

Application to FSANZ for the Inclusion of wheat event UM-12115T-1 with increased Nicotianamine in Standard 1.5.2 Genetically Modified Food

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[Personal Information Removed as CCI]

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Executive Summary

This application seeks regulatory approval for the food use of genetically modified bread wheat (*Triticum aestivum* L.) event UM-12115T-1. The event was developed via *Agrobacterium tumefaciens*-mediated transformation of the Australian wheat cultivar 'Gladius' to constitutively express the OsNAS2 (Nicotianamine Synthase 2) gene derived from rice (*Oryza sativa*).. The constitutive expression of *OsNAS2* increases the biosynthesis of nicotianamine (NA), a naturally occurring plant chelator important for the uptake, long-distance transport, and grain loading of transition metals such as iron and zinc.

A comprehensive molecular characterisation utilising PacBio HiFi long-read sequencing confirmed the precise genomic architecture of the modification. The transformation resulted in two independent, stable insertion loci located on Chromosome 1D (Locus 1) and Chromosome 4D (Locus 2). Because these loci are located on separate chromosomes, they are not genetically linked. Subsequent introgression of the trait into elite wheat varieties via conventional breeding will result in independent segregation, yielding three distinct genetic configurations: lines containing both Locus 1 and Locus 2, lines containing only Locus 1, and lines containing only Locus 2. This application encompasses all three variants. Both loci contain a single copy of the intended transfer-DNA expression cassettes. While Locus 2 is a simple insertion, Locus 1 contains a discrete, rearranged fragment of functionally inert vector backbone resulting from a complex integration event. The structural integrity of both insertion loci is fixed and has been demonstrated to be stably inherited through eight generations of self-pollination from T0 to T8. Furthermore, an extensive bioinformatic assessment of 94 putative open reading frames spanning the integration junctions and backbone sequences confirmed the absence of biologically relevant sequence homology to known allergens or protein toxins.

Wheat event UM-12115T-1 contains two introduced proteins: the intended trait protein, OsNAS2, hygromycin phosphotransferase (HPT) a selectable marker used during plant transformation. In addition, Locus 1 contains an unintended, non-functional fragment of the vector backbone comprising the Neomycin-kanamycin phosphotransferase (Kan) binary vector selectable marker. Liquid Chromatography-Parallel Reaction Monitoring-Mass Spectrometry demonstrated that the OsNAS2 protein is present at very low levels in vegetative tissues and edible flour. Conversely, the HPT and Kan selectable marker proteins were not detectable in either the grain or leaf tissues, confirming the Kan backbone sequence is transcriptionally and translationally inert *in planta*.

OsNAS2, HPT and Kan do not share sequence homology with known allergens or toxins, and none of the proteins are predicted to enter the secretory pathway, thereby precluding host-specific unexpected post-translational modifications such as glycosylation. The proteins are highly susceptible to proteolytic degradation. Direct *in vitro* assays demonstrated that OsNAS2 is completely degraded within 30 seconds of exposure to simulated gastric fluid containing pepsin. Additionally, OsNAS2 is thermolabile and is rapidly inactivated at standard food processing temperatures. Deterministic dietary exposure assessments for both human populations in Australia and New Zealand, as well as for livestock, confirm that potential exposure to the OsNAS2 protein is exceptionally low, strictly remaining in the low nanogram per kilogram of body weight per day range.

A rigorous multi-environment compositional assessment was conducted across multiple locations and growing seasons to establish the metabolic and nutritional equivalence of UM-12115T-1 against a near-isogenic wild type control and commercial reference varieties. The combined-site analysis confirmed that UM-12115T-1 grain is strictly equivalent to conventional wheat in all key proximates, dietary fibre, amino acid profiles, fatty acid composition, and estimated metabolizable energy. The genetic modification does not disrupt secondary metabolism or elevate anti-nutritional profiles, with phytic acid levels remaining comparable to those of conventional varieties. Furthermore, no novel accumulation of toxic heavy metals was detected. The intended downstream metabolic effect of elevated NA concentration was consistently expressed and reliably maintained across diverse

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agronomic environments. These elevated NA levels in UM-12115T-1 remain well within the natural physiological ranges already established by conventional high-NA wheat germplasm.

The comprehensive molecular, biochemical, and compositional data presented in this application definitively demonstrate that wheat event UM-12115T-1 is structurally stable, safe for consumption, and compositionally equivalent to traditional commercial wheat. The event introduces no novel toxicological, allergenic, or nutritional hazards and will interact with the food supply chain in a manner fundamentally identical to conventional *Triticum aestivum*.

PART 1: ADMINISTRATIVE AND GENERAL INFORMATION

This section is completed in accordance with Chapter 3.1 (General Requirements for Applications) of the Food Standards Australia New Zealand Application Handbook (FSANZ, 2025).

A. Form of the Application

This application has been prepared in English and is submitted electronically in a word-searchable format. In accordance with formatting requirements, the application includes the following components:

- An executive summary, provided as a separate electronic file
- A table of contents to assist with navigation
- Sequential page numbering on each page
- A structure with headings that clearly identify all parts of the relevant guidelines being addressed.

B. Applicant Details

(a)	Applicant's name/s	[CCI]
(b)	Company/organisation name	The University of Melbourne
(c)	Address (street and postal)	The University of Melbourne Grattan Street, Parkville Victoria 3010 Australia
(d)	Telephone number	[CCI]
(e)	Email address	[CCI]
(f)	Nature of the applicant's business	The University of Melbourne is a comprehensive, research-intensive public institution whose primary business is the delivery of higher education and the advancement of academic research. Additionally, its operations extend into the commercialisation of its intellectual property through strategic industry partnerships, research translation, and the incubation of innovative spin-out companies.
(g)	Details of other individuals, companies or organisations associated with the application	[CCI]

C. Purpose of the Application

This application seeks to amend the *Australia New Zealand Food Standards Code* to allow for the inclusion of wheat event UM-12115T-1 in **Standard 1.5.2-Genetically Modified Food**.

Specifically, wheat event UM-12115T-1 contains two independent, stable insertion loci located on Chromosome 1D (Locus 1) and Chromosome 4D (Locus 2). While wheat is predominantly a self-fertilising species, the deployment of this trait will involve introgression into locally adapted elite wheat varieties through conventional breeding. Because Locus 1 and Locus 2 are situated on separate chromosomes, they are not genetically linked and will segregate independently during these crossing programs.

As a result of this independent assortment, this application seeks approval to encompass the original wheat event containing both inserted loci, as well as the viable transgenic lines generated through conventional crossing that contain either locus independently. Therefore, this application covers three distinct *OsNAS2* genetic configurations derived from the event:

- Lines containing both Locus 1 and Locus 2
- Lines containing only Locus 1
- Lines containing only Locus 2.

C.1 Food regulatory measure(s) to be varied

The application seeks a variation to the Australia New Zealand Food Standards Code, specifically:

- **Standard 1.5.2–Food produced using gene technology:** Approval is sought to include wheat event UM-12115T-1 in Schedule 26–GM Food, thereby permitting its sale and use as a food produced using gene technology

This is the primary regulatory measure that must be varied to enable the lawful sale and use of GM wheat event UM-12115T-1, including its three potential segregant configurations, in the food supply of Australia and New Zealand.

C.2 Purpose relevant to specific application guidelines

This application relates to matters dealt with under Chapter 3 of the FSANZ Application Handbook (2025), specifically Guidelines 3.5.1

Purpose Relevant to Guideline 3.5.1–Foods produced using gene technology

The purpose of this application, in relation to Guideline 3.5.1, is to provide FSANZ with the comprehensive technical information required to conduct a thorough safety assessment of wheat event UM-12115T-1. This includes:

- detailed information on the host organism and donor sequences
- a description of the genetic modification process and molecular characterisation of the inserted sequences
- data on the stability of the genetic modification
- characterisation and safety assessment of the expressed *OsNAS2* gene and other proteins present in the wheat event, including bioinformatics analyses for toxicity and allergenicity
- compositional analysis comparing UM-12115T-1 to conventional wheat; and

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- other information relevant to assessment of the safety of wheat event UM-12115T-1.

This information will enable FSANZ to evaluate the safety of UM-12115T-1 as a food produced using gene technology and its suitability for inclusion in Schedule 26.

D. Justification for the Application

D1 Regulatory Impact

D.1.1 Costs and benefits of the application

(a) Costs and benefits to the consumer

The primary benefit to consumers is confidence in a safe, secure and legally compliant food supply chain. UM-12115T-1 is a wheat line intended, in the first instance, to support feed-related research, market development and early commercial applications focused on high-efficiency monogastric and supplementary cattle feed. However, because wheat is an established food commodity that moves through interconnected production, storage, transport and handling systems, seeking FSANZ approval is both prudent and appropriate. It ensures that grain derived from UM-12115T-1 is subject to pre-market food safety assessment and regulatory oversight consistent with the realities of bulk grain supply chains.

In particular, the application addresses credible pathways by which grain may enter food channels, including inadvertent co-mingling through shared infrastructure at pre-farm gate, on-farm and post-farm gate stages, the blending of grain within commodity systems, and the possible redirection of surplus grain into broader market pathways during periods of structural oversupply. Obtaining FSANZ approval ensures that, in the unlikely event of unintended presence in food, the material would be an authorised and safety-assessed product rather than an unapproved GM presence. This is a material benefit to consumers because it reduces regulatory uncertainty, strengthens supply chain assurance, and ensures that foreseeable exposure scenarios are covered by an appropriate food safety framework.

Comprehensive safety assessments further support this conclusion, indicating that potential dietary exposure to the introduced proteins would be negligible to effectively zero, with OsNAS2 present at a low level in vegetative tissues and flour and the selectable markers HPT and Kan are not detected. Accordingly, the application should be understood as a consumer protection and risk management measure that provides regulatory assurance for a wheat commodity with a foreseeable interface with the human food supply, while recognising that the initial commercial emphasis is feed, research scale-up and market development rather than immediate promotion for direct human food use.

(b) Costs and benefits to industry and business

A primary reason for seeking regulatory approval at this stage is the need to cultivate UM-12115T-1 over substantially larger areas to support research, market development and pre-commercial validation activities. This is constrained under the current Limited and Controlled Release Licence DIR 165. Expanded cultivation is required to generate the grain volumes necessary to undertake efficacy, operational and value-chain studies at commercially meaningful scale, including larger animal feeding trials and broader assessment of the line's agronomic, nutritional and market potential.

For example, the preliminary poultry trials were conducted on a limited scale. Consultation with industry indicates that subsequent validation will require trials involving significantly more birds to assess performance under conditions more representative of commercial production. Increased grain production will also be necessary to support robust feeding studies in swine and to evaluate potential nutritional and operational benefits in ruminant systems. In addition, obtaining food approval now

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provides an important enabling platform for future market flexibility, recognising that wheat is a dual-purpose commodity and that downstream market pathways may evolve over time as research, stewardship systems and commercial opportunities develop.

[This section has been amended to exclude certain proprietary data and financial modelling designated as Confidential Commercial Information (CCI)]. Approval for scaled research and development has the potential to generate valuable economic and operational benefits for the Australian livestock and feed-milling sectors. Preliminary *in vivo* broiler studies indicate that diets incorporating UM-12115T-1 may deliver a positive improvement in feed conversion ratios. Under current market conditions, this improved feed efficiency translates to meaningful reductions in the cost of live weight production. If these performance benefits are confirmed at an industry scale and adopted broadly across the Australian chicken meat sector, the resulting economic efficiencies could be significant. Furthermore, poultry producers, processors, and ultimately consumers may benefit from optimised feed utilisation and improved overall flock health outcomes, including indications of enhanced survivability observed in preliminary studies.

Potential costs to industry are expected to be low because cultivation, handling and market development would occur within established stewardship and segregation systems. Grain production would be managed under a dedicated crop management plan designed to maintain traceability and appropriate separation from conventional commodity channels during the research and scale-up phases. Any broader commercial pathway would also be expected to align with the Grain Trade Australia Market Choice Framework and the principles of Excellence Through Stewardship. Together, these systems provide a practical basis for managing supply chain integrity, minimising unintended presence, and reducing potential operational, marketing and trade risks.

(c) Costs and benefits to government

The primary benefit to government and regulatory agencies is the proactive management of foreseeable regulatory and supply chain risks associated with a GM wheat line entering larger-scale cultivation, research and market development. Because wheat is a major food and feed commodity moving through shared infrastructure, approval under the FSANZ framework provides an important regulatory safeguard by ensuring that grain derived from UM-12115T-1 has been assessed for human food safety before broader scale-up occurs.

This reduces the likelihood of administrative, compliance, enforcement and incident-response burdens that could arise if grain from an unapproved GM wheat line were detected in food-related channels. In practical terms, prior approval helps avoid the costs associated with investigation, market intervention, possible recalls, trade management and stakeholder communication that may otherwise arise from the presence of an unapproved event in the food supply. No material ongoing costs to government are anticipated beyond the standard cost-recovered administrative and assessment activities associated with consideration of the application by FSANZ and, where relevant, associated oversight by the Office of the Gene Technology Regulator.

D.1.2 Impact on international trade

Approval of UM-12115T-1 has the potential to support international trade by reducing the regulatory and commercial risks associated with the movement of grain through shared bulk handling systems and by providing a foundation for coordinated domestic and international regulatory management. As wheat is traded through interconnected commodity channels, unintended presence of grain derived from UM-12115T-1 in domestic or export pathways remains a foreseeable possibility even where stewardship and segregation measures are applied. In the absence of prior food approval, the detection of an unapproved GM wheat event could create significant trade, compliance and

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reputational risks, particularly in markets that apply strict requirements to the import of genetically modified products.

