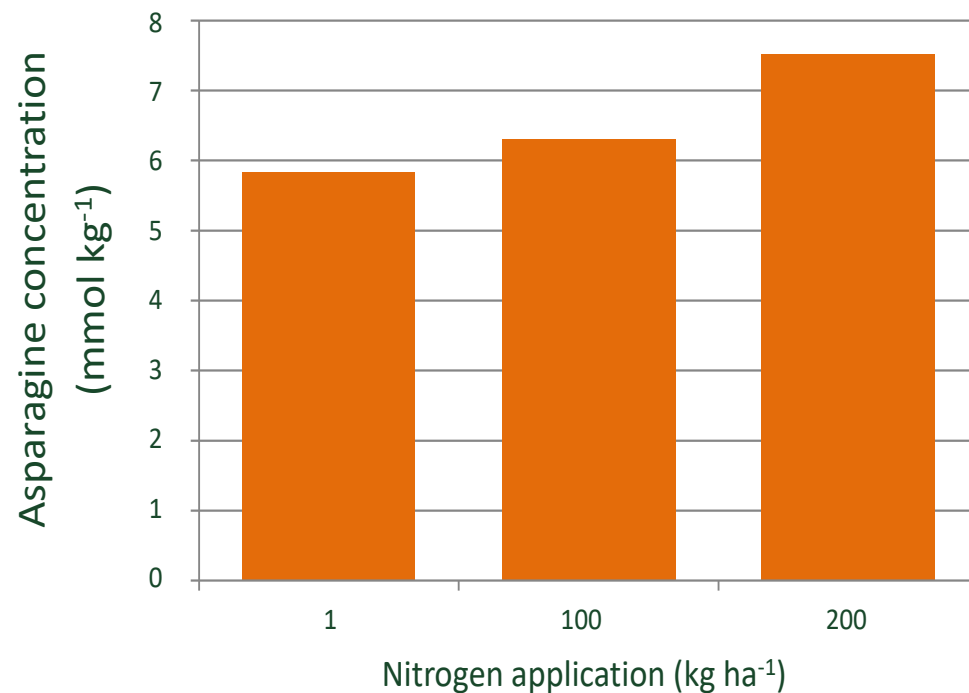
Tanya Curtis

Crop management: Effect of nitrogen availability on acrylamide-forming potential in rye

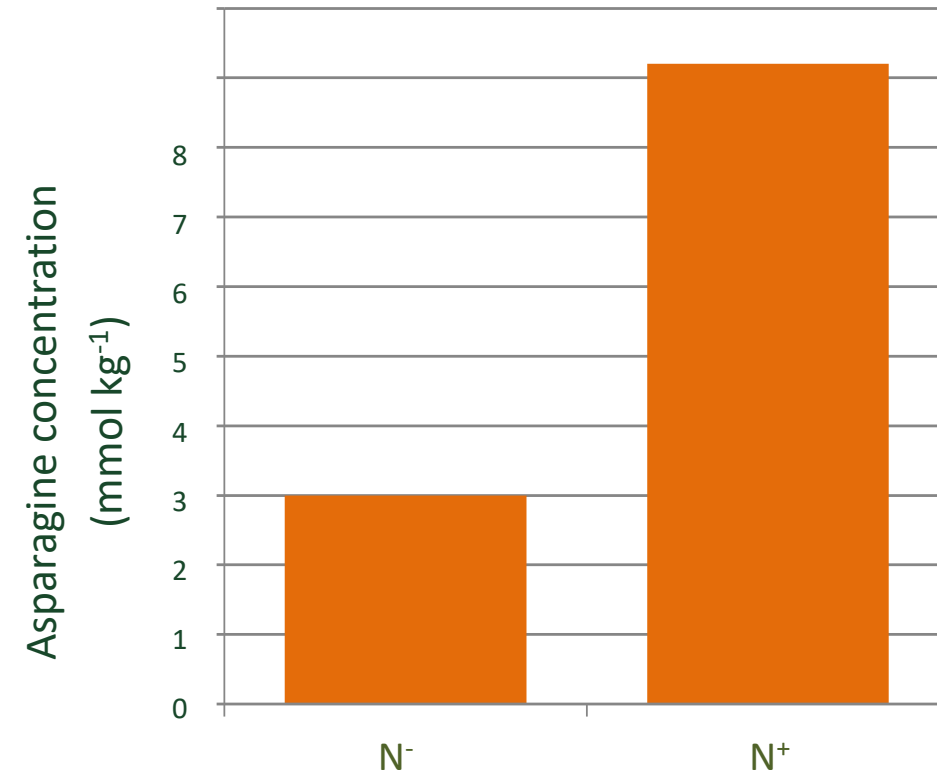


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Field



Glasshouse



Nitrogen application increases the free asparagine concentration of wheat and rye grain and other cereals, causing a concomitant increase in acrylamide-forming potential. Nitrogen fertilizer is required to maintain the yield and quality of the crop, but excessive application should be avoided. This has been incorporated into the European Commission's cereal compulsory Code of Practice.



Jen Postles

Crop management: ensuring sulphur sufficiency is a key factor in preventing high levels of free asparagine accumulation in wheat grain

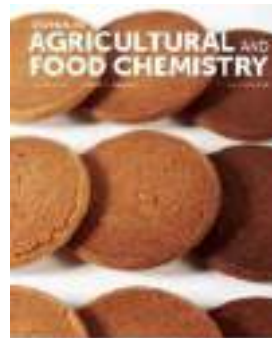


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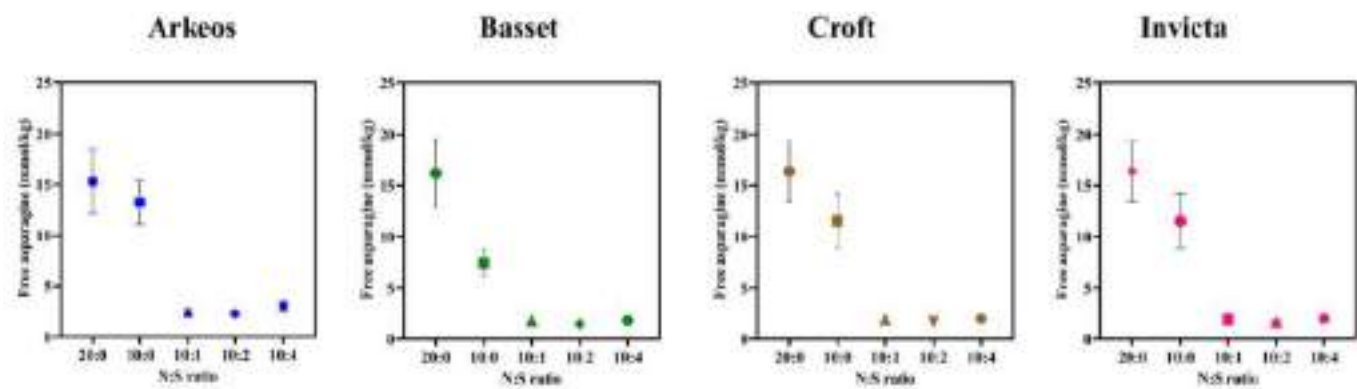


- Field trials aimed at refining our advice on N and S application and other minerals' Woburn UK, 2019-2021.
- Different ratios of N and S.
- Also looked at phosphate and potassium.
- Use of imaging methods during cultivation and after harvest to predict asparagine levels.
- Our results indicated that a N:S ratio of 10:1 was sufficient to prevent large increases in free asparagine, whereas withholding or supplying potassium or phosphorus alone did not cause changes in free asparagine when sulphur was applied.

Joe Oddy



Effects of the N:S fertiliser application ratio on free asparagine in the grain and acrylamide and colour formation in biscuits



Free asparagine concentrations in grain from different varieties of wheat grown with different ratios of N and S.



Biscuits prepared from flour (variety Basset) containing high, medium and low concentrations of free asparagine.

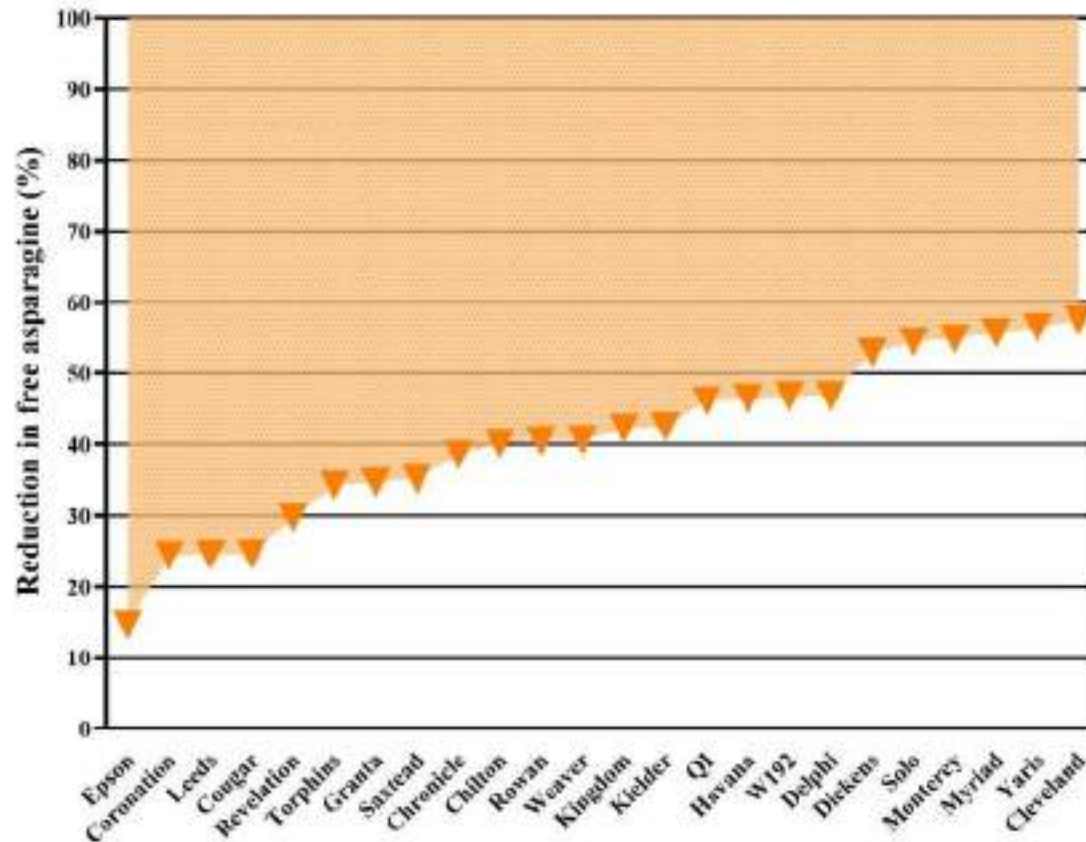
Acrylamide in the biscuits correlated with free asparagine concentration and with colour (top surface hue angle): R² values from linear models of 89 % and 91 %, respectively (p < 0.001). Current Benchmark for biscuits is 350ppb!