FSANZ approval therefore provides an important baseline regulatory status. It demonstrates that grain derived from UM-12115T-1 has undergone a human food safety assessment in its country of origin and supports the progression of corresponding regulatory and market access activities in relevant overseas jurisdictions. When combined with structured stewardship, segregation, and alignment with the Grain Trade Australia Market Choice Framework prior to any broader commercial release, this approach helps reduce the risk of shipment disruption, market rejection, and reputational harm to Australian grain and livestock export sectors. In this respect, domestic approval is not only relevant to food safety compliance within Australia, but also to the orderly management of trade risk for a wheat commodity that may move across multiple end-use channels and international markets.

E. Information to Support the Application

This application has been prepared in accordance with the *Food Standards Australia New Zealand Handbook* (as at, 1 October 2025), Guideline 3.5.1. Collectively, results support this application for amendment to the *Australia New Zealand Food Standards Code* to allow inclusion of the wheat event UM-12115T-1 in accordance with Standard 1.5.2-GM Foods.

E.1 Data Requirements

In preparing this submission, the University of Melbourne has followed the general data requirements in Guideline 3.1.1 of the Handbook. All data are fully referenced with details of the source, author and year of production, and studies have been conducted using validated or standardised methods with documented sensitivity and specificity. Where population-relevant data were required, Australian and New Zealand populations were used as the reference. Statistical analyses were applied appropriately, and electronic copies of all references, studies and reports have been included.

E.2 Information relevant to Guideline 3.5.1–Foods produced using gene technology

Information has been provided on the identity of the host organism (*Triticum aestivum* L.) and the donor sequences used in the genetic modification, along with the history of safe use of *T. aestivum* L in food applications. The dossier describes the molecular characterisation of the inserted sequences, demonstrates their stability, and confirms the absence of unintended changes.

Wheat event UM-12115T-1 has been characterised in terms of the proteins expressed, with bioinformatics analyses, allergenicity assessment and toxicity evaluations included. Compositional analyses compare wheat event UM-12115T-1 to conventional wheat across relevant nutrients.

E.3 Assurance of data quality

All studies and supporting information have been presented in sufficient detail to allow independent evaluation of methods and results. The evidence demonstrates that wheat event UM-12115T-1 is compositionally well-characterised and safe for use in foods. Collectively, the data confirm that wheat event UM-12115T-1 is as safe and appropriate for consumption as conventional wheat.

F. Assessment Procedure

The University of Melbourne anticipates that this application will be considered under the **General Procedure** for Administrative Assessment process by Food Standards Australia New Zealand.

The General Procedure is considered appropriate because:

1. The application does not raise any novel issues of public health and safety that would warrant assessment under the Major Procedure.
2. The technical and safety information provided addresses the requirements of Guideline 3.5.1 (Foods produced using gene technology) of the FSANZ Application Handbook (2025); and
3. The scope of the proposed variations is confined to Standard 1.5.2—GM Food, with the primary variation sought being to Schedule 26.
4. On this basis, the University of Melbourne respectfully submits that the General Procedure provides an efficient and proportionate pathway for FSANZ to evaluate the safety, identity, and nutritional suitability of wheat event UM-12115T-1.

G. Confidential Commercial Information (CCI)

This application contains certain information that the University of Melbourne requests be treated as Confidential Commercial Information (CCI) in accordance with section 114 of the *FSANZ Act*. A separate redacted version of the application has been prepared for public release.

The information claimed as CCI is limited to material that, if disclosed, would reveal proprietary details of the University of Melbourne's development of this GM wheat. Such information is highlighted in the dossier and clearly identified in the confidential schedule provided to FSANZ.

Consistent with FSANZ's information publication scheme, the University does not seek to restrict the publication of non-confidential elements of the application, including general descriptions of the product, its purpose, and its proposed uses. Personal information such as signatures, names and contact details of company personnel and consultants is not considered CCI and will be managed by FSANZ in accordance with its privacy policy.

H. Other Confidential Information

No additional confidential material is included in this submission document.

I. Exclusive Capturable Commercial Benefit

The University of Melbourne acknowledges that the proposed amendment to the Standard will likely result in an exclusive capturable commercial benefit being accrued to The University of Melbourne as defined in Section 8 of the *FSANZ Act*.

J. International and Other National Standards

J.1 International Standards

The safety assessment detailed in this application aligns with the internationally recognised standards and guidelines established by the Codex Alimentarius Commission for evaluating genetically modified

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foods. Specifically, the data, methodologies, and safety conclusions presented for UM-12115T-1 comply with the "Principles for the Risk Analysis of Foods Derived from Modern Biotechnology" (CAC/GL 44-2003) and the "Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants" (CAC/GL 45-2003). In accordance with these Codex standards, the evaluation of UM-12115T-1 wheat encompasses a comprehensive molecular characterisation of the genetic modification, robust toxicity and allergenicity assessments of the introduced proteins (OsNAS2, HPT and Kan), and a detailed compositional analysis. This rigorous framework ensures that UM-12115T-1 is assessed to be as safe and nutritious as its conventional wheat counterparts.

J.2 Other National Standards or Regulations

At present, no applications for the regulatory approval of UM-12115T-1 wheat have been submitted to any other national or international jurisdictions. The Australian regulatory process is the first to be initiated for this specific event (Table 1). Concurrent with this food safety assessment application to FSANZ, the University of Melbourne is submitting an application for a commercial release licence to the Australian Office of the Gene Technology Regulator (OGTR) to permit the cultivation of the crop. While future regulatory submissions may be made to authorities with comparable regulatory processes in key international export markets, such as the United States Food and Drug Administration (FDA), Health Canada, or the European Food Safety Authority (EFSA), no such assessments are currently underway or pending outside of Australia.

Table 1: Current Applications and Approval Status for UM-12115T-1

Country	Competent National Authority	Type of Authorisation	Approval Status
Australia	Office of the Gene Technology Regulator (OGTR)	Application for a DIR licence for the Commercial Release of GM plants	Under Evaluation (2026)

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K. Statutory Declaration – Australia

See attached statutory declaration provided separately. [CCI]

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L. Checklists Provided With Application

General Requirements

General Requirements (3.1.1)		
Check	Page No.	Mandatory requirements
		A Form of application
☒	4	☒ Application in English ☒ Executive Summary (separated from main application electronically) ☒ Relevant sections of Part 3 clearly identified ☒ Pages sequentially numbered ☒ Electronic copy (searchable) ☒ All references provided
☒	4	B Applicant details
☒	5	C Purpose of the application
		D Justification for the application
☒	6	☒ Regulatory impact information ☒ Impact on international trade
☒	8	E Information to support the application
		☒ Data requirements
		F Assessment procedure
☒	9	☒ General ☐ Major ☐ Minor ☐ High level health claim variation
		G Confidential commercial information
☒	9	☒ CCI material separated from other application material ☒ Formal request including reasons ☒ Non-confidential summary provided
		H Other confidential information
☒	9	☒ Confidential material separated from other application material ☒ Formal request including reasons
☒	9	I Exclusive Capturable Commercial Benefit
		☒ Justification provided
		J International and other national standards
☒	9	☒ International standards ☒ Other national standards
☒	11	K Statutory Declaration
		L Checklist/s provided with application
☒	12	☒ 3.1.1 Checklist ☒ All page number references from application included ☒ Any other relevant checklists for Chapters 3.2–3.7

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☒	25	A.2 History of use of host and donor organisms
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Part 2: Specific Data Requirements for Safety Assessment

The following information is provided to support an application for a new genetically modified food. The details presented are in accordance with Section 3.5.1. of the FSANZ Application Handbook as at, 1 October 2025.

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SENSITIVE_UOM-25-PROT-RPT-01-02 Allergen and Toxin Evaluation of ORFs
SENSITIVE_UOM-25-PROT-RPT-02-02 Protein Safety
SENSITIVE_UOM-26-AGR-RPT-01-02_Agronomic Assessment 2025
SENSITIVE_UOM-26-AGR-RPT-02-01_Event Assessment 2019-2023
SENSITIVE_UOM-26-COMP-RPT-01-1.0 Composition Analysis
SENSITIVE_UOM-25-FEED-RPT-01-01 Poultry Trial 2025
SENSITIVE_UOM-26-FEED-RPT-01-01 Poultry Trial 2026

Abbreviations, Acronyms and Definitions¹

Abbreviation	Definition
ADF	Acid detergent fibre
ADP	Adenosine diphosphate
ANOVA	Analysis of Variance
ATP	Adenosine triphosphate
AUG	Start codon
Backbone DNA	DNA associated with construct backbone
bp	Base pair
CBI	Confidential Business Information
CDS	Coding sequence
Chr	Chromosome
C-t	Carboxy terminal region
DW	Dry weight
DNA insert	DNA sequence from pUbi-OsNAS2 integrated into the wheat genome
dNTP	Deoxy nucleotide triphosphate
dsRNA	Double-stranded RNA
ELISA	Enzyme Linked Immunosorbent Assay
ETS	Excellence Through Stewardship
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FARRP	Food Allergy Resource Research Program and the University of Nebraska Lincoln
FDA	Food and Drug Administration
FW	Fresh weight
GE, GM, GMO	Genetically engineered/modified/modified organism
<i>hph</i>	Hygromycin phosphotransferase antibiotic resistance gene
HPT	Protein encoded by the <i>hph</i> gene
UM-12115T-1	OECD unique identifier for the wheat event selected for commercial approval
Kb	Kilobase
kDa	Kilodaltons
LC-MS	Liquid Chromatography Mass Spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization - Time of flight
Mt	Metric tonnes
NA, NAS	Nicotianamine; Nicotianamine Synthase
NDF	Neutral Detergent Fibre
NGS	Next generation sequencing
OECD	Organisation for Economic Cooperation and Development
OGTR	Office of the Gene Technology Regulator
ORF	Open Reading Frame
<i>OsNAS2</i>	
PacBio	Pacific Biosciences of California
PCR	Polymerase chain reaction

¹ NOTE: Abbreviations of units of measure and of physical and chemical quantities are used according to the standard format described in Instructions to Authors in the Journal of Biological Chemistry (<http://www.jbc.org/>).

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Abbreviation	Definition
RT-qPCR	Reverse transcription-qualitative polymerase chain reaction
SDAP	Structural Database of Allergenic Proteins
TF	Transcription factor
US	United States of America
WHO	World Health Organization
WT	Wild type

A. Technical Information on the Food Produced Using Gene Technology

A.1. Nature and Identity of the Genetically Modified Food

A.1(a) A description of the GM organism from which the new GM food is derived. The description must include the nature and purpose of the genetic modification.

The transgenic wheat event UM-12115T-1 was developed to enhance grain iron and zinc concentrations through the constitutive expression of the *OsNAS2* (*Nicotianamine Synthase 2*) gene from *Oryza sativa*. The *OsNAS2* gene product is a key enzyme in the biosynthesis of Nicotianamine (NA), a natural plant chelator essential for the uptake and transport of metal micronutrients such as iron and zinc.

A precise molecular characterisation of the inserted DNA is a fundamental requirement for the regulatory assessment of any genetically modified crop. This process aims to determine the number of insertion sites, the integrity and structure of the inserted DNA, and the nature of the insertion site within the host genome. Genetically modified wheat events were created via *Agrobacterium*-mediated transformation of the Australian wheat cultivar 'Gladius' to express two genes (Table 2).

The *Agrobacterium*-mediated transformation followed an in-house protocol that is similar to that described by Sparks, Doherty and Jones (2014) and Hensel et al. (2017). The selected event has been given a unique identifier in accordance with OECD 'Guidance for the Designation of a Unique Identifier for Transgenic Plants'. The OECD Unique Identification Code for the wheat event is UM-12115T-1.

Table 2: Summary of Genes, Intended Traits, and Benefits in UM-12115T-1 Wheat

Gene	Trait	Benefit
<i>OsNAS2</i> (LOC_Os03g19420)	Nicotianamine is a non-protein amino acid involved in metal uptake, transport and homeostasis in all plants and constitutes the biosynthetic precursor of mugineic acid family phytosiderophores (MAs) in graminaceous plant species. NA is also characterised as a metal chelator in short and long-distance transport of divalent cations such as Fe ²⁺ , Zn ²⁺ , Mn ²⁺ , Cu ²⁺ and ferric iron (Fe ³⁺).	Overexpression of the rice <i>OsNAS2</i> gene (LOC_Os03g19420) in rice, and constitutive expression of the rice <i>OsNAS2</i> gene in wheat, has been shown to significantly increase Fe and Zn concentrations in grain (Johnson et al., 2011; Singh et al., 2016; Moreno-Moyano et al., 2016; Beasley et al., 2019a; Beasley et al., 2019b; Beasley et al., 2022).
<i>Hygromycin phosphotransferase</i>	Antibiotic resistance	Antibiotic resistance during <i>in vitro</i> selection of transformants

¹The HPT protein has been previously assessed by FSANZ in cotton (see A509 and A615) and safflower (A1156)

Details of the identity of genes and expression products are provided in *Supplement Report UOM-25-PROT-RPT-02-02 Protein Safety*.

A.1(b) The name, number or other identifier of each of the new lines or strains of GM organism from which the food is derived.

In accordance with OECD '[Guidance for the Designation of a Unique Identifier for Transgenic Plants](#)', the OECD Unique Identification Code for the wheat event is UM-12115T-1. For the purposes of this regulatory approval, this identifier encompasses the original dual-locus event as well as the independent single-locus segregants (Locus 1 only and Locus 2 only) derived through conventional breeding.

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A.1(c) The name the food will be marketed under (if known).

This wheat will be marketed under a variety of labels depending on the background wheat variety and licenced user of the event.

A.2. History of use of the host and donor organisms

A.2(a) For the donor organism(s) from which the genetic elements are derived:

A.2(a)(i) Any known pathogenicity, toxicity or allergenicity of relevance to the food

The genetic elements introduced into UM-12115T-1 are derived from *Oryza sativa* (rice), *Zea mays* (maize), *Agrobacterium tumefaciens*, Cauliflower mosaic virus (CaMV), and common bacterial species including *Klebsiella* sp., *Escherichia coli*, *Enterococcus faecalis*, and *Pseudomonas fluorescens*.

- ***Oryza sativa* and *Zea mays*:** Both rice (donor of the *OsNAS2* gene) and maize (donor of the *Ubi 1* promoter) are staple food crops. Neither species is considered pathogenic or toxic. While rice and maize can act as rare allergens in some individuals, they are not classified among the major allergenic foods, and the specific genetic elements utilised do not encode recognised allergens.
- ***Agrobacterium tumefaciens* and CaMV:** *A. tumefaciens* (donor of the *nos* terminator) is a soil-borne bacterial plant pathogen, and CaMV (donor of the 35S promoter and poly-A signals) is a plant virus. Neither is pathogenic, toxic, or allergenic to humans or animals.
- **Bacterial Donors (*Klebsiella* sp., *E. coli*, *E. faecalis*, *P. fluorescens*):** These bacteria are the sources of the *hph* selectable marker and plasmid backbone elements (e.g., *nptIII*). While certain strains of *E. coli*, *Klebsiella*, and *Enterococcus* can be human pathogens, the specific genetic sequences transferred are highly characterised elements (such as antibiotic resistance markers and origins of replication). They are not virulence factors and do not confer any pathogenic, toxic, or allergenic properties to the food (OGTR 2024; Lu et al., 2007).

A.2(a)(ii) History of use of the organism in the food supply or history of human exposure to the organism through other than intended food use (e.g., as a normal contaminant)

The donor organisms from which the genetic elements are derived possess an extensive and well-established history of safe use in the food supply or widespread human exposure. Both *Oryza sativa* (rice) and *Zea mays* (maize) are major agricultural crops with a long history of safe global consumption as staple human foods. The Cauliflower mosaic virus (CaMV) naturally infects commonly consumed brassica species, such as cabbage, broccoli, and cauliflower, resulting in a history of safe, ubiquitous human exposure to CaMV genetic elements through the conventional food supply. Furthermore, the bacterial donors, which include *Escherichia coli*, *Klebsiella* spp., *Enterococcus faecalis*, and *Pseudomonas fluorescens*, are widespread in the natural environment, including soil and water, and are common commensal bacteria within the gastrointestinal tracts of humans and animals. Similarly, *Agrobacterium tumefaciens* is a prevalent and naturally occurring soil bacterium. Consequently, humans are routinely exposed to these microorganisms and their associated genetic material through normal environmental contact, established intestinal flora, and the consumption of fresh agricultural produce, thereby establishing a robust history of safe human exposure (EFSA GMO Panel, 2004).

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A.2(b) A description of the host organism into which the genes were transferred:

A.2(b)(i) Its history of safe use for food

The host organism, bread wheat (*Triticum aestivum* L.), is one of the world's most widely cultivated and consumed agricultural crops, possessing a history of safe dietary use spanning millennia (OECD, 2024). It provides a substantial portion of the dietary calories and protein consumed globally.

It is noted that wheat grain contains gluten proteins (gliadins and glutenins) that can trigger Celiac disease (CeD) in genetically susceptible individuals (Amnuaycheewa et al., 2022; Bromilow et al., 2017). Wheat is also a recognised major food allergen responsible for IgE-mediated allergic reactions, such as baker's asthma or wheat-dependent exercise-induced anaphylaxis. Comprehensive bioinformatic assessments confirm that the genetic modification in UM-12115T-1 does not introduce novel allergens or Celiac-toxic peptides. Aside from pre-existing sensitivities in specific subpopulations, *T. aestivum* has a well-established history of safe consumption for the public.

ORDER:	<i>Poales</i>
FAMILY:	<i>Poaceae</i>
GENUS:	<i>Triticum</i>
SPECIES:	<i>T. aestivum</i> L
COMMON NAME:	Wheat, Bread Wheat

A.2(b)(ii) The part of the organism typically used as food

Wheat grain is the primary part consumed as food and as animal feed. Vegetative material may also be directly grazed by livestock or harvested for hay. Young seedlings may also be added to drinks such as smoothies.

A.2(b)(iii) The types of products likely to include the food or food ingredient

Wheat grain is processed into a vast array of food products. The primary derivative is flour (both wholemeal and refined white flour), which serves as a foundational ingredient in baked goods (breads, cakes, biscuits, pastries), pasta, noodles, and breakfast cereals (Beasley et al., 2022). Wheat-derived ingredients, such as bran, starch, and vital wheat gluten, are also widely utilised as thickeners, binders, and functional ingredients in highly processed foods, sauces, and confectioneries.

In addition to human food applications, wheat is a foundational component of commercial animal feedstocks, playing a critical role in Australia's intensive livestock industries. Wheat grain, frequently including downgraded crops that do not meet premium human milling specifications, as well as varieties cultivated expressly for feed, serves as a primary dietary energy source for monogastric animals, most notably poultry (broilers and layers) and swine. In Australian commercial diets, wheat provides a high concentration of metabolizable energy (predominantly via starch) and essential amino acids.

Because monogastric animals lack the endogenous enzymes required to efficiently degrade the non-starch polysaccharides (NSPs) inherent in wheat grain, primarily arabinoxylans, poultry and swine feed formulations are routinely supplemented with exogenous enzymes such as xylanases. This processing step is essential to optimise nutrient digestibility, reduce gut viscosity, and mitigate anti-nutritional effects (Kim et al., 2005). Furthermore, the fibrous by-products of human flour milling, including wheat bran, pollard, and millrun, are extensively captured and repurposed as valuable nutritional co-

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products in monogastric diets to support gastrointestinal health and optimize feed conversion efficiency (Stas et al., 2024).

A.2(b)(iv) Whether special processing is required to render food derived from the organism safe to eat

No special processing is required to render wheat grain safe to eat. However, standard processing is necessary to make the food palatable and digestible. This typically involves milling the grain into flour and applying thermal processing (e.g., baking, boiling, or extrusion) to produce finished food items. This conventional thermal processing is sufficient for normal consumption (OECD, 2024). Notably, the introduced novel proteins in UM-12115T-1 (OsNAS2, HPT) and the non-functional Kan protein have been demonstrated to be highly thermolabile and are rapidly inactivated and degraded at standard cooking temperatures (Lu et al., 2007; Özen, 2007), further reinforcing that no novel or special processing methods are required to ensure food safety.

It is noted, however, that up to 1 % of the population may be affected by an autoimmune disease associated with exposure to gluten (i.e., coeliac disease; Caio et al., 2020).

A.3. The nature of the genetic modification

A.3(a) A description of the method used to transform the host organism

The wheat event UM-12115T-1 was developed using *Agrobacterium*-mediated transformation of the bread wheat cultivar 'Gladius' with the binary vector pUbi-OsNAS2 to produce the proteins OsNAS2 and HPT. This plasmid is described in detail in Section A3(b) with the HPT protein assessed previously by FSANZ in numerous applications (see Table 2). The transformation protocol followed the methodologies of Sparks et al., 2014 and Hensel et al., 2017 described below and outlined in Figure 1.

The genetic transformation of the wheat cultivar 'Gladius' was achieved using an *Agrobacterium tumefaciens*-mediated DNA delivery system. This method harnesses the natural ability of *A. tumefaciens* to transfer a specific segment of DNA (the T-DNA) from the introduced binary vector, pUbi-OsNAS2, into the nuclear genome of the host plant cells.

Explant Preparation

The primary target tissues for transformation were immature embryos harvested from the 'Gladius' wheat cultivar. Immature seeds were collected approximately 12 to 16 days post-anthesis. Following surface sterilisation of the seeds, translucent immature embryos ranging from 0.8 to 2.0 mm in length were surgically isolated. To prevent precocious germination during tissue culture, the embryo axis was removed, leaving the scutellum tissue as the explant.

***Agrobacterium* Inoculation**

A hypervirulent strain of *A. tumefaciens* (AGL1), engineered to contain the pUbi-OsNAS2 binary vector, was utilised for the inoculation. The bacteria were grown in liquid culture, pelleted, and resuspended in an inoculation medium. This medium was supplemented with acetosyringone, a phenolic compound that induces the virulence (vir) genes of the *Agrobacterium* to facilitate T-DNA transfer. A surfactant, such as Silwet L-77, may also be added to the suspension to assist with bacterial adhesion to the embryo surface. The isolated 'Gladius' embryos were submerged in this *Agrobacterium* suspension for approximately one hour.

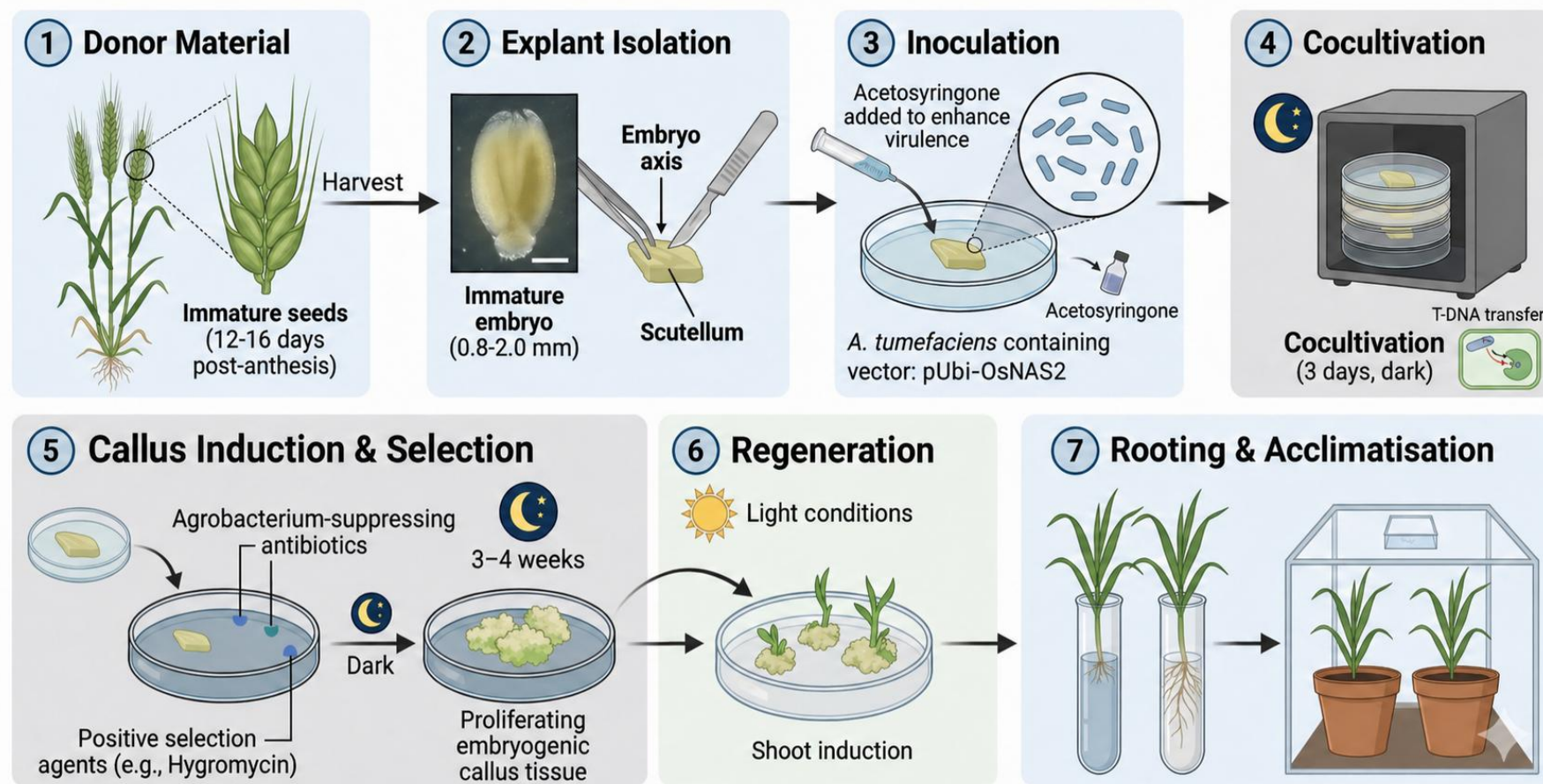


Figure 1. The Development and Selection of UM-12115T-1 Wheat Transformed with *pUbi-OsNAS2*

The protocol for *Agrobacterium* mediated transformation of wheat was adapted from published literature (Sparks et al., 2014 and Hensel et al., 2017). Schematic overview of 'Gladius' wheat transformation utilising pUbi-OsNAS2 [AI-Generated Image; Google Gemini 7 April 2026].

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Cocultivation

Following inoculation, the excess *Agrobacterium* suspension was removed, and the embryos were transferred, scutellum side up, onto a solid cocultivation medium. The explants were incubated in the dark at 22-23°C for three days to allow the T-DNA from the pUbi-OsNAS2 vector to transfer and integrate into the plant cells.

Callus Induction and Selection

After the three-day cocultivation period, the embryos were transferred to an induction medium to promote the formation of embryogenic callus. This medium contained specific growth regulators (such as 2,4-D) and an antibiotic (e.g., Timentin) designed to selectively halt any further growth of the *Agrobacterium* without harming the plant tissue. Additionally, a selective agent corresponding to the selectable marker gene present on the pUbi-OsNAS2 vector was included in the medium. The cultures were maintained in the dark for 3 to 4 weeks, during which time only the transformed cells containing the T-DNA successfully developed into embryogenic calli.