Acrylamide (µg kg⁻¹): 13523 6114 488

Crop management: the importance of disease control



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Graph representing the impact of disease control on free asparagine content in the grain of 24 winter wheat varieties. Each triangle represents the percent reduction in free asparagine content in grain from plots treated with fungicide compared with grain from untreated plots.

The fungicide treatment was given from growth stages 30 to 65.

The main diseases observed in the untreated plots were *Septoria tritici*, Yellow rust and Brown rust.

To follow 'good phytosanitary practices to prevent fungal infection' has been included in the European Commission's compulsory Code of Practice for wheat products

Tanya Curtis
(data)



Navneet Kaur
(plot)



The importance of sulphur sufficiency and disease control



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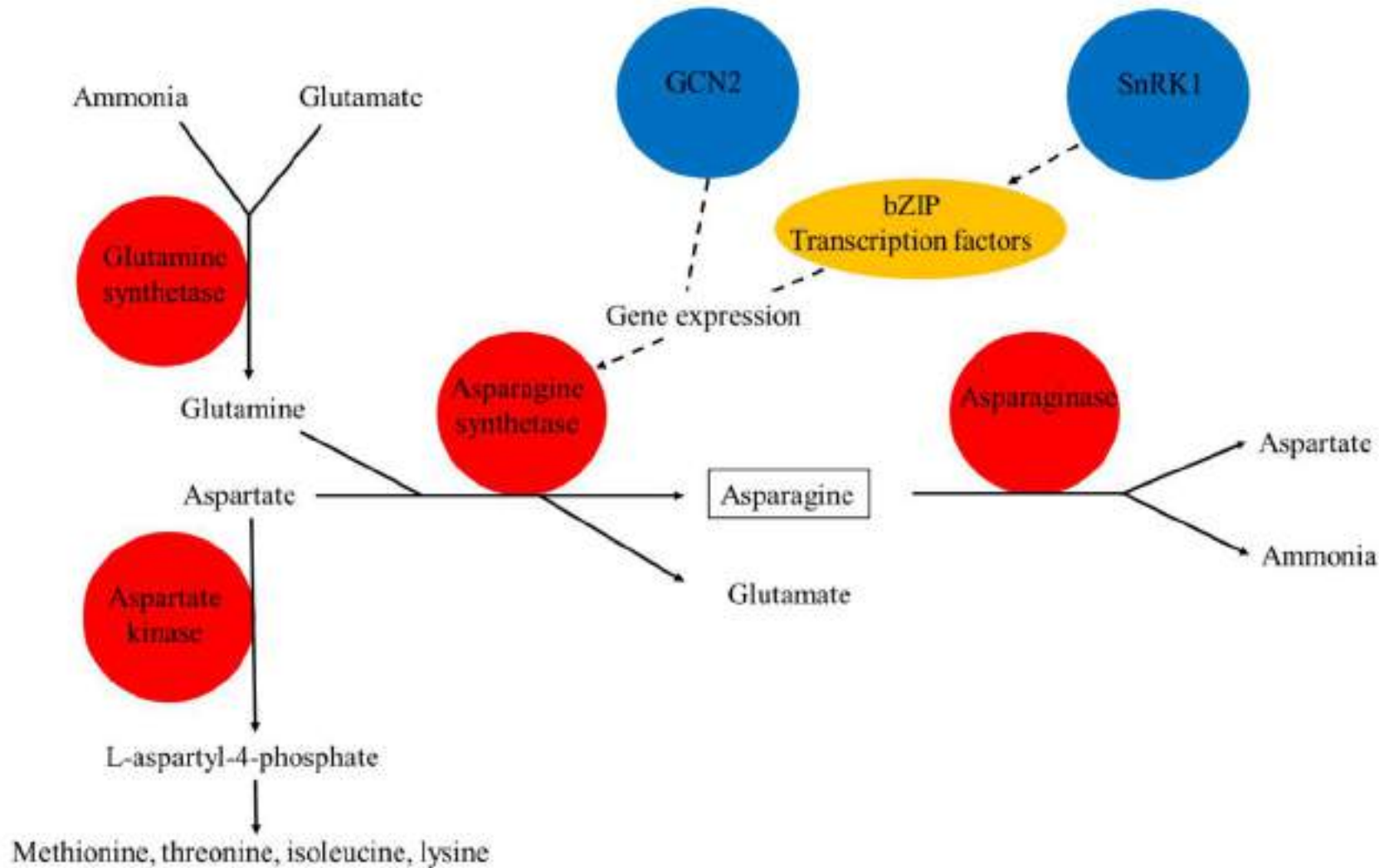


- The practice of ensuring sulphur sufficiency and effective disease control should be adopted to mitigate the problem of acrylamide formation in wheat products.
- Excessive and late application of nitrogen should be avoided. In wheat, in particular, if nitrogen is applied when sulphur is limiting it will accumulate as free asparagine, increasing acrylamide risk without achieving the desired result of improving protein quality.
- Regulatory authorities should take the consequences for acrylamide-forming potential into account when considering the risks and benefits of mineral fertiliser and fungicide usage.

Potential genetic targets for interventions to reduce free asparagine accumulation



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- Genes encoding enzymes for biosynthesis or degradation of asparagine.
- Genes/proteins involved in the signalling pathways that control asparagine accumulation.

Genetic targets: Wheat asparagine synthetase genes



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TaASN1 on Chromosome 5



TaASN2 on Chromosome 3



TaASN3.1 on Chromosome 1



TaASN3.2 on Chromosome 1



TaASN4 on Chromosome 4



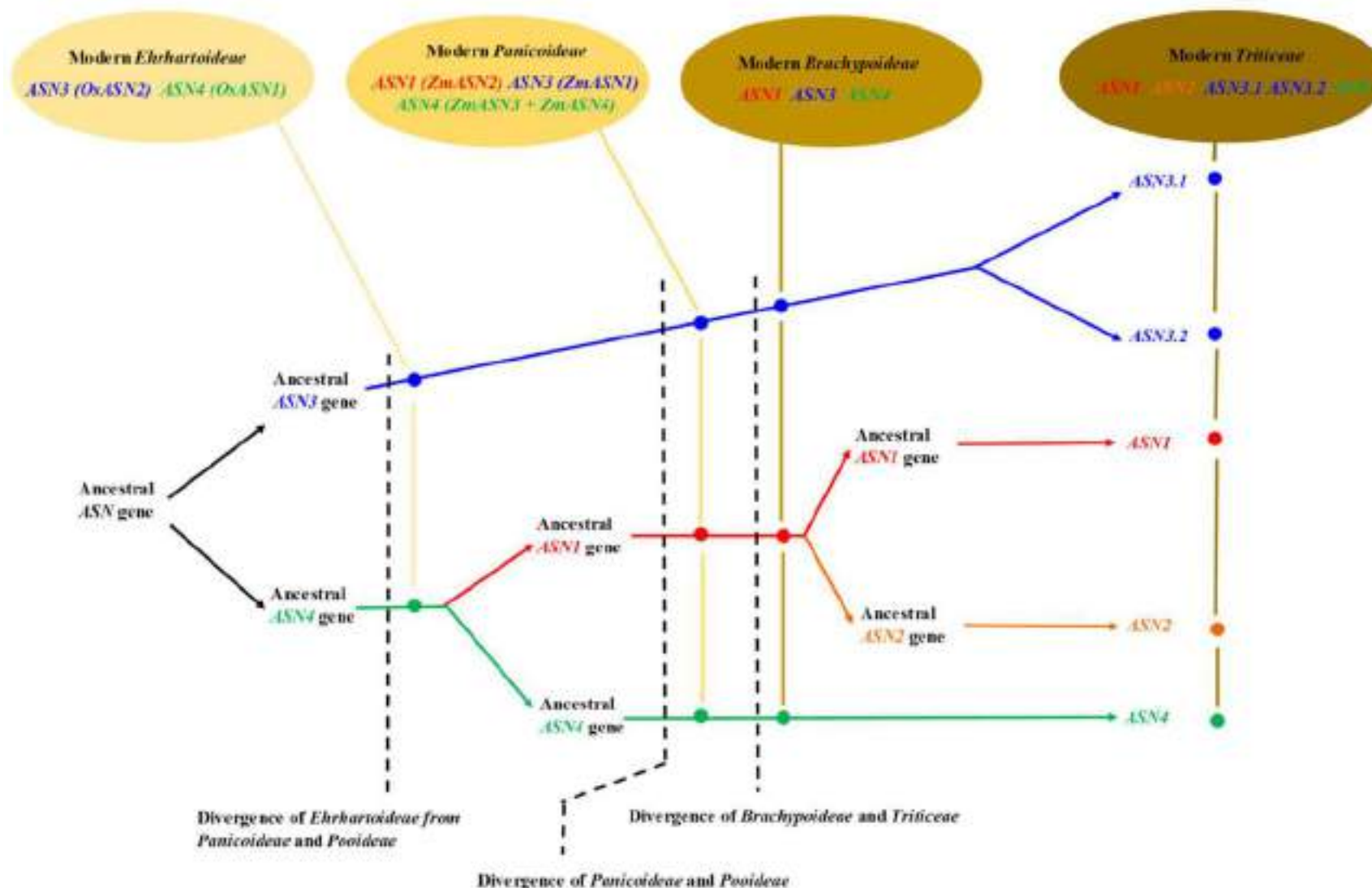
Key: Exon Intron

- Five genes per genome: *TaASN1*, *TaASN2*, *TaASN3.1*, *TaASN3.2* and *TaASN4*.
- Each gene is present as a single copy on each genome (bread wheat is a hexaploid with three genomes: A, B and D).
- Some varieties lack a *TaASN2* gene on Chromosome 3B.
- This gene family structure is unique to the Triticeae: maize and rice, for example, do not have a *TaASN2* equivalent.