Regeneration and Plant Establishment

The resulting embryogenic calli were transferred to a regeneration medium and moved into light conditions to stimulate shoot development. Emerging shoots were subjected to further rounds of selection to minimise the occurrence of non-transformed escapes. Viable, green plantlets with well-developed root structures were subsequently transferred to a rooting medium. Once root systems were fully established, the putative transgenic plants were carefully transferred to soil, initially kept in high-humidity conditions to acclimatise, and finally grown to maturity in a containment glasshouse for downstream molecular and phenotypic validation

A.3(b) A description of the construct and the transformation vectors used

The transgenic wheat event UM-12115T-1 was developed via *Agrobacterium*-mediated transformation utilising the binary vector pUbi-OsNAS2, which is a modified derivative of the pMDC32 Gateway-compatible plant transformation vector (Curtis and Grossniklaus, 2003). As illustrated in the genetic map (Figure 2) and schematic outline (Figure 3), the vector contains a defined Transfer-DNA (T-DNA) region demarcated by Right Border (RB) and Left Border (LB) cleavage sequences. Within this T-DNA region, the vector harbours two distinct expression cassettes. The principal trait cassette consists of the maize *Ubi1* promoter driving the expression of the *Oryza sativa* *OsNAS2* gene, which encodes an enzyme vital for NA biosynthesis, and is followed by a 3' nopaline synthase (*nos*) terminator sequence. The secondary cassette functions as a selectable marker, employing an enhanced CaMV35S promoter to drive the hygromycin phosphotransferase (*hph*) gene, which is terminated by a CaMV35S Poly A sequence. This selectable marker allows for the *in vitro* selection of plant transformants by conferring tolerance to the antibiotic hygromycin. A comprehensive itemisation of all structural elements, including plasmid backbone components located outside the T-DNA that are necessary for bacterial maintenance, such as the kanamycin resistance gene and the pVS1 and pBR322 origins of replication, is systematically detailed in Table 3.

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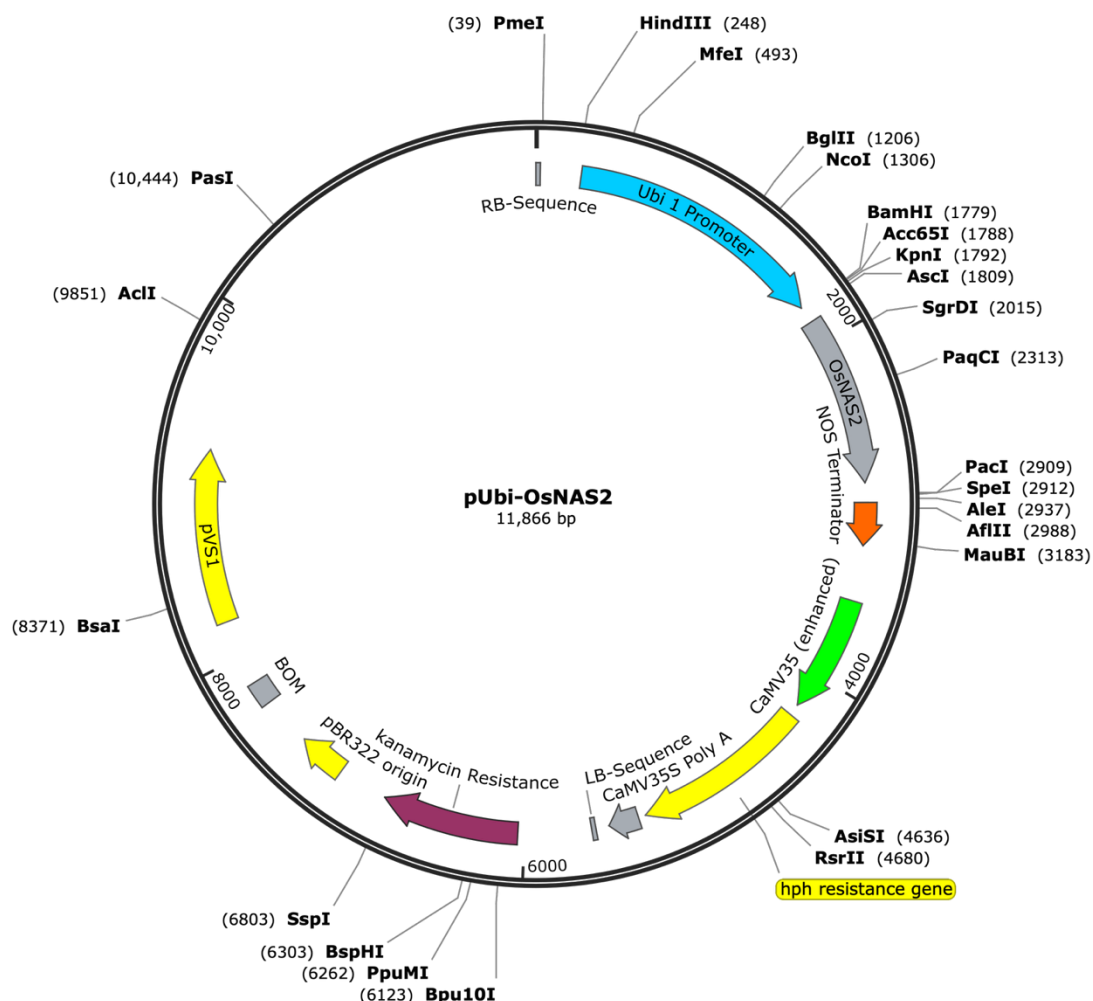
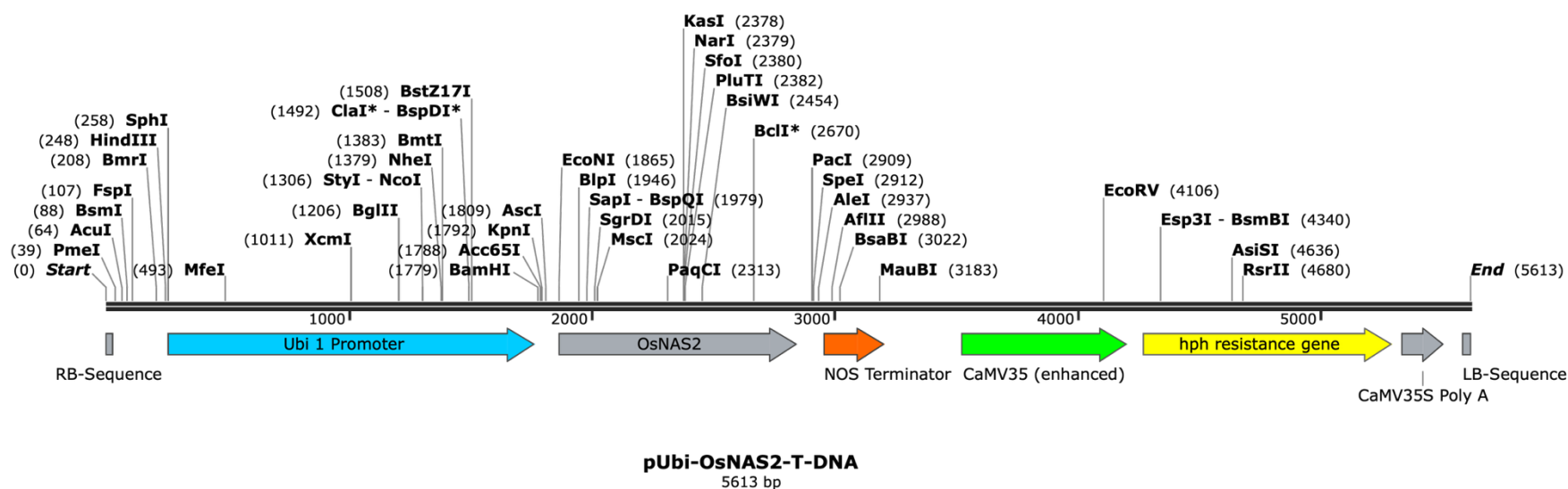


Figure 2. Plasmid Map of *pUbi-OsNAS2*

The diagram illustrates the structural elements contained within the plant binary expression vector *pUbi-OsNAS2*. The Transfer-DNA (T-DNA) region, delimited by the Right Border (RB) and Left Border (LB) sequences, encompasses two primary expression cassettes. The target trait cassette features the maize *Ubi1* promoter driving the expression of the *Oryza sativa* Nicotianamine synthase 2 (*OsNAS2*) gene, succeeded by a 3' nopaline synthase (*nos*) terminator. The selectable marker cassette consists of an enhanced CaMV35S promoter directing the hygromycin phosphotransferase (*hph*) antibiotic resistance gene, terminated by a CaMV35S Poly A signal. Essential plasmid backbone elements located outside the T-DNA boundaries for bacterial maintenance include the kanamycin resistance gene and the pVS1 and pBR322 origins of replication. Unique restriction enzyme recognition sites (e.g., *HindIII*, *NcoI*, *PacI*) are also indicated along the sequence.

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**Figure 3. Schematic outline of transformation vector pUbi-OsNAS2**

Features include: RB-Sequence = Right Border; LB-Sequence = Left Border; Ubi 1 Promoter = *Maize Ubi1 promoter*; OsNAS2 = *Oryza sativa Nicotianamine synthase 2*; nos = 3' nonpolyA; CaMV35 (enhanced) = 35S promoter; *hph* = hygromycin phosphotransferase gene; Unique 6+ restriction sites are also labelled (e.g., *HindIII*, *NcoI*, *PacI*).

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Table 3. Genetic Elements of *pUbi-OsNAS2*

Genetic Element	Origin	Position	Size (bp)	Intended Function
1. Right Border (RB)	Synthetic DNA pMDC32	1-25	25	Secondary cleavage site releases ssDNA inserts from pMDC32
2. Intervening sequence	Synthetic DNA pMDC32	26-259	234	Sequence used for DNA cloning
3. Ubi 1 Promoter	<i>Zea mays</i>	260-1767	1508	Drives expression of the <i>OsNAS2</i> gene
4. Intervening sequence	Synthetic DNA pMDC32	1768-1864	97	Sequence used for DNA cloning
5. <i>OsNAS2</i> gene	<i>Oryza sativa</i>	1865-2845	981	Expresses the <i>OsNAS2</i> gene a key enzyme in the biosynthesis of nicotianamine
6. Intervening sequence	Synthetic DNA pMDC32	2846-2958	113	Sequence used for DNA cloning
7. Nopaline Synthase gene terminator (<i>nos</i>)	<i>A. tumefaciens</i>	2959-3206	248	3' non-translated region of the nopaline synthase gene; terminator and polyadenylation signal for the <i>OsNAS2</i> gene transcript
8. Intervening sequence	Synthetic DNA pMDC32	3207-3526	320	Sequence used for DNA cloning
9. CaMV35S enhanced	Cauliflower mosaic virus	3527-4204	678	Expresses the <i>hph</i> gene for resistance to the antibiotic hygromycin
10. Intervening sequence	Synthetic DNA pMDC32	4205-4271	67	Sequence used for DNA cloning
11. <i>hph</i> Hygromycin resistance gene	<i>Klebsiella</i> sp. via <i>E. coli</i>	4272-5294	1023	Hygromycin phosphotransferase antibiotic resistance gene and selectable marker
12. Intervening sequence	Synthetic DNA pMDC32	5298-5336	39	Sequence used for DNA cloning
13. CaMV35S Poly A	Cauliflower mosaic virus	5337-5511	175	Terminator for <i>hph</i> gene transcript
14. Intervening sequence	Synthetic DNA pMDC32	5512-5588	77	Sequence used for DNA cloning
15. Left border (LB)	Synthetic DNA pMDC32	5589-5613	25	Secondary cleavage site releases ssDNA inserts from pMDC32
16. Intervening sequence	Synthetic DNA pMDC32	5614-6037	424	Sequence used for DNA cloning
17. Kanamycin resistance gene	<i>Enterococcus faecalis</i> pJH1	6038-6832	795	Kanamycin resistance (<i>aphIII/nptIII/3'5'</i> -aminoglycoside phosphotransferase type III from <i>Enterococcus faecalis</i> pJH1)
18. Intervening sequence	Synthetic DNA pMDC32	6833-7122	290	Sequence used for DNA cloning
19. Origin of replication for pBR322 (pBR322 ori)	Synthetic DNA pMDC32	7123-7403	281	Origin of replication
20. Intervening sequence	Synthetic DNA pMDC32	7404-7692	289	Sequence used for DNA cloning
21. BOM	Synthetic DNA pMDC32	7693-7833	141	basis of mobility region from pBR322
22. Intervening sequence	Synthetic DNA pMDC32	7834-8212	379	Sequence used for DNA cloning
23. Intervening sequence pVS1	<i>Pseudomonas fluorescens</i> pVS1	8213-9213	1001	Origin of replication for the <i>Pseudomonas</i> plasmid pVS1
24. Intervening sequence	Synthetic DNA pMDC32	9214-11866	2653	Sequence used for DNA cloning

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A.3(c) A full molecular characterisation of the genetic modification in the new organism

This Section provides information that addresses the requirements for Part A.3(c) A full molecular characterisation of the genetic modification in the new organism, including:

- (i) Identification of all transferred genetic material and whether it has undergone any rearrangements
- (ii) A determination of the number of insertion sites, and the number of copies at each insertion site
- (iii) Full DNA sequence of each insertion site, including junction regions with the host DNA
- (iv) A map depicting the organisation of the inserted genetic material at each insertion site; and
- (v) Details of an analysis of the insert and junction regions for the occurrence of any open reading frames (ORFs).

Further information is provided in the *Supplement Report UOM-25-MOL-01-01*.

A.3(c)(i) Identification of all transferred genetic material and whether it has undergone any rearrangements

Comprehensive molecular characterisation utilising PacBio HiFi long-read sequencing definitively identified the genetic material transferred from the pUbi-OsNAS2 transformation vector into the UM-12115T-1 genome, which resulted in two independent insertion loci (Figure 2; Table 3).

1. Identification of Transferred Genetic Material

The transferred genetic material consists of the following defined elements:

- **Intended T-DNA (Present at both Locus 1 and Locus 2):** Both insertion sites contain the intended *OsNAS2* expression cassette (comprising the maize Ubi1 promoter, *Oryza sativa OsNAS2* gene, and nos terminator) and the selectable marker cassette (comprising the CaMV 35S promoter and the *hph* hygromycin resistance gene).
- **Vector Backbone Elements (Present only at Locus 1):** In addition to the intended T-DNA, Locus 1 contains a discrete, unintended fragment of the vector backbone comprising the kanamycin resistance gene (*nptIII*), the pBR322 origin of replication (*ori*), and the basis of mobility (*bom*) sequence. Because this backbone fragment lacks eukaryotic promoters and terminators, it is incapable of expression in the plant host and is entirely non-functional. Genome-wide screening confirmed no other vector backbone sequences were transferred.

2. Assessment of Rearrangements

- **Locus 2 (Chromosome 4D) – No Rearrangements:** This locus represents a simple, intact insertion of the intended T-DNA. Flanking sequence analysis confirmed clean junctions with the endogenous wheat genome, with no evidence of structural rearrangements, partial integrations, or unintended sequence modifications.
- **Locus 1 (Chromosome 1D) – Structural Rearrangement:** This locus underwent a complex structural rearrangement during the integration process. While the *OsNAS2* and hygromycin cassettes integrated largely intact (noting a truncation at the CaMV35S Poly A signal boundary), the accompanying fragment of the vector backbone integrated in a reversed orientation. The sequential order of these backbone elements within the plant genome (*bom* → *ori* → *nptIII*) is inverted relative to their original configuration on the transformation plasmid. This structural inversion is a defined rearrangement characteristic of host-mediated DNA repair mechanisms (such as Non-Homologous End Joining) acting upon double-strand breaks in the vector prior to or during integration.

A.3(c)(ii) A determination of the number of insertion sites, and the number of copies at each insertion site

Alignment of the sequencing data to the annotated plasmid reference definitively established the presence of two independent insertion loci within the UM-12115T-1 genome (Figure 4). Furthermore, the background read alignment observed in the wild-type track over the OsNAS2 sequence region highlights the expected synteny with endogenous wheat NAS genes, confirming the assay's robust sensitivity and highlighting the conserved nature of the introduced trait.

The first insertion, designated as Locus 1, is located on Chromosome 1D. This is a single, complex insertion event resolved on a single high-confidence contig (ptg0000821) containing one copy of the intended T-DNA elements along with the integrated vector backbone sequences (Figure 5). The second insertion, Locus 2, is located on Chromosome 4D. This represents a single, simple insertion event resolved on a separate high confidence contig (ptg0002121). Locus 2 contains exactly one copy of the intended T-DNA and is entirely free of plasmid backbone sequences (Figure 6). The high quality of the de novo assembly, with an N50 of 73.7 Mbp, ensures these results are definitive (Table 4).

Uniform long-read coverage across both loci confirms that the insertions are stable and fixed as single-copy integration events at their respective chromosomal locations (Figure 7).



Figure 4. A snapshot of alignment of event UM-12115T-1 and wild type wheat samples to the pUbi-OsNAS2 construct

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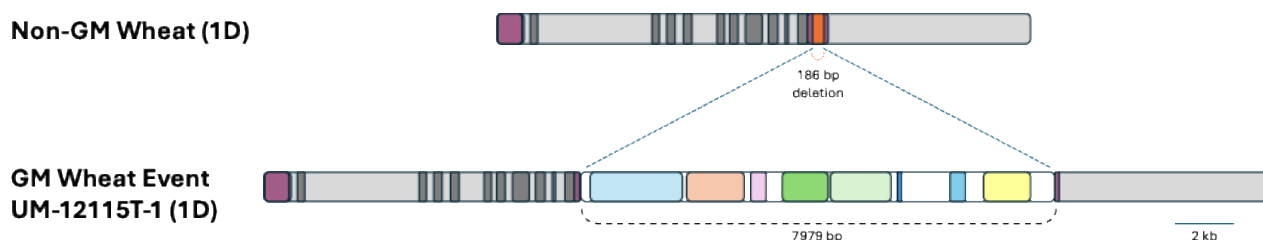


Figure 5. Schematic map of the 7,979 bp insertion locus on Chromosome 1D.

The diagram shows the inserted genetic elements relative to the flanking wheat genomic DNA, including the 3' UTR and exons of the endogenous PEAMT1 gene. A 186 bp deletion of the wild-type sequence at the insertion site is indicated. [Some Figure Information Removed-CCI]

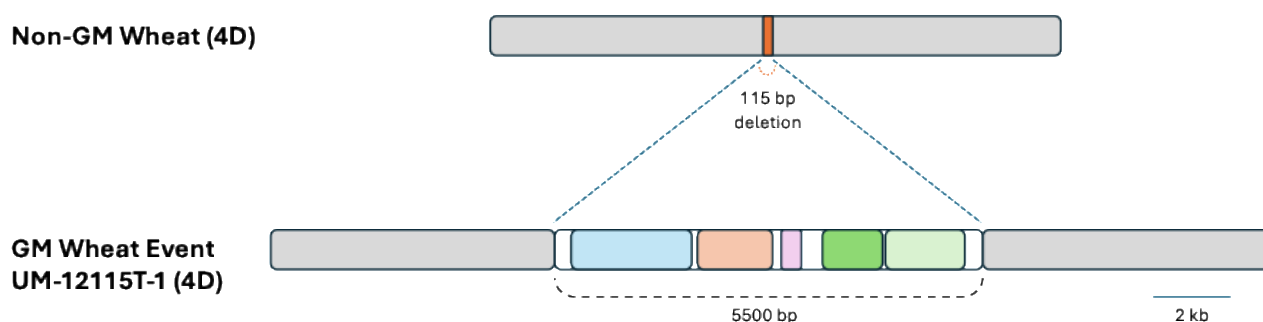


Figure 6. Schematic map of the 5,504 bp insertion locus on Chromosome 4D.

The diagram shows the inserted genetic elements relative to the flanking intergenic DNA. A 115 bp deletion of the wild-type sequence at the insertion site is indicated. [Some Figure Information Removed-CCI]

Table 4. Assembly Statistics (Transgene Sample)

Metric	Value	Interpretation
N50	73,746,696 bp	An approximately 73.7 Mb N50 indicates chromosome-scale scaffolding, far exceeding typical fragmentation thresholds for insertion analysis
Total Length	14,637,245,976 bp	The total assembly size (approx. 14.6 Gb) aligns with the expected size of the hexaploid wheat genome (approx. 16-17 Gb)
Longest Contig	471,587,485 bp	The largest single continuous sequence is approx. 471 Mb
GC Content	46.08 %	Consistent with <i>Triticum aestivum</i> genomic composition
L50	48	Half the genome is contained in the 48 largest contigs

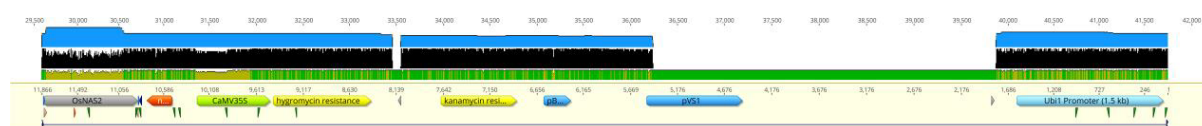


Figure 7. Event UM-12115T-1 coverage when aligned to pUbi-OsNAS2

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A.3(c)(iii) Full DNA sequence of each insertion site, including junction regions with the host DNA

PacBio HiFi sequencing enabled chromosome-scale *de novo* assembly, resolving each insertion site to nucleotide precision and fully sequencing the junction regions connecting the introduced DNA to the host wheat genome. Typical of *Agrobacterium*-mediated transformation, both insertions resulted in minor, localised deletions of native genomic DNA. The integration at Locus 1 comprises a 7,9798 bp continuous DNA fragment inserted into the 3' untranslated region of the endogenous wheat Phosphoethanolamine N-methyltransferase 1 (*PEAMT1*) gene. This integration was accompanied by a 186 bp deletion of the native genomic sequence at the insertion point (Figure 8). Mapping of the junctions confirmed a precise alignment of the Right Border with the upstream genomic flank and uninterrupted contiguity at the Left Border into the downstream genomic sequence (Figure 9 and Figure 10).

The integration at Locus 2 comprises a 5,504 bp continuous DNA fragment inserted within an intergenic region of Chromosome 4D, associated with a 115 bp deletion of the native intergenic sequence (Figure 11). Flanking sequence analysis demonstrated clean and unambiguous junctions, with the expected Right Border termination transitioning immediately into native DNA, and a fully resolved Left Border aligning completely with the downstream flanking sequence (Figure 12 and Figure 13).

The use of T8 generation plant material further demonstrates that these junction sequences and the overall insertion structure are stable and heritable.

[Redacted – Confidential Commercial Information (CCI)]

Figure 8. Chromosome 1D Insertion Site with the 186 bp Deletion

The Chromosome 1D reference sequence was obtained from The International Wheat Genome Sequencing Consortium RefSeq v2.1, and IWGSC Annotation v2.1 (Zhu et al., 2021). Sequences highlighted in green represent the flanking sequence of the insertion.

[Redacted – Confidential Commercial Information (CCI)]

Figure 9. Right Border Junction for the Insertion in Chromosome 1D

Sequences in: Green = genomic DNA flanking sequence from chromosome 1D; pink = Right Border sequence; Light blue = Ubi1 promoter

[Redacted – Confidential Commercial Information (CCI)]

Figure 10. Left Border Junction for the Insertion in Chromosome 1D

Sequences in: Green = genomic DNA flanking sequence from chromosome 1D; yellow = kanamycin resistance gene

[Redacted – Confidential Commercial Information (CCI)]

Figure 11. Chromosome 4D Insertion Site with 115 bp Deletion

The Chromosome 4D reference sequence was obtained from The International Wheat Genome Sequencing Consortium RefSeq v2.1, and IWGSC Annotation v2.1 (Zhu et al., 2021). Sequences highlighted in green represent the flanking sequence of the insertion.

[Redacted – Confidential Commercial Information (CCI)]

Figure 12. Right Border Junction for the Insertion in Chromosome 4D

Sequences in: Green = genomic DNA flanking sequence from chromosome 4D; pink = Right Border sequence; Light blue = Ubi1 promoter

[Redacted – Confidential Commercial Information (CCI)]**Figure 13. Left Border Junction for the Insertion in Chromosome 4D**

Sequences in: Green = genomic DNA flanking sequence from chromosome 4D; Pink = left border sequence; Grey = CaMV35S poly A site associated with the *hph* gene; Yellow = the hygromycin phosphotransferase gene (*hph*).

A.3(c)(iv) A map depicting the organisation of the inserted genetic material at each insertion site

Details of the organisation of the inserted genetic material at each integration site are described above. Specifically: Detailed organisation of the genetic elements in each insert (Figure 5 and Figure 6).

A.3(c)(v) Details of an analysis of the insert and junction regions for the occurrence of any open reading frames (ORFs)

The sequence of both inserts and the wheat flanking regions were subjected to an ORF analysis (see Section B1(d) and the *Supplement Report UOM-25-PRT-RPT-01-02 Allergen and Toxin Evaluation of ORFs*). None of the peptides that might be hypothetically produced from these ORFs were identified as homologs of known toxins or allergens (see Section B1(d)).

A.3(d) A description of how the line or strain from which food is derived was obtained from the original transformant (i.e. provide a family tree or describe the breeding process) including which generations have been used.

Agrobacterium mediated transformation is an established method for the introduction of plasmid vectors into plants. Wheat event UM-12115T-1 was developed using a transformation procedure adapted from published methods (Sparks et al., 2014 and Hensel et al., 2017; see Figure 1).

A schematic representation of the development of event UM-12115T-1 and the generations used for analysis is presented in Figure 14.

The original event UM-12115T-1 will be crossed into elite, locally adapted wheat varieties through conventional breeding programs. Because Locus 1 (Chromosome 1D) and Locus 2 (Chromosome 4D) are unlinked, they will segregate independently during these crosses. Consequently, the commercial derivatives of UM-12115T-1 will comprise three distinct genetic variants: lines possessing both loci, and lines possessing either Locus 1 or Locus 2 independently. Commercial varieties containing any of these three *OsNAS2* configurations will be used for feed and food.