Asparagine synthetase gene evolution in the cereals



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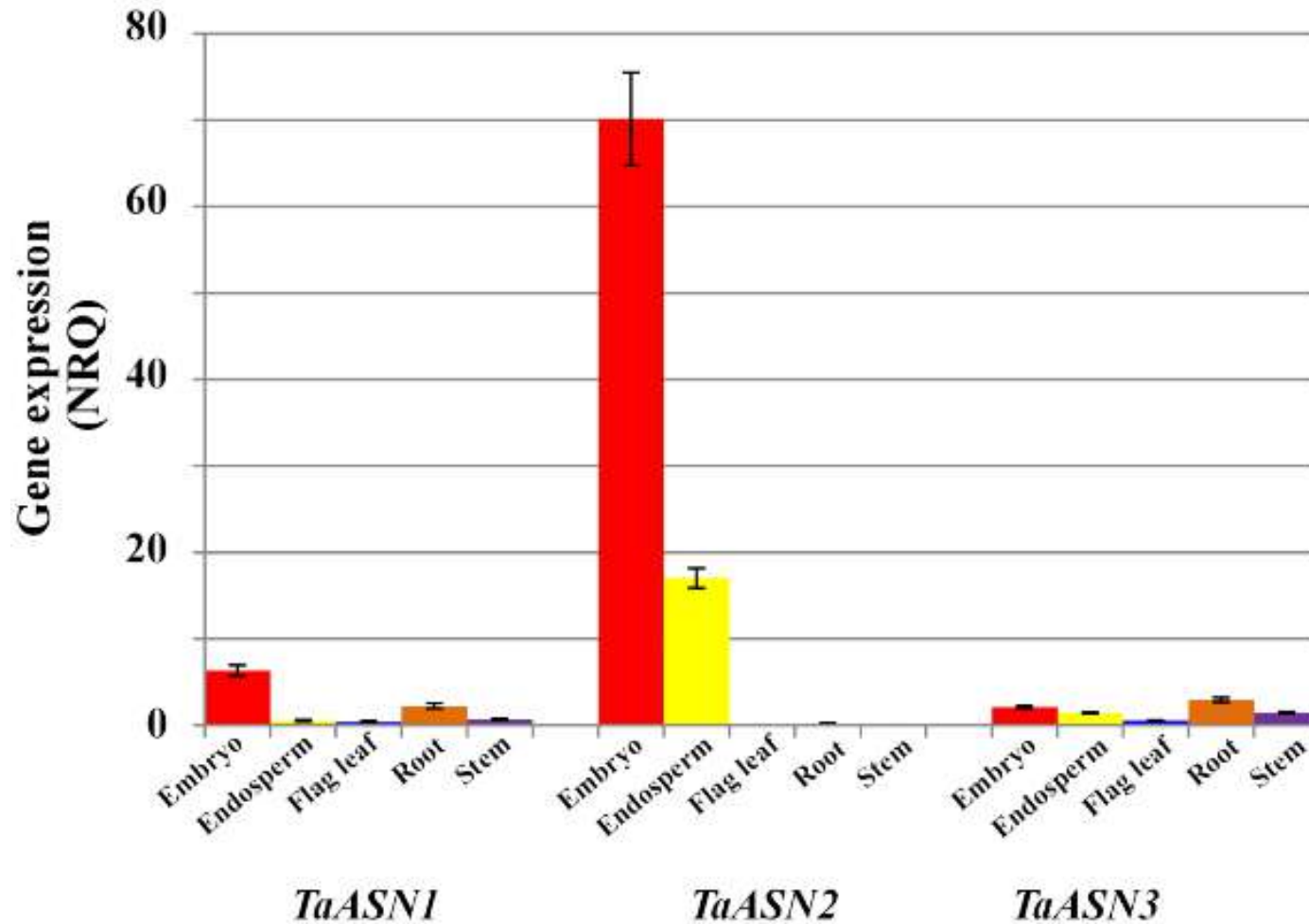


- A family of 5 genes in 4 groups is present in all of the *Triticeae*
- Maize, sorghum (*Panicoideae*) and Brachypodium have 3 groups, with some gene duplication in maize
- Rice (*Ehrhartoideae*) has only two genes
- ASN2, which is the most highly expressed ASN gene in wheat grain, is only present in the *Triticeae*
- The significance of this for the control of free asparagine levels in rice, maize and sorghum grain requires investigation

Differential expression of asparagine synthetase genes in different tissues of wheat at the midpoint of grain development



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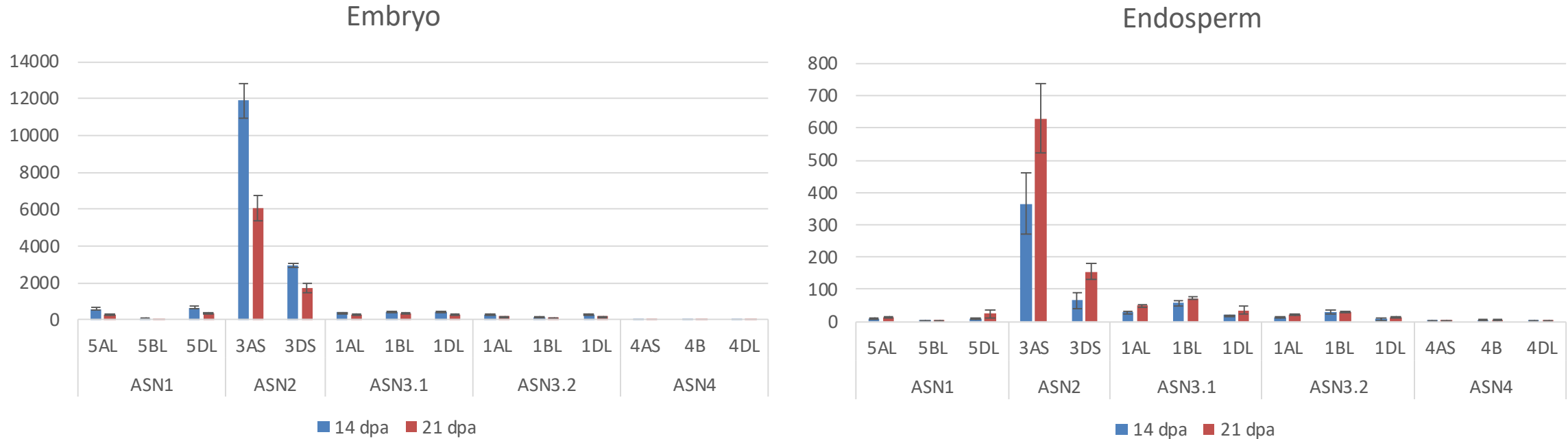
Expression (NRQ means and standard errors) of *TaASN1*, *TaASN2* and *TaASN3* in different tissues of wheat, including the embryo (bran fraction) and endosperm (white flour fraction).

TaASN2 is an obvious target for genetic intervention

Runhong Gao



Asparagine synthetase gene expression: RNA-seq data

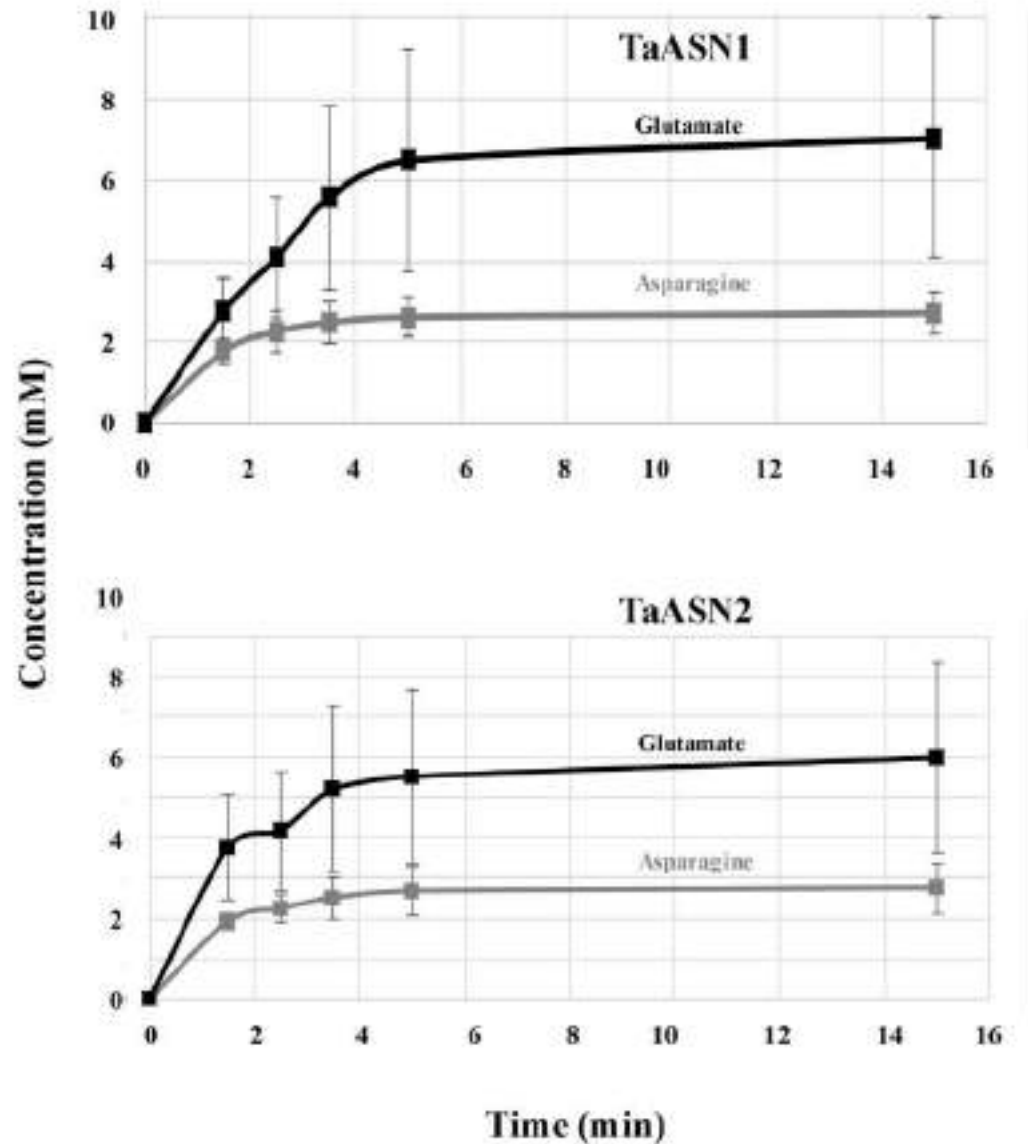


- Higher levels of expression in the embryo than in the endosperm (> 10-fold difference).
- *TaASN2* was the most highly expressed gene in both tissues.
- The A genome *TaASN2* gene was much more highly expressed than the D genome copy (> 3-fold difference). The *TaASN-B2* gene is absent in these genotypes.
- Reaffirmed *TaASN2* as the best target! Knocking out the *TaASN-A2* gene on its own may be effective.