Application to FSANZ for the Inclusion of wheat event UM-12115T-1 with increased Nicotianamine in Standard 1.5.2 Genetically Modified Food

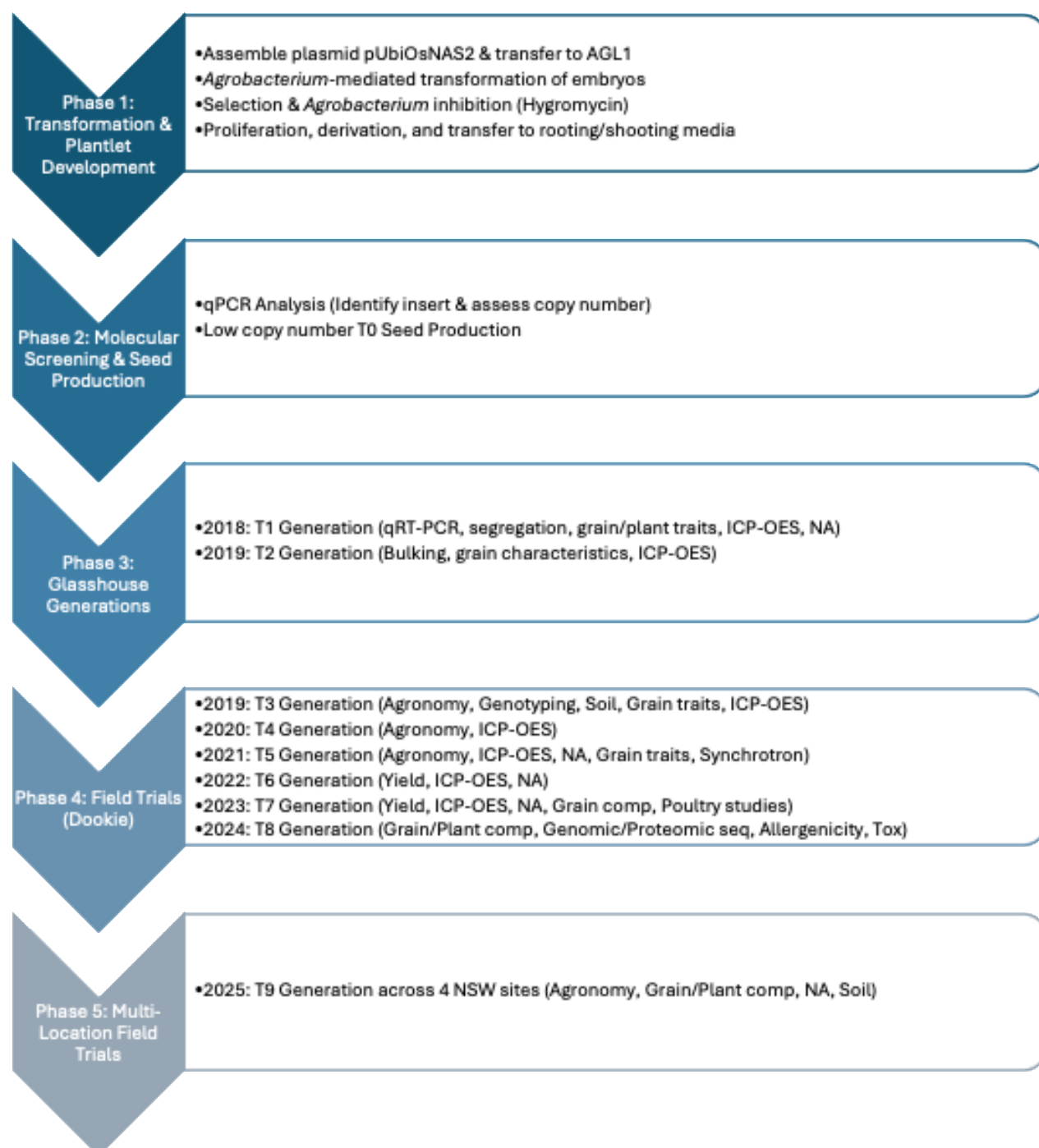


Figure 14. Schematic representation of the development of event UM-12115T-1 and the generations used in the different studies.

A.3(e) Evidence of the stability of the genetic changes, including:

- (i) The pattern of inheritance of the transferred gene(s) and the number of generations over which this has been monitored
- (ii) The pattern of inheritance and expression of the phenotype over several generations and, where appropriate, across different environments

A.3(e)(i) The pattern of inheritance of the transferred gene(s) and the number of generations over which this has been monitored

The molecular stability of wheat event UM-12115T-1 was established through the comprehensive analysis of the lineage from the primary T0 transformant through eight generations of self-pollination (T0 to T8). High-molecular-weight genomic DNA sourced from the T8 generation was analysed using PacBio HiFi long-read sequencing, which confirmed that the genetic modifications are integrated at two independent loci on Chromosome 1D (Locus 1) and Chromosome 4D (Locus 2). The structural integrity of these insertions was demonstrated by the resolution of each locus into single, uninterrupted, high confidence contigs (ptg0000821 and ptg0002121) within the *de novo* assembly (see Figure 7). Mapping of HiFi reads across the insertion sites showed uniform coverage without discordant pairs or split-read signatures, verifying that the junctions and internal sequences are fixed as distinct haplotypes and stable across the T8 population.

Several non-functional vector backbone sequences were detected at Locus 1, which include the *nptIII* (kanamycin resistance) gene and bacterial origins of replication. These elements were determined to be functionally inert in the plant host, as they lack the necessary eukaryotic regulatory signals for expression. This conclusion is supported by a comprehensive bioinformatic evaluation of all 94 putative Open Reading Frames (ORFs) generated at the insertion sites, which found no homology to known toxins or allergens and no evidence of functional chimeric transcripts (see below). Furthermore, proteomic analysis via LC-MS/MS confirmed that while the target *OsNAS2* protein is consistently expressed, the selectable marker proteins from the backbone remain undetectable in the grain, providing high confidence that the backbone integration does not impact the safety or phenotypic stability of the event (Table 3).

A.3(e)(i) The pattern of inheritance and expression of the phenotype over several generations and, where appropriate, across different environments

The inheritance and expression of the micronutrient enhancement phenotype were monitored through continuous field propagation from 2019 to 2023, spanning the T4 to T8 generations. Comparison of these seasons demonstrates that while the genetic insertion is molecularly fixed, the magnitude of the biofortification trait is subject to standard Genotype by Environment (G×E) interactions.

During the 2022 field season (T4 generation), the intended trait was strongly expressed, with event UM-12115T-1 achieving a mean grain zinc concentration of 46.40 mg/kg, a significant increase over the 33.80 mg/kg observed in the wild type control ($P < 0.001$). Significant increases were concurrently noted for iron, with the GM line reaching 46.20 mg/kg compared to 40.20 mg/kg in the wild type control. In contrast, the 2025 multi-site evaluation (T8 generation) showed that the event was compositionally equivalent to the wild type control across four distinct environments.

The variance in trait magnitude between the 2022 and 2025 seasons does not indicate genetic instability or trait silencing. Instead, it reflects the high sensitivity of metal homeostasis to localised environmental factors, such as soil mineral availability, pH, and moisture profiles, which are known to modulate the biosynthesis of metal-chelating ligands. The stability of the underlying genetic change is verified by the T8 molecular characterisation, which confirmed the structural integrity and contiguity of the insertion loci. Furthermore, the agronomic performance remained highly consistent throughout all evaluations. Regression analysis reveals that the yield performance of the GM event is highly predictive of the Control performance ($R^2=0.9937$; $P<0.001$; see *Supplement Report UOM-26-AGR-RPT-02-01 Event Assessment 2019-*

2023). The regression slope of 1.18 indicates that the GM line responds positively to higher-yielding environments without suffering yield drag in lower-yielding years (Table 5).

Table 5. Longitudinal summary of grain yield performance for GM Event UM-12115T-1 versus the Wild Type Control across five generations (2019–2023)

[Redacted – Confidential Commercial Information (CCI)]

This environmental modulation of the phenotype provides a positive safety narrative for a weight-of-evidence approach: it demonstrates that the *OsNAS2* expression operates within the natural physiological boundaries of wheat and does not lead to an uncontrollable or toxic accumulation of minerals under varied agro-ecological conditions. Stability is confirmed by the consistency of the T8 genomic architecture, the detectable translation of the *OsNAS2* protein at low levels in grain (see Section B.1(a)), and the maintenance of phenotypic equivalence in traits like productive tiller number across all environments (Figure 15).

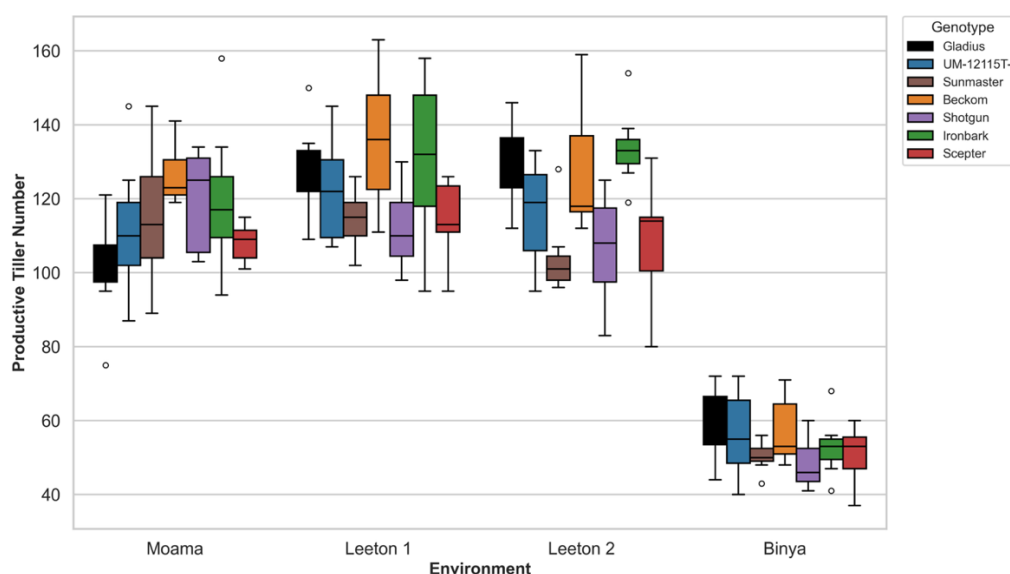


Figure 15. Distribution of productive tiller number for GM event UM-12115T-1 and reference varieties in 2025.

Box plots represent the count of fertile tillers 75 cm of row, serving as a primary indicator of vegetative vigour and potential weediness. No outliers indicating excessive tillering or invasive potential were detected for the GM event UM-12115T-1 relative to the wild type control or standard commercial varieties. Data is from 4 different environments (see *Supplement Report UOM-26-AGR-RPT-02-01 Event Assessment 2019-2023*).

Ultimately, the consistent achievement of yield parity and superior performance across the 2025 trial environments confirms that the *OsNAS2* expression does not impose a deleterious metabolic load, providing definitive evidence that the genetic modification is both stably integrated and physiologically harmonised with the wheat host's primary metabolic pathways.

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The stability of wheat event UM-12115T-1 is supported by a cohesive weight of evidence linking its molecular architecture to its agronomic performance. The genomic evidence from the T8 generation confirms that the insertion sites are structurally fixed and heritable. This molecular permanency directly explains the high phenotypic correlation across 2019-2023 ($R^2=0.9937$; $P<0.001$) and consistent nutritional enhancement observed across multiple years and environments. By resolving the insertion to nucleotide precision and demonstrating stable protein expression while excluding functional backbone activity, these data provide a robust foundation for the conclusion that the genetic modification in UM-12115T-1 is safe and predictably maintained throughout the life cycle of the crop.

A.3(g) An analysis of the expressed RNA transcripts, where RNA interference has been used

Not applicable to this application.

B. Characterisation and Safety Assessment of New Substances

B.1. Characterisation and Safety Assessment of New Substances

B.1(a) a full description of the biochemical function and phenotypic effects of all new substances (e.g. a protein or an untranslated RNA) that are expressed in the new GM organism, including their levels and site of accumulation, particularly in edible portions

Identity, function and expression of newly expressed proteins in wheat event UM-12115T-1

Wheat event UM-12115T-1 was developed to express two introduced proteins: nicotianamine synthase 2 from rice (*Oryza sativa*; OsNAS2), which confers the intended increase in nicotianamine biosynthesis, and hygromycin phosphotransferase (HPT), which was used as a selectable marker during transformation. OsNAS2 is the only introduced protein associated with the intended product phenotype. HPT functioned during *in vitro* selection of transformed cells and is not associated with the intended increase in nicotianamine of the final product. In addition, the non-functional Neomycin-kanamycin phosphotransferase (Kan) binary vector selectable marker was introduced in locus 1.

Together, these represent the biologically relevant new substances in event UM-12115T-1.

Additional information is provided in the *Supplement Report UOM-25-PROT-RPT-02-02*.

Identity and function of the OsNAS2 protein

OsNAS2 is a nicotianamine synthase (NAS; EC 2.5.1.43), a well-characterised plant aminopropyltransferase that catalyses the conversion of three molecules of S-adenosyl-L-methionine (SAM) into one molecule of NA, with 5'-methylthioadenosine as a co-product (Dreyfus et al., 2009; Inoue et al., 2003; Schuler and Bauer, 2011; Figure 16). Nicotianamine is a naturally occurring non-proteinogenic amino acid ubiquitous in higher plants and consumed routinely in plant-derived foods. It is a central chelator in plant metal homeostasis and plays a key role in the uptake, intracellular trafficking, long-distance transport, and grain loading of iron (Fe), zinc (Zn), and other divalent metal ions (Curie et al., 2009; Schuler and Bauer, 2011; Seregin and Kozhevnikova, 2023).

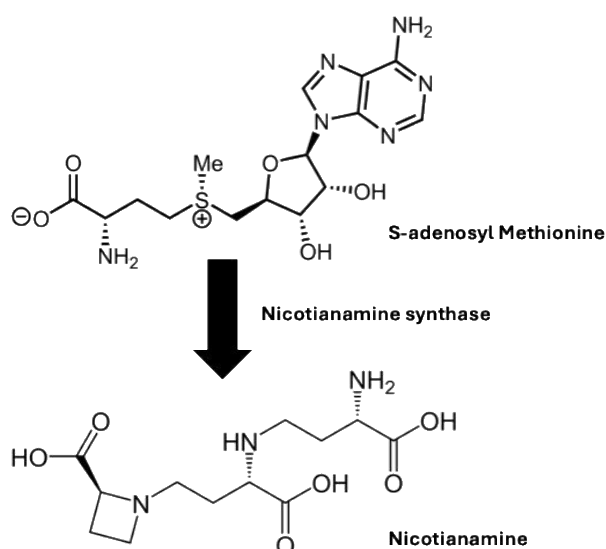


Figure 16. The Biosynthetic Pathway of Nicotianamine

Nicotianamine synthase catalyses the intramolecular condensation of three molecules of S-adenosyl-L-methionine (SAM) to form Nicotianamine, a non-proteinogenic metal chelator central to plant iron and zinc homeostasis. In this reaction, NAS transfers and rearranges amino-butyl moieties derived from SAM, generating the cyclic tripeptide backbone of nicotianamine while releasing three molecules of 5'-methylthioadenosine (MTA) as by-products. The process is ATP-independent and typically occurs in the cytosol, providing a committed step in the biosynthesis of nicotianamine that links SAM metabolism to metal transport and distribution in plants.