Production of glutamate and asparagine by wheat asparagine synthetases TaASN1 and TaASN2



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The two enzymes appear to be very similar.

Both enzymes continue to synthesise glutamate even when aspartate has run out and they cannot make asparagine

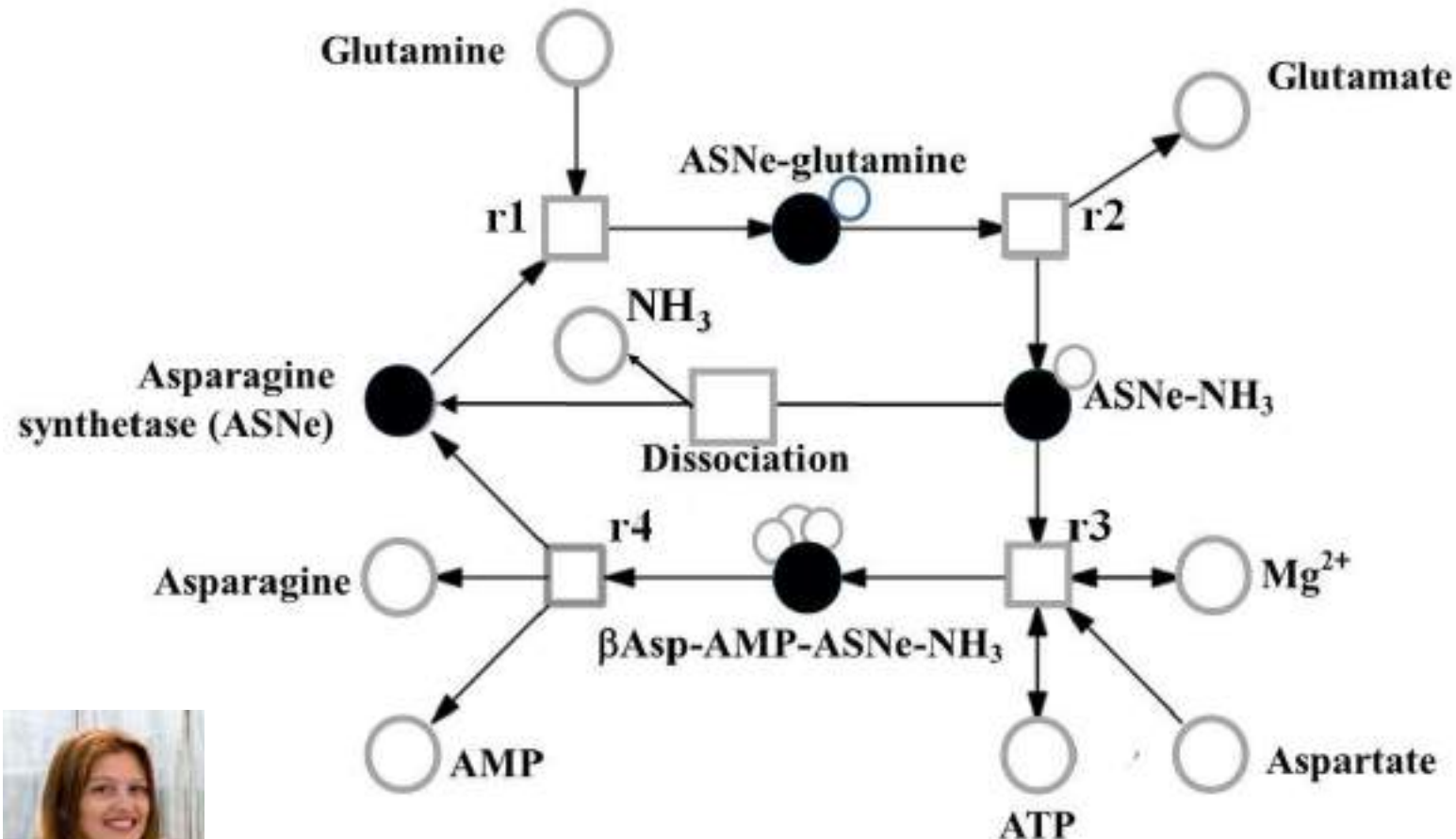


Hongwei Xu

Model representing the reaction catalysed by asparagine synthetase



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The model comprises metabolites (circles), and reactions (squares).

Water and pyrophosphate are not included.

The model was generated assuming that the reactions followed mass action kinetics.

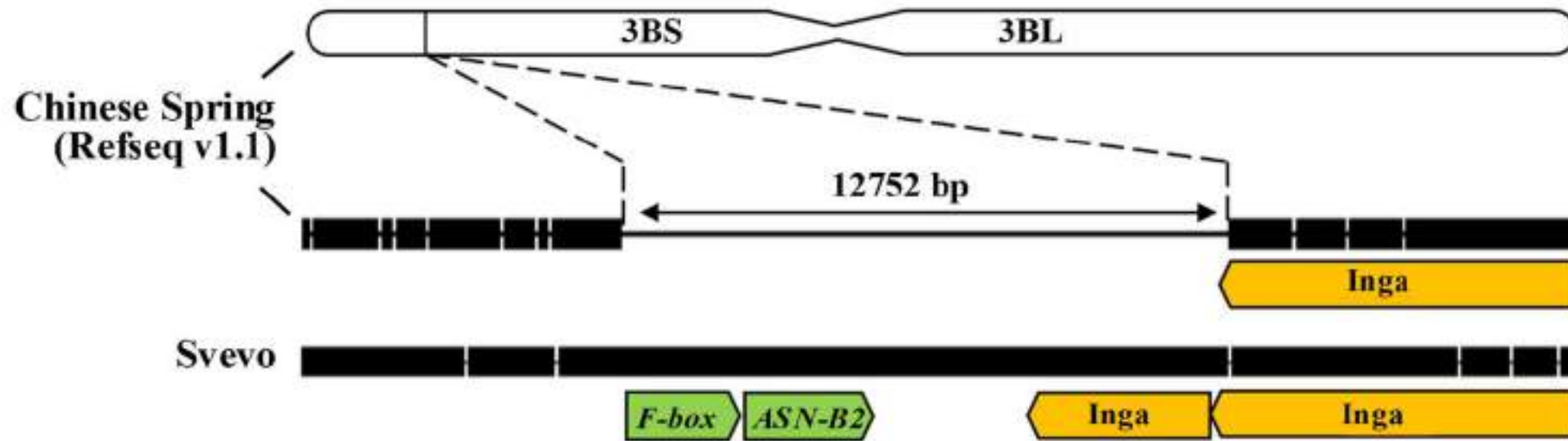


Tanya Curtis

A natural deletion in Chromosome 3B of variety Chinese Spring includes the *TaASN-B2* gene



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Alignment of the region of Chromosome 3B of variety Chinese Spring where the *TaASN-B2* gene has been deleted, and alignment with the corresponding region in variety Svevo, in which the *TaASN-B2* gene is present.

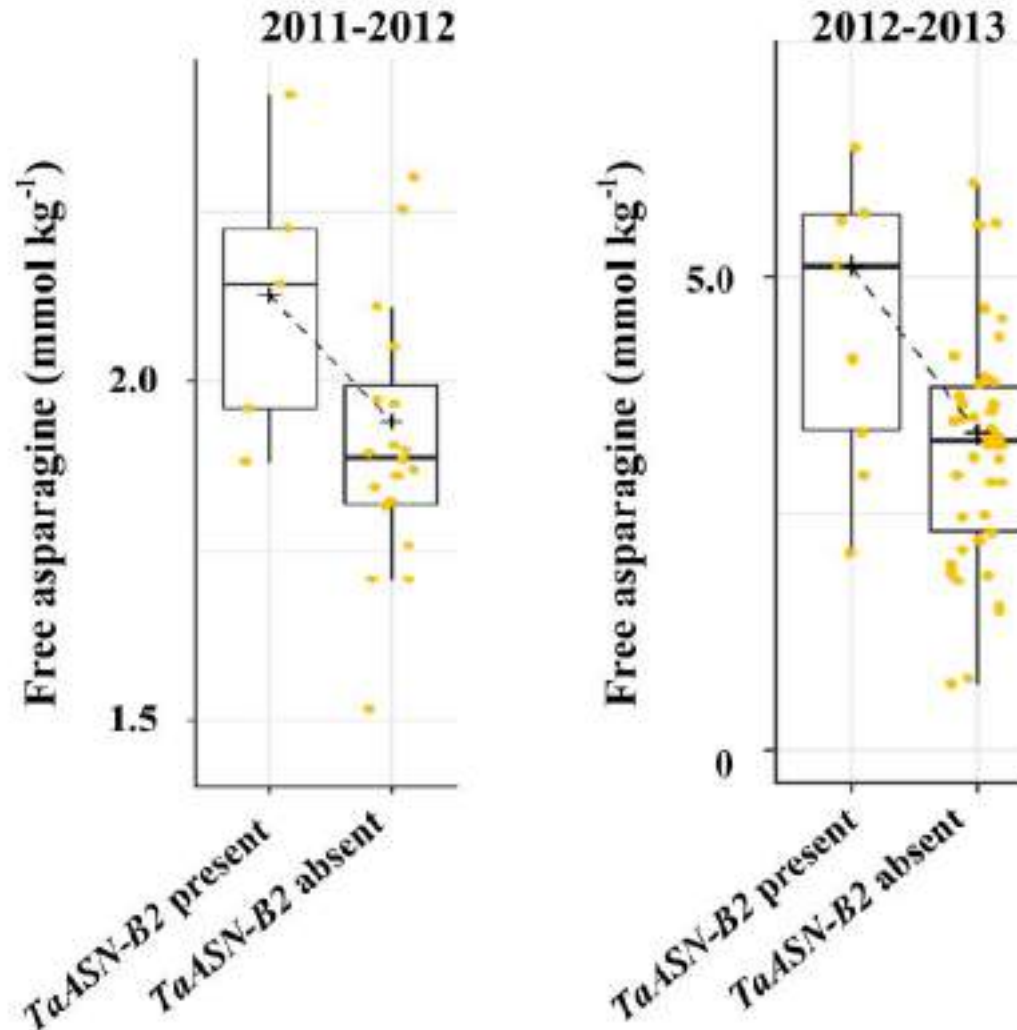


Joe Oddy

Effect of the *TaASN-B2* deletion on free asparagine levels in the grain of 50 varieties of winter wheat over two years of field trials



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- The presence of the *TaASN-B2* gene was associated with higher free asparagine concentrations.
- This breaks down under sulphur deficiency (not shown).
- Most but not all UK and some US wheat varieties, across all milling types, already lack a *TaASN-B2* gene. This limits the scope for exploiting this natural genetic event.
- Nevertheless, it is something that breeders could be doing now.