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The biochemical role of OsNAS2 in wheat event UM-12115T-1 is to increase metabolic flux through an endogenous and highly conserved plant pathway that is central to mineral homeostasis. As a specialised chelating agent for transition metals, NA is required by plants for the long-distance transport of Fe and Zn, facilitating the movement of these micronutrients into the developing grain. The ubiquity of NA highlights the physiological importance of this mechanism. Research analysing 166 plant foods detected the compound in 162 of them, failing to find it only in asparagus, rice, wheat, and corn. In contrast to these trace or zero-level crops, certain legumes serve as highly concentrated sources, with soybeans yielding 45 mg/100g, lentils 36 mg/100g, and chickpeas 31.5 mg/100g (Day, 2020). By overexpressing the OsNAS2 gene, the event enhances the plant's existing capacity to mobilise and deposit divalent cations into edible tissues without introducing unfamiliar reaction chemistry or novel classes of metabolites.

Accordingly, the biochemical function of OsNAS2 in UM-12115T-1 is to increase flux through an endogenous and highly conserved plant metabolic pathway. The introduced enzyme does not create a novel biochemical pathway, produce an unfamiliar class of metabolite, or introduce new reaction chemistry into wheat. Rather, it increases the biosynthesis of NA, thereby enhancing the plant's existing capacity to chelate and mobilise micronutrients. In graminaceous species such as wheat, NA also serves as a precursor of mugineic acid family phytosiderophores, which contribute to Fe acquisition and translocation (Curie et al., 2009; Schuler and Bauer, 2011). The biochemical role of OsNAS2 is therefore fully consistent with known cereal physiology and with the intended trait of improved Fe and Zn accumulation in grain.

The phenotypic effects associated with OsNAS2 expression in cereals are well established. Increased NAS activity elevates NA pools and supports greater movement of Fe and Zn into the developing grain. In rice and wheat, expression of OsNAS2 has been shown to increase grain NA, Fe and Zn concentrations and, in wheat, to improve mineral distribution into flour-relevant tissues and increase Fe bioavailability in white flour (Beasley et al., 2019a; Johnson et al., 2011; Singh et al., 2017). The intended biological effect of OsNAS2 expression is enhanced nicotianamine biosynthesis; however, compositional assessments of UM-12115T-1 demonstrated equivalence to conventional wheat, and increased grain iron and zinc concentrations were not observed in the evaluated event.

Analytical characterisation confirmed that the expressed protein in UM-12115T-1 corresponds to the intended OsNAS2 protein (see *Supplement Report UOM-25-PROT-RPT-02-02 Protein Safety*). Targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS) identified multiple unique OsNAS2-derived peptides in transgenic tissues, providing sequence-level confirmation of protein identity. OsNAS2 was detected exclusively in transgenic leaf and seed samples and was not observed in any wild type controls, confirming that the protein is specifically associated with the transgenic genotype and not with the non-transgenic background. Representative dDIA and DDA spectra for the **LEYDLLVR** peptide (Figure 17 and Figure 18) confirm the sequence identity of this surrogate peptide and support its use for downstream quantitative analyses. Additional sequence and structural features of OsNAS2 are provided in Figure 19.

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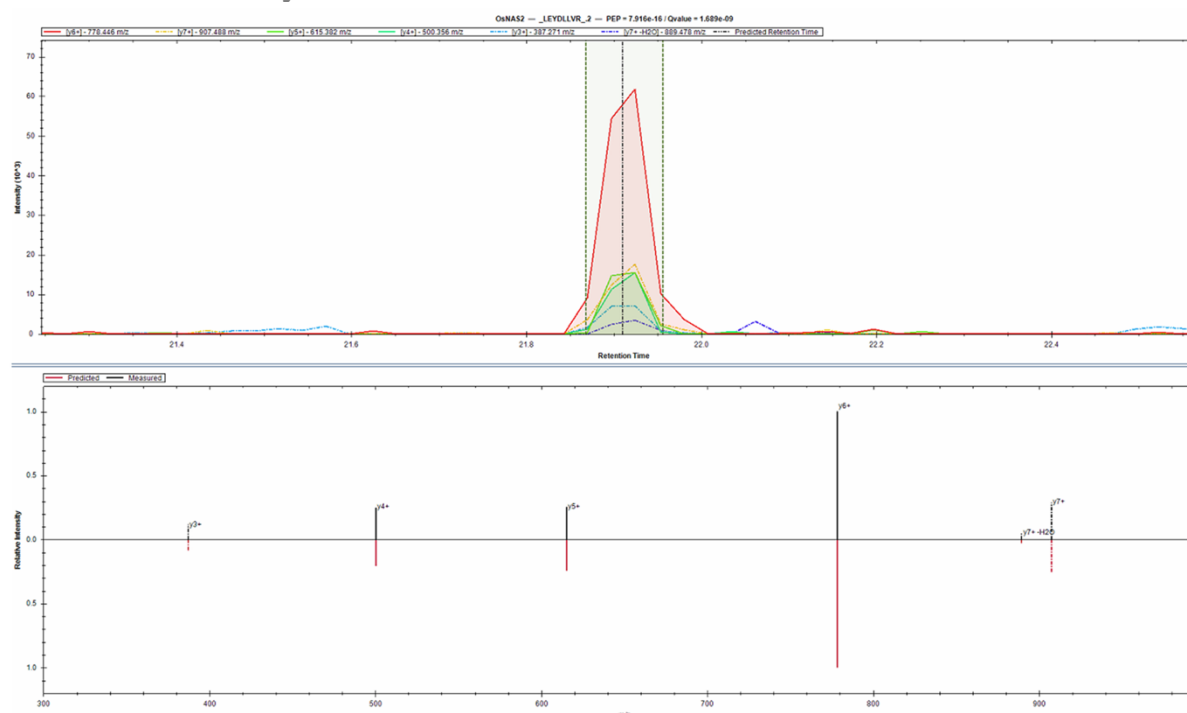


Figure 17. dDIA Spectrum of the Unique OsNAS2 Peptide LEYDLLVR

This figure displays the mass spectral fragmentation pattern of the unique peptide LEYDLLVR obtained using Data-Independent Acquisition (dDIA). The spectrum confirms the amino acid sequence of this specific peptide, which serves as a surrogate marker for the identification of the OsNAS2 protein. The comprehensive fragmentation data provided by this acquisition mode validates the presence of the target peptide within the complex sample matrix.

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Figure 18. DDA Spectrum of the Unique OsNAS2 Peptide LEYDLLVR

This figure illustrates the mass spectrum of the OsNAS2-derived peptide LEYDLLVR generated via Data-Dependent Acquisition (DDA). The spectrum details the specific ion fragments characteristic of the peptide's sequence, providing independent confirmation of its identity. Together with the dDIA data, this spectral evidence establishes the reliability of LEYDLLVR as a unique identifier for the detection of the novel protein in transgenic wheat tissues.

>OsNAS2

MEAQNQEVAALVEK**IAGLHAAISK**LPSPSPSAEVDALFTDLVTACVPA**SPVD**VAKLGPEAQAMREELIRLCSAAEGHLEAH
YADMLAAFDNPLDHLARFPYYGNYV**NLSKLEYDLLVR**YVPGIAPTRVAFVSGSGLPFSSVLAAHHLDPDAVFDNYDRCGAA
NERARRLFRGADEGLGARMFAHTADVATLTGELGAYDVVFLAALVGMAAEEKAGVIAHLGAHMADGAALVVRSAHGA
RGFLYPVDLEDIRRGGFDFVLAVYHPDDEVINSVIVARKADPRRGGLAGARGAVPVVSPCKCKCKMEAAAGAFQKAEEF
AAKRLSV*

Figure 19. Features of the OsNAS2 Protein

The OsNAS2 amino acid sequence (UM-12115T-1 and as expressed from *E. coli*). Sequences in **red** represent the unique peptide sequences identified using targeted DDA and DIA mass spectrometry workflows. A single potential N-glycosylation consensus motif sequence was predicted at location 107 (**purple**) and a single potential O-glycosylation site at position 49 (**green**), but neither considered a likely candidate for glycosylation.

From a cellular processing perspective, OsNAS2 is not expected to enter the secretory pathway. *In silico* analysis indicates that the protein lacks a signal peptide and is not predicted to undergo secretory-pathway-

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dependent glycosylation. This is consistent with its role as an intracellular metabolic enzyme and supports the conclusion that the protein expressed in wheat corresponds to the intended functional enzyme without unexpected post-translational modification of relevance to food safety.

Identity and function of the HPT protein

The second introduced protein in event UM-12115T-1 is hygromycin phosphotransferase, referred to as HPT. This protein is also commonly described in the literature as HPT or APH(4)-Ia. HPT is an aminoglycoside phosphotransferase of bacterial origin that was used as a selectable marker during plant transformation. Its function is to detoxify hygromycin B during *in vitro* culture, thereby allowing transformed cells to survive on selective medium while non-transformed cells do not (Miki and McHugh, 2004; Rao et al., 1983; Stogios et al., 2011).

Biochemically, HPT catalyses transfer of the γ -phosphate group from ATP or GTP to the 4-hydroxyl group of hygromycin B, producing a phosphorylated derivative that no longer inhibits ribosomal translocation (Rao et al., 1983; Stogios et al., 2011). This enzymatic function is highly substrate specific. Structural and biochemical studies show that the enzyme is adapted to hygromycin B and does not confer resistance to clinically important aminoglycosides such as kanamycin or gentamicin (Stogios et al., 2011). The function of HPT is therefore specific to transformation selection and does not confer a trait relevant to food composition, nutrient value, or agronomic performance under normal cultivation conditions.

The phenotypic effect of HPT is limited to the transformation and regeneration stage of plant development. In the absence of hygromycin selection pressure, HPT does not provide an agronomic or nutritional advantage and has no intended role in the final food product. Its presence in the transformation cassette is therefore considered incidental to development of the event and not related to the intended product phenotype. A summary of key structural and functional features of HPT is provided in Figure 20.

Sequence comparison showed that the HPT protein encoded in UM-12115T-1 is identical at the amino acid level to the HPT protein assessed previously in published safety studies, supporting the relevance of the existing digestibility and acute oral toxicity data for this marker protein (Lu et al., 2007; see Figure 11 in *Supplement Report UOM-25-PROT-RPT-02-02*).

>Hygromycin Resistance Gene

MKKPELTATSVEKFLIEKFDSVSDLMQLSEGEESRAFSFDVGGRGYVLRVNSCADGFYKDRYVYRHFASAALPIPEVLDIGEF
 SESLTYCISRRSQGVTLQDLPETELPAVLQPVAEAMDAIAAADLSQTSFGFPFGPQGIGQYTTWRDFICAADPHVYHWQT
 VMDDTVSASVAQALDELMLWAEDCPEVRHLVHADFGSNNVLTDNGRITAVIDWSEAMFGDSQYEVANIFFWRPWLAC
 MEQQTRYFERRHPELAGSPRLRAYMLRIGLDQLYQSLVDGNFDDAAWAQGRCDIVRSGAGTVGRTQIARRSAAVWTD
 GCVEVLADSGNRRPSTRPRAKK*

Figure 20. Features of the HPT Protein

Potential O-glycosylation sites (green) as predicted by NetOGlyc 4.0. None are considered likely candidates for glycosylation.

Identity and function of the Kan protein

The Neomycin-Kanamycin Phosphotransferase type III protein (NPTIII), formally designated under the Enzyme Commission nomenclature as ATP:kanamycin 3'-O-phosphotransferase (EC 2.7.1.95), is a bacterial aminoglycoside-modifying enzyme (Woegerbauer 2007). The mature APH(3')-IIIa monomer is a protein of 264 amino acids with an approximate molecular mass of 29 kDa. Structurally, it features a conserved bilobal kinase-like three-dimensional fold that shares a high degree of structural and architectural homology with eukaryotic serine/threonine and tyrosine protein kinases, despite bearing little primary sequence identity. The native gene encoding this enzyme, frequently annotated in scientific literature as *aphA-3* or *nptIII*, was originally isolated from an R-plasmid of the Gram-positive commensal and opportunistic pathogen

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Enterococcus faecalis (formerly known as *Streptococcus faecalis*). In the context of genetically modified crops and FSANZ regulatory assessments, the *nptIII* sequence is typically encountered as a bacterial selectable marker located on the plasmid vector backbone (such as in pCAMBIA derivatives), utilised primarily for maintaining the plasmid in microbial hosts during laboratory cloning phases rather than for intentional integration into the plant genome.

Functionally, the enzyme confers antibiotic resistance by catalysing the transfer of a phosphate group from an adenosine triphosphate (ATP) donor to specific hydroxyl targets on the antibiotic structure, primarily the 3'-hydroxyl group of 4,6-disubstituted aminoglycosides and the 5'-hydroxyl group of 4,5-disubstituted variants (Wright and Thompson 1999; Woegerbauer 2007). Under normal physiological conditions, aminoglycosides are highly positively charged, which allows them to bind tightly to the negatively charged backbone of bacterial nucleic acids to arrest protein synthesis. The covalent addition of a negatively charged phosphate group by NPTIII introduces severe steric hindrance and unfavourable electrostatic repulsion, completely abrogating the antibiotic's ability to bind to the bacterial ribosome. Because of the extreme flexibility of its active site, NPTIII can phosphorylate and inactivate a broad spectrum of antibiotics.