Knocking out *TaASN2* (and *TaASN1*) to produce low asparagine wheat: Genome editing with CRISPR-Cas9



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1F -> GGGGTGCGGCGACGAGTCCAGG

1 ATGTGCGGCATACTAGCGGTGCTGGGGTGC GCGCGACGAGTCCAGGGGAAGAGGGTCCACSTGCTAGAGCTCTCGCGCAG
1 M C G I L A V L G C G D E S Q G K R V H V L E L S R R

2F -> GGACTGGAGCGGCGCTGCACCCAGG

81 CCTCAAGCACCGGGGGCGGCGACTGGAGCGGCGCTGCACCCAGGTGCGCGACAACTACCTCTGCCACCAGCGCGCTCGCCATCA
28 L K H R G P D W S G L H Q V A D N Y L C H Q R L A I I

GGAGGCGCGCTGCTCGGCGAGATG <- 3R

161 TCGACCCGGCCTCCGGCGACCGAGCCGCTCTACAACGAGGACAAAGTCCATCGCCGTTGCCGTCAACGGGGAGGTCTACAAC
55 D P A S G D Q P L Y N E D K S I A V A V N G E V Y N

GGCCTGGCCGTCCTACTGACGCTCC <- 4R

241 CATGAGGAGCTTCGGGCACGGCTCTCCGGACACAGGTTCGGGACCGGCACTGACTGCGAGSTCATCGCCCATCTG
81 H E E L R A R L S G H R F R T G S D C E V I A H L



Four gRNAs were designed to target the first exon of *TaASN2*

A gene encoding the 4 gRNAs was introduced into wheat by particle bombardment, along with a gene encoding the Cas9 nuclease and a selective marker gene (*Bar*).

An A genome null (Line 178) and two total nulls (Lines 23 and 59) were selected for analysis.

Most of the edits in the plants are deletions, but there are substitutions and single base pair insertions as well. The edited genes encode highly truncated and/or dysfunctional proteins.

Line 59 also has edits in the A and D genome *TaASN1* genes, so it is a partial null for *TaASN1*.



Sarah Raffan



CRISPR/Cas9 editing in *TaASN1* genes of Line 59



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A genome *TaASN1* (*TaASN-A1*)

The nucleotide sequence is from the ATG translation start codon in the first exon of the gene. The gRNA3 binding site is underlined. The single nucleotide (T) insertion in the *TaASN-A1* gene of Line 59 is shown in red.

```
1  ATGTGCGGCATACTGGCGGTGCTGGGCTGCGCCGATGACACCCAGGGGAAGAGAGTGCGC
61  GTGCTCGAGCTCTCGCGCAGGCTCAAGCACCGCGGCCCCGACTGGAGCGGCATGCACCAG
121 GTCGGCGACTGCTACCTCTCCCACCAGCGCCTCGCCATCATCGACCCTGCCTCTGTGCGAC
181 CAGCCCCTCTACAACGAGGACAAGTCCATCGTCGTCACTGTGAATGGAGAGATCTACAAC
```

B genome *TaASN1* gene (*TaASN-B1*)

The nucleotide sequence is from the ATG translation start codon in the first exon of the gene. The gRNA3 binding site is underlined. The 36 bp deletion in the *TaASN-B1* gene of Line 59 is shown in red.

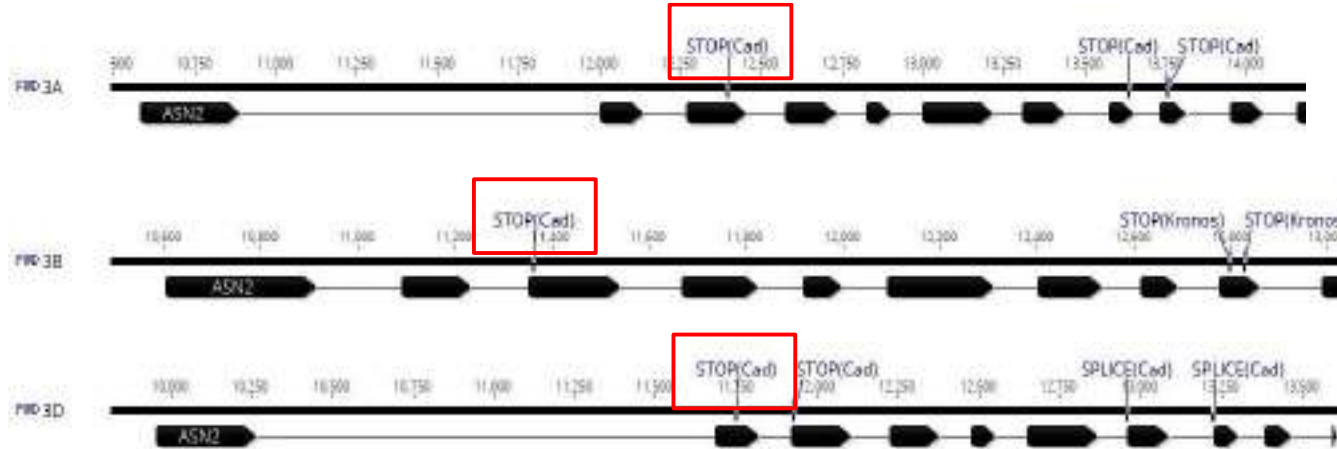
```
1  ATGTGCGGCATACTGGCGGTGCTGGGCTGCGCTGATGACACCCAAGGGGAAGAGAGTGCGC
61  GTGCTTGAGCTCTCGCGCAGGCTCAAGCACCGCGGCCCCGACTGGAGCGGCATGCACCAG
121 GTTGGCGACTGCTACCTCTCCCACAGCGCCTCGCCATCATCGACCCTGCCTCTGGCGAC
181 CAGCCGCTCTACAACGAGGACAAGTCCATCGTCGTCACAGTGAATGGAGAGATCTACAAC
```

The D genome *TaASN1* gene (*TaASN-D1*) is not edited in Line 59.

Knocking out *TaASN2*: stacking mutations from TILLING lines (produced by chemical mutagenesis)



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- Target Induced Local Lesions In Genome (TILLING)
- An EMS-mutagenised population of bread wheat cv. Cadenza has been developed by the University of California Davis, Rothamsted Research, The Earlham Institute, and John Innes Centre.
- Every plant in the population has been exome-sequenced.
- Lines have been identified carrying mutations in the *TaASN2* genes in each of the A, B and D genomes
- The A and D genome mutations have been stacked in variety Claire by our partners at RAGT to generate partial and total null *TaASN2* lines (Claire already lacks *TaASN-B2*).
- Mutants produced using chemical mutagenesis will carry thousands of uncharacterised mutations, as well as the mutations in the target gene(s). This may affect yield or other traits.
- However, there are no regulatory issues with this type of mutagenesis.

Europe's first CRISPR wheat field trial, 2021-2025



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ENVIRONMENTAL PROTECTION ACT 1986, SECTIONS 111 AND 112

CONSENT TO RELEASE GENETICALLY MODIFIED ORGANISMS REFERENCE 200001

1. In accordance with section 111 of the Environmental Protection Act 1986, the Secretary of State for Environment, Food and Rural Affairs hereby grants consent to Rothamsted Research to perform the release of the genetically modified organisms described in paragraph 5, in accordance with the particulars set out in paragraph 5, and subject to the conditions and conditions set out in the Schedule attached.

2. Genetically Modified Organisms to be released

The genetically modified organisms (GMOs) are wheat. The consent is given on the basis that the wheat has been genetically modified using CRISPR-Cas9 to remove protein mutations in the TaLSD2 gene, resulting in a wheat with a higher protein content.

3. Particulars of the consent to release

Maximum size of the release: The area covered by the GM wheat must not exceed 1000 square metres and may take the form of one or more plots within the trial site.

(a) Purpose of the release

To investigate the effect of knocking out the TaLSD2 gene on the wheat phenotype and to determine the effect of the gene on the wheat grain.

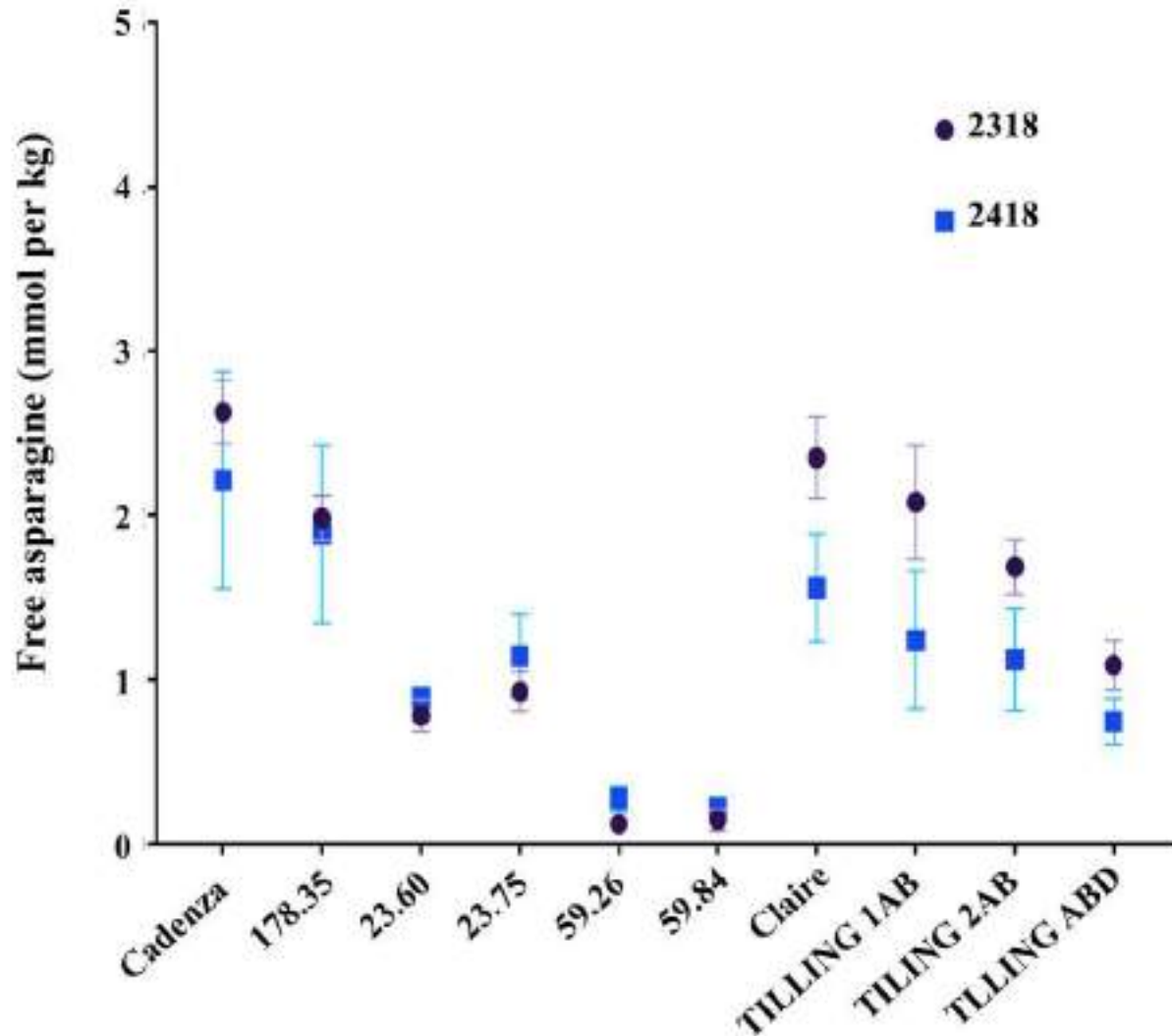
(b) Location of the release (trial site)



Free asparagine concentrations (mean with SD) in flour prepared from the 2022-2023 and 2023-2024 field trial samples



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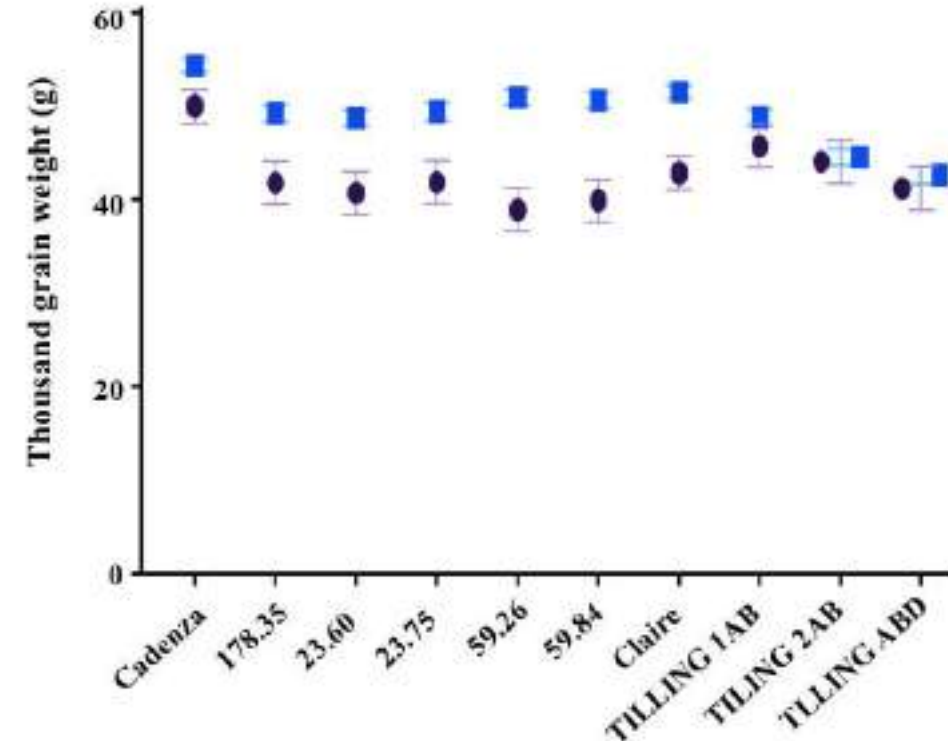
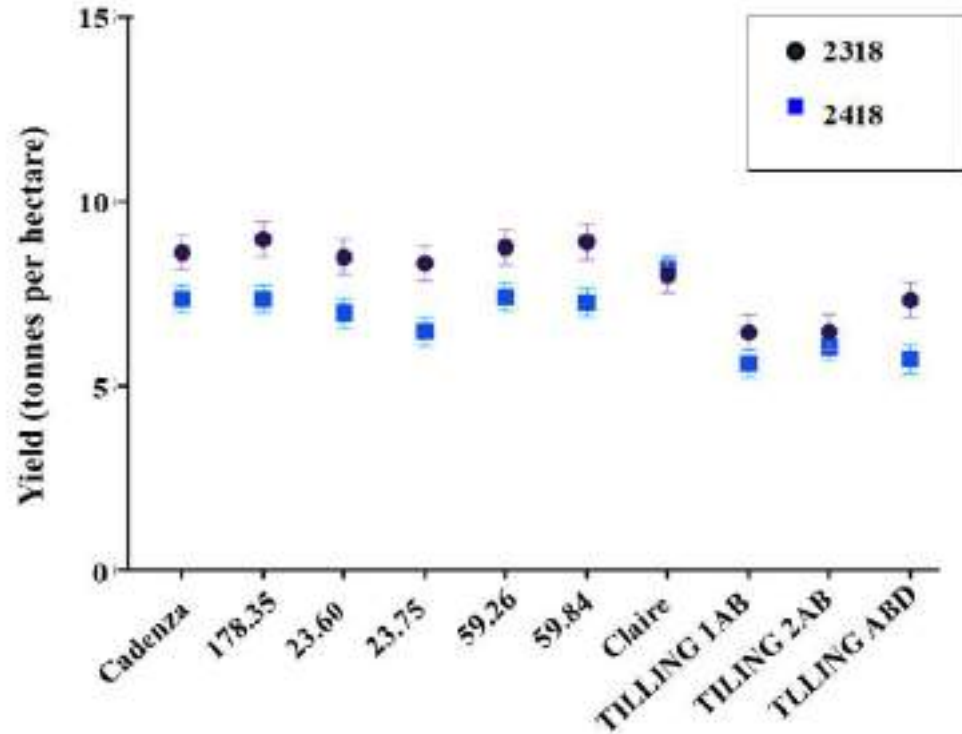


- CRISPR lines: Line 178.35 is a partial null for *TaASN2*. The 23 and 59 lines are total nulls for *TaASN2*. The 59 lines are also partial nulls for *TaASN1*. All in Cadenza.
- TILLING lines. TILLING 1AB and 2AB are partial nulls for *TaASN2*. TILLING ABD is a total null for *TaASN2*. All in the Claire background.
- Free asparagine concentration in Line 23 (*TaASN2* null) was approx. 30 - 50 % that of Cadenza, while that in Line 59 (*TaASN1/2* null) was 5 - 10 % of Cadenza.
- Free asparagine concentration in the ABD *TaASN2* null TILLING line was similar to the equivalent CRISPR Line 23.
- Differences between lines were significant.

Yield and thousand grain weight (TGW) data from the 2022-2023 and 2023-2024 field trials



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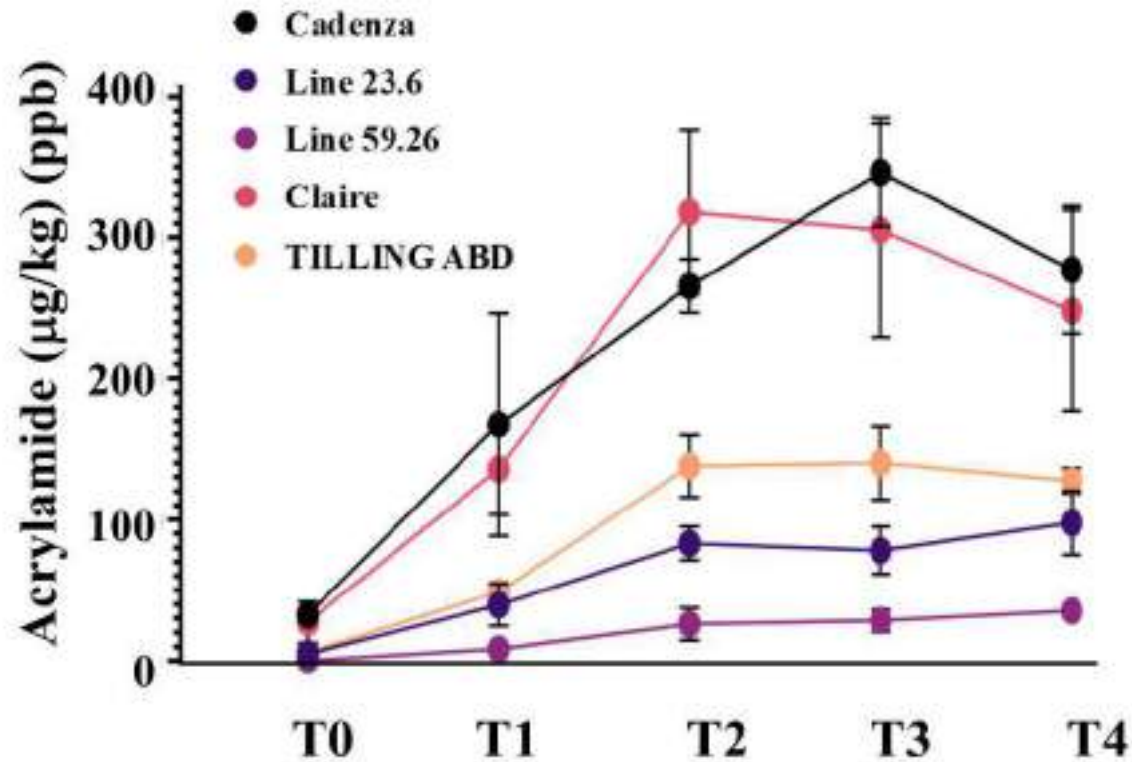


- The yield of the edited lines was not significantly different from Cadenza, but TGW was lower, so the edited lines produced more but smaller grains (still in the normal range for wheat).
- The TILLING nulls showed a yield penalty compared with Claire, presumably due to the genetic baggage of uncharacterised mutations that they carry. It may be possible to reverse this with more back-crossing.

Acrylamide in bread and toast



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- Acrylamide in untoasted bread (T0) from Line 59.26 was below detection levels, compared with 32.38 $\mu\text{g kg}^{-1}$ in bread made from the Cadenza control.
- The concentration of acrylamide in bread made from Line 23.60 flour was 14 % that for Cadenza,
- The concentration in the TILLING ABD lines was 21 % that for Claire.
- In T3 toast made from Line 59 bread, the concentration of acrylamide was 8 % that of Cadenza. The concentration for Line 23.60 was 23 % that of Cadenza. The concentration in TILLING ABD T3 toast was 46 % that for Claire.
- The differences between the lines were highly significant.

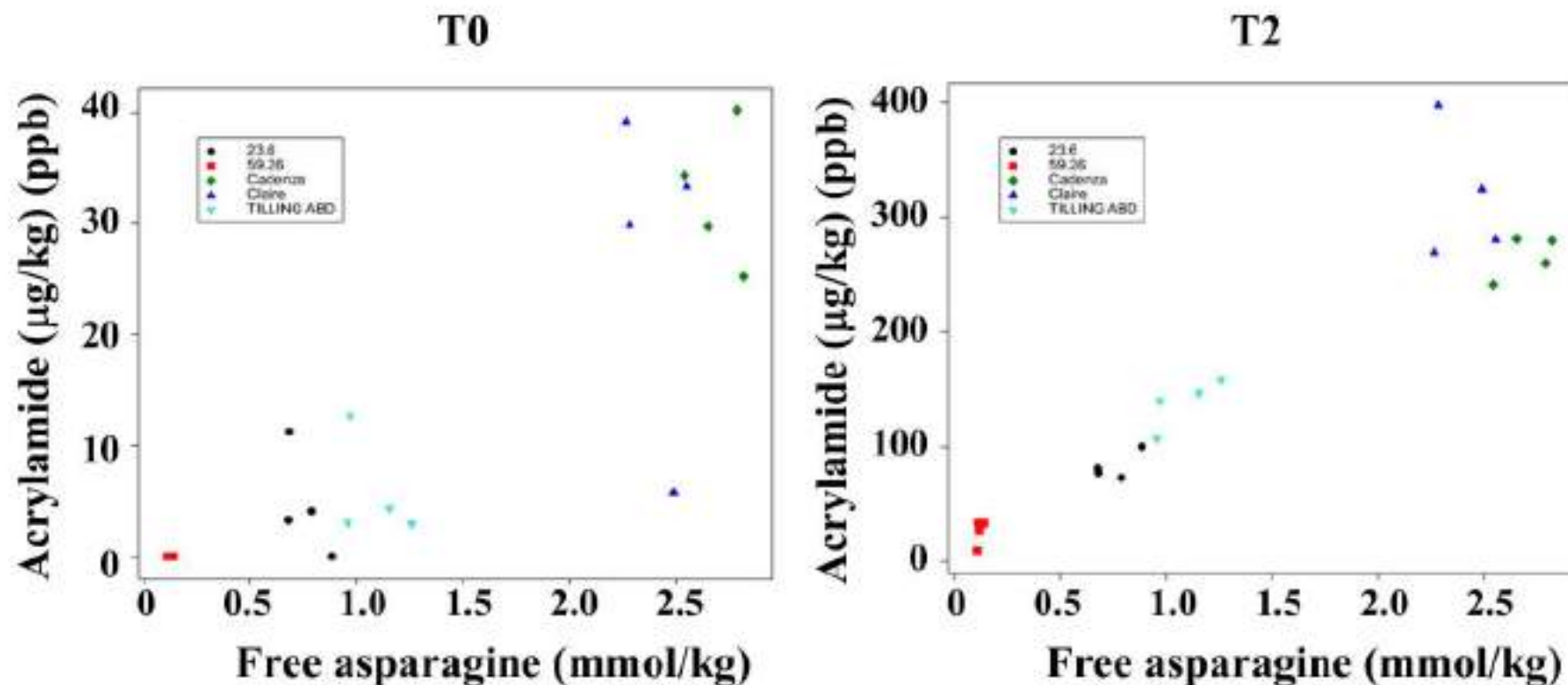


Navneet Kaur and Shpresa Musa

Bread and toast (T0 and T2); asparagine versus acrylamide



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T0



T2

- Toasting led to a large increase in acrylamide formation.
- The relationship between free asparagine and acrylamide tightened after toasting.
- Note the different scales on the two graphs.

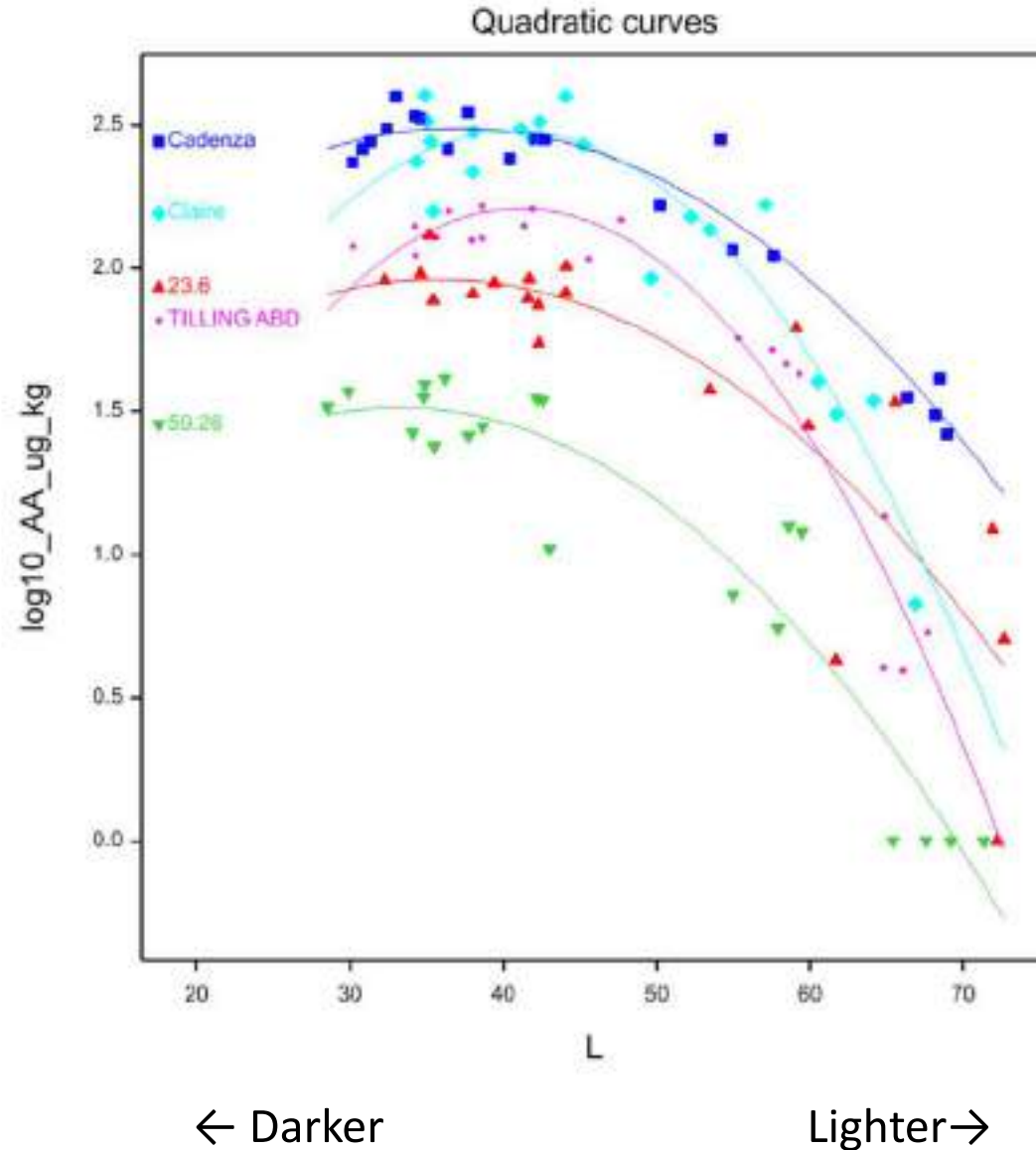


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Acrylamide versus colour (L) in toast



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- Reducing free asparagine concentration changes the relationship between colour and acrylamide formation.
- This suggests that using low asparagine grain could enable food manufacturers to retain the colours, flavours and aromas that define their products and brands while complying with acrylamide regulations.

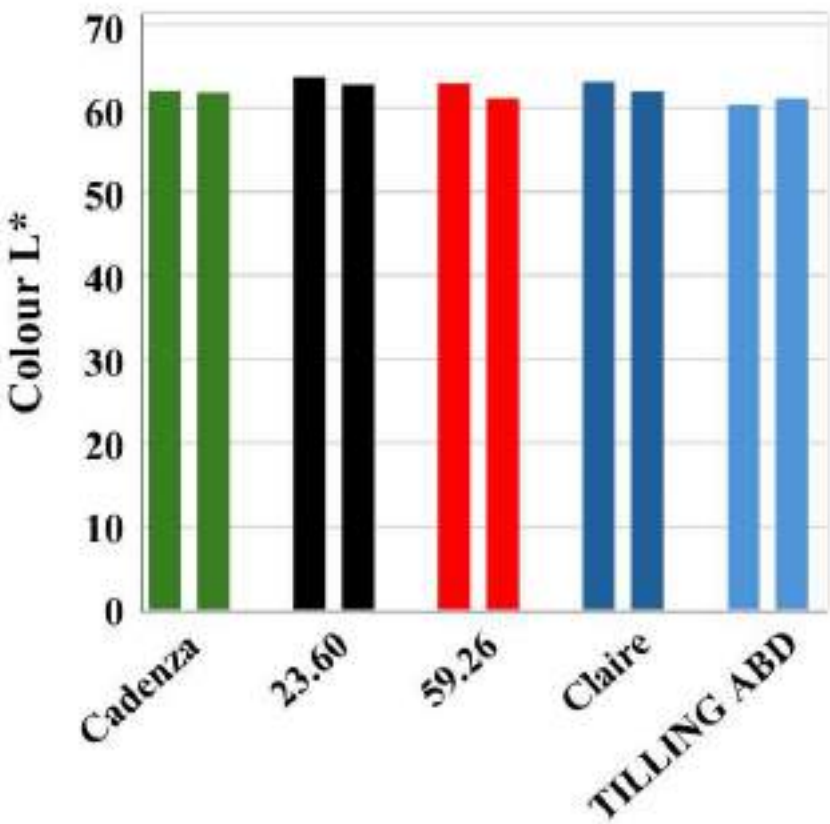
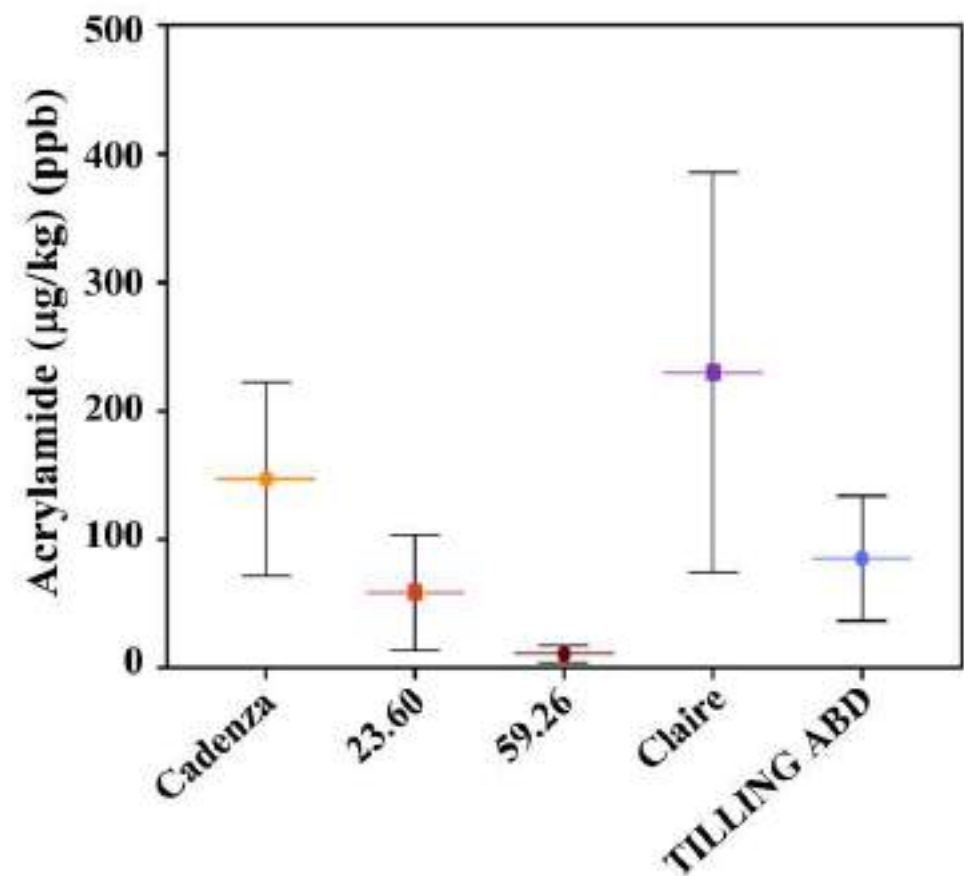


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Acrylamide and colour in biscuits



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ACRYRED

Ongoing projects: Proteomic analysis of low asparagine lines



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Katharina Belt

With Katharina Belt and Harvey Millar, University of Western Australia.

Quantitative proteomics applied to compare mature grain protein profiles of Cadenza and Claire with CRISPR and TILLING lines.

Conclusions:

- Across genotypes, the grain proteome was highly conserved, with no evidence for widespread reprogramming of storage or metabolic protein composition in low-Asn lines.
- Nevertheless, some conserved differences were detected in specific protein groups, including glutenins, gliadins and β -amylase.
- These are being followed up with wheat flour quality testing.
- The study highlights proteomics as a powerful systems-level tool to identify subtle molecular changes and to evaluate their functional relevance for food production.

Ongoing projects: PROBITY



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- We now have plants of the *TaASN2* knockout line (Line 23) that are PCR-negative for all the transgenes. Absence of any foreign DNA has been confirmed by whole genome sequencing.
- Plants must be free of foreign DNA to qualify as precision bred organisms (PBOs) under the UK's new legislation on genome edited crops.
- The UK opened up the process for approving PBOs for marketing in November 2025 and we have submitted an application.
- The aim is to bulk these up for field trials on commercial farms and produce up to 100 tonnes for processing in real-world food pilot plants (the PROBITY project).
- We are also screening for transgene-free plants from Line 59.
- The EU is also moving on genome editing crops (New Genomic Techniques), but at glacial speed!

Ongoing projects: Linking pathogen infection with asparagine accumulation



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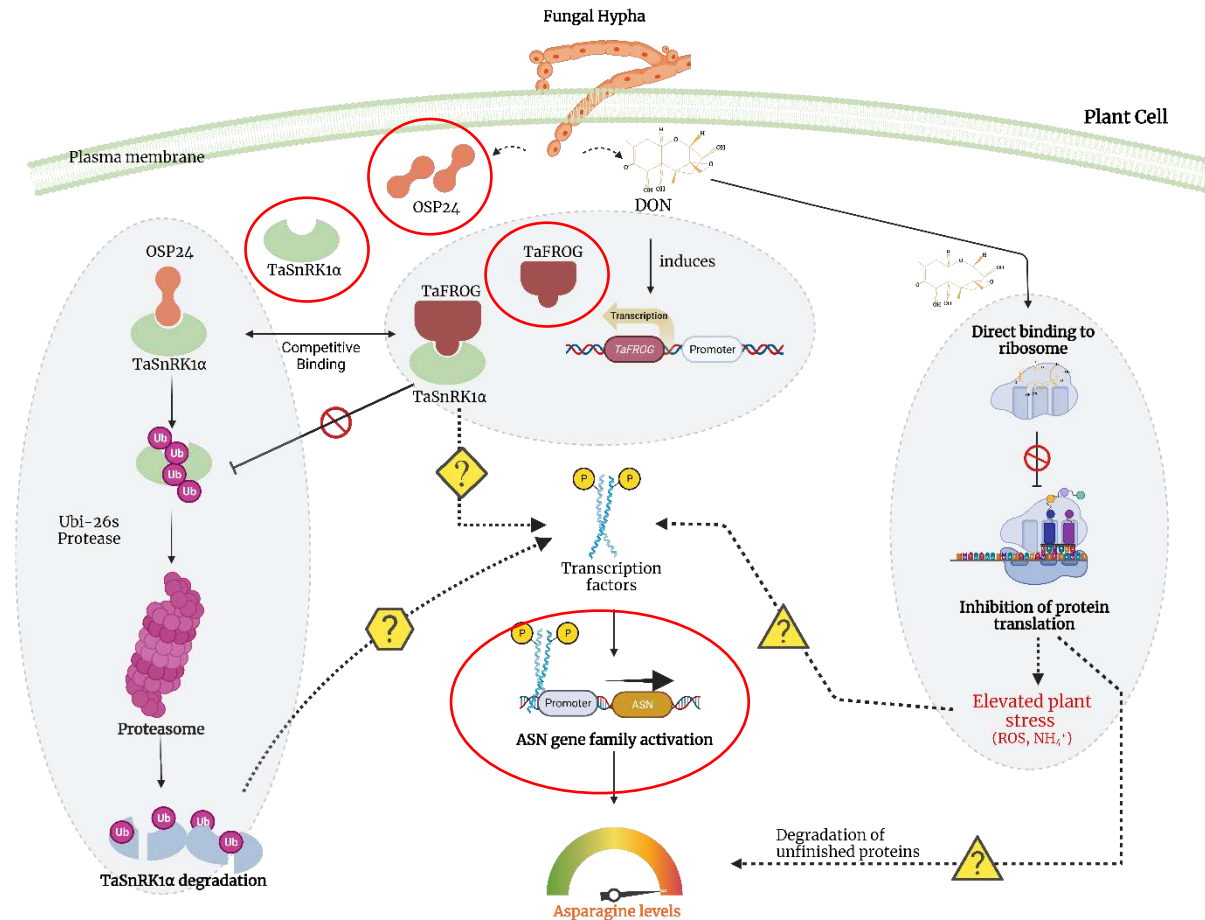
Navneet Kaur

Kim Hammond-Kosack

Martin Urban

Fiona Doohan, University College
Dublin

Nigel G. Halford



Our working model of the signalling hub linking *Fusarium graminearum* infection with asparagine synthetase gene expression and asparagine synthesis.

We are looking at the roles of:

- **SnRK1**, a protein kinase involved in metabolic regulation, signalling and stress responses, including the control of asparagine synthetase gene expression.
- **OSP24**, a fungal effector that interacts with SnRK1 and accelerates SnRK1 degradation by the ubiquitin-26S proteasome.
- **TaFROG**, a wheat protein that competes with OSP24 for binding with SnRK1, thereby protecting it from degradation.
- **Deoxynivalenol (DON)**: the mycotoxin produced by Fg

Perochon et al. (2019) *Plant Sci.* **288**, 110217.

Jiang et al. (2020) *Nat. Commun.* **11**, 4382.

Kaur and Halford (2023) *Foods* **12**, 3264.

Fusarium graminearum mutants (PH1 strain)



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Fg-PH1

Wild type



Fg-PH1-ΔTri5

No DON mycotoxin



Fg-PH1-ΔOsp24

No Osp24 effector



Fg-PH1-ΔTri5ΔOsp24

No mycotoxin or Osp24



Navneet Kaur

- We are compiling RNAseq, proteomic and proximity labelling data to refine our model and identify targets for intervention.
- Asparagine biosynthesis-related genes group within a single cluster in the RNAseq analysis, suggesting co-ordinated expression and possible co-regulation.
- The asparagine synthetase gene that is involved is *TaASN4*.
- See Kaur et al. poster

Concluding remarks



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- As further big gains from processing/quality control become difficult to achieve, the provision of consistently low asparagine grains will become increasingly important. **We need breeders to engage on the issue and deliver low asparagine wheat varieties.**
- Ensuring sulphur sufficiency and strong disease control are key crop management factors (but grain from sulphur-fed and fungicide-treated wheat still contains a lot of free asparagine).
- Using varieties that naturally lack the B genome *TaASN2* gene could be a relatively easy gain for wheat breeders, but most varieties already lack it, so the scope for improvement is limited.
- Other QTL identified so far offer the prospect of only limited progress.

Concluding remarks



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- Large reductions in the free asparagine concentration of wheat grain are possible using genome editing or chemical mutagenesis (TILLING).
- No significant effects on yield have been evident in three years of field trials of low asparagine GE wheat, although TGW is lower (more but smaller grains). Some evidence of a yield drag in TILLING lines, but more back-crossing may reverse that.
- The availability of low acrylamide wheat could enable food businesses to comply with evolving regulations on acrylamide without costly changes to production lines or reductions in product quality.
- It could also have a significant impact on dietary acrylamide intake for consumers.
- BUT: Breeders are still nervous about using GE plants, despite the change in the UK's regulations, and will only adopt the new technology if the regulatory framework is supportive.

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