OsNAS2 Protein Expression in Wheat Event UM-12115T-1

Expression of OsNAS2 in UM-12115T-1 was confirmed at both transcript and protein level. Relative transcription analysis demonstrated strong expression of the introduced *OsNAS2* gene in transgenic wheat and negligible background signal in the non-GM comparator (Table 6 and Figure 21).

Table 6. Primer Sequences and Amplification Parameters

[Redacted – Confidential Commercial Information (CCI)]

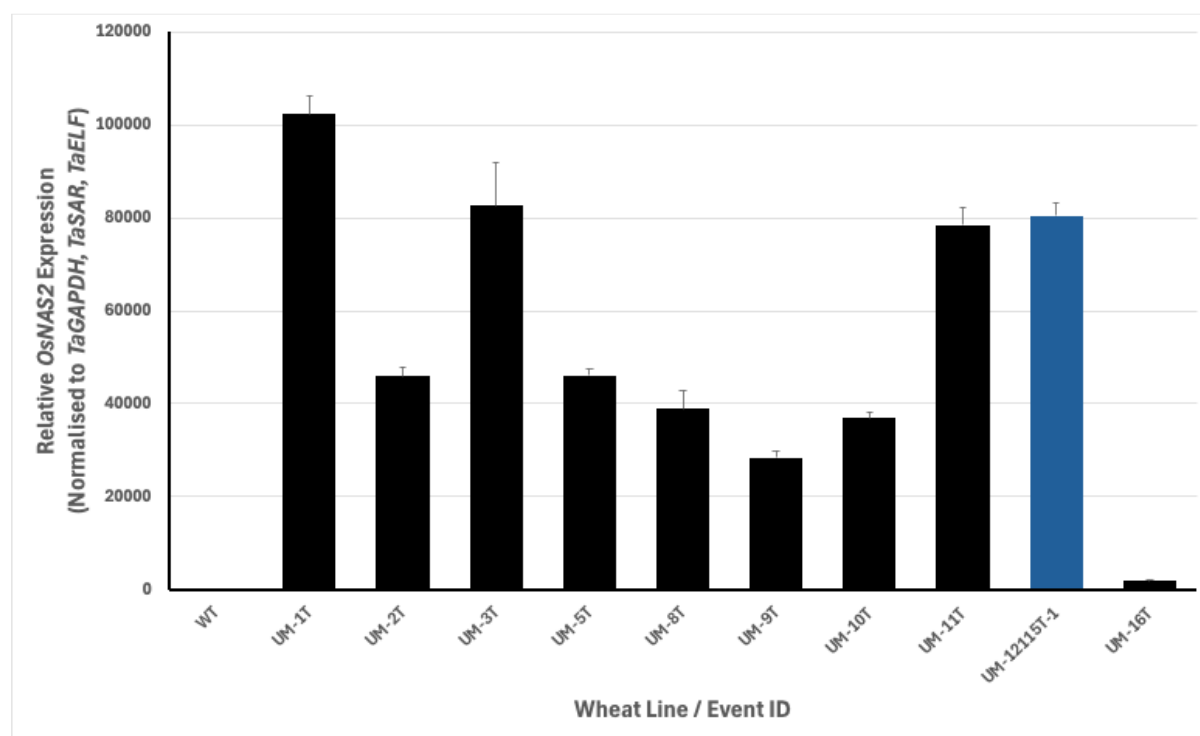


Figure 21. Transcriptional analysis of the *OsNAS2* transgene in genetically modified wheat

Total RNA was extracted from ten independent transgenic events (UM-1T–UM-16T) and one wild-type control (WT). Expression levels were measured via qRT-PCR and normalised to the geometric mean of three endogenous reference genes (*TaGAPDH*, *TaSAR*, and *TaELF*). Data are presented as mean relative expression units $\pm$ Standard Error of the Mean (SEM) (n=3 technical replicates).

Protein expression was characterised in both edible flour and vegetative leaf tissue using a targeted LC-MS/MS workflow that combined discovery-based and quantitative analyses. OsNAS2 was unequivocally detected in transgenic tissues through identification of multiple unique peptides, and quantification was achieved using a stable-isotope-labelled peptide standard (Figure 22 and Figure 23).

Quantitative analysis showed that OsNAS2 is present at low levels in flour and at higher levels in leaf tissue. In flour derived from UM-12115T-1, OsNAS2 was quantified at a mean concentration of 0.024 ng/mg extractable protein. In leaf tissue, the mean concentration was approximately 0.32 ng/mg extractable protein, indicating that expression in leaf was around 13-fold higher than in flour. OsNAS2 was not detected in the corresponding wild type controls. These data demonstrate that the introduced trait protein is present in the edible portion, but at low concentration relative to vegetative tissue. A summary of the quantitative expression data and assay sensitivity is provided in Table 7.

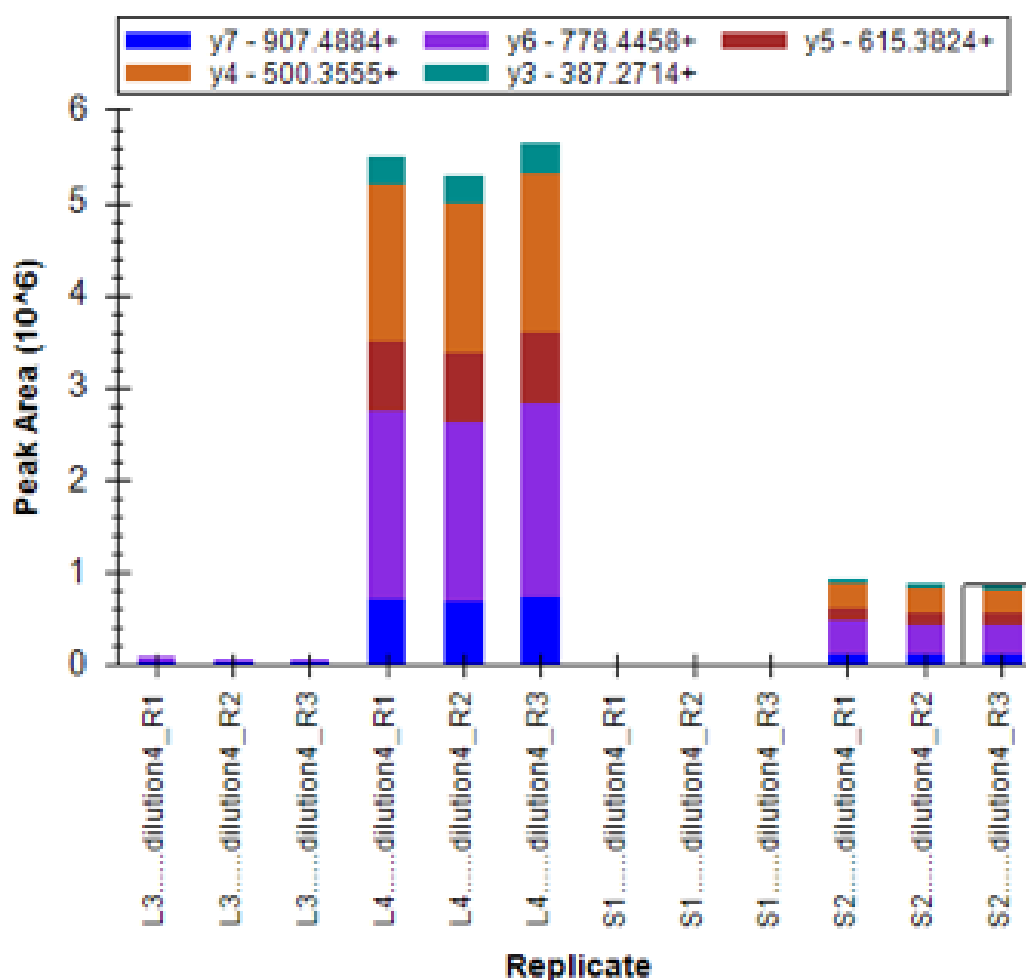


Figure 22. Peak area measurement of the endogenous light LEYDLLVR peptide

This graph displays the integrated peak areas for the endogenous (light) LEYDLLVR peptide, calculated by summing the signal intensities of five specific fragment ions: y3, y4, y5, y6, and y7. The analysis compares four sample groups: wild type 'negative' leaf (L3), transgenic 'positive' leaf (L4), wild type 'negative' seed (S1), and transgenic 'positive' seed (S2). Data are presented for three independent replicates (R1, R2, and R3) within each group, demonstrating the specific detection of the OsNAS2-derived peptide in the transgenic lines relative to the wild type controls.

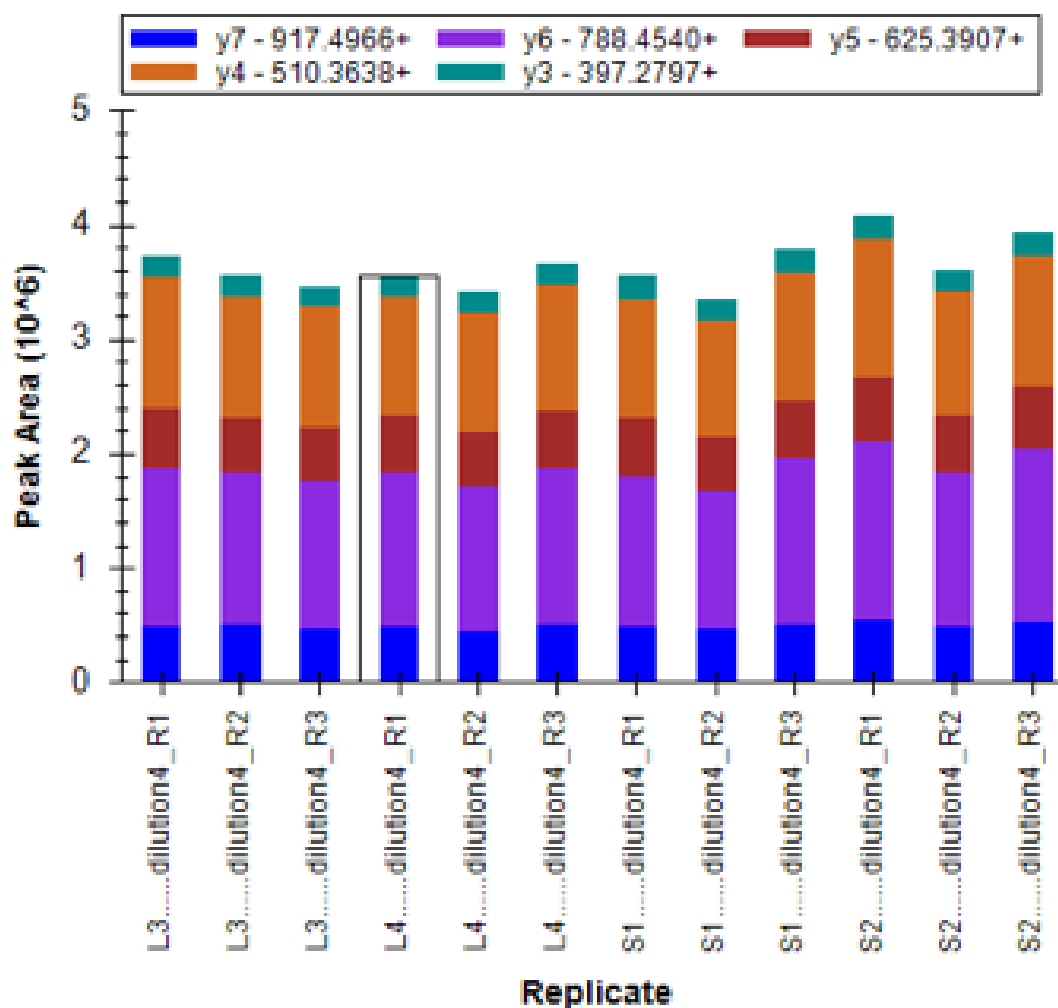


Figure 23. Peak area measurement of the Heavy Isotope Labelled LEYDLLVR[¹³C₆¹⁵N₄] Peptide Standard

This graph presents the summed peak areas for the synthetic heavy peptide standard, LEYDLLVR[¹³C₆¹⁵N₄], which served as the internal reference for absolute quantification. The total area was derived from the same five fragment ions (y3, y4, y5, y6, and y7) monitored for the endogenous peptide, enabling direct comparison. The standard was spiked into all samples, including wild type 'negative' leaf (L3), transgenic 'positive' leaf (L4), wild type 'negative' seed (S1), and transgenic 'positive' seed (S2), across three replicates (R1, R2, and R3).

The site of accumulation of OsNAS2 is therefore best described as low-level accumulation in edible flour and higher accumulation in vegetative tissue. From a food safety and nutritional perspective, the biologically relevant consequence of this expression pattern is not high dietary exposure to the protein itself, but increased nicotianamine synthesis. This finding aligns with the biochemical function of OsNAS2, even though this did not result in increased grain micronutrient concentrations as assessed. This interpretation is consistent with the established function of NAS proteins in cereals and with published evidence that *OsNAS2* expression increases NA biosynthesis and/or Fe and Zn accumulation in grain and flour-relevant tissues (Beasley et al., 2019a; Singh et al., 2017).

HPT and Kan Expression in UM-12115T-1

Expression of HPT and Kan in UM-12115T-1 was assessed using the same LC-MS/MS analytical workflow used for OsNAS2. No HPH or Kan protein was detected in any analysed sample, including edible flour and leaf tissue. Thus, although the *hph* and *nptIII* coding sequences are physically present in the event, the

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marker proteins are not present at measurable levels in the tested tissues under the conditions used. It is important to note that while the *hph* gene was actively driven by a plant promoter for *in vitro* selection during transformation, the *nptIII* (Kan) sequence lacks plant regulatory elements and was solely functional in the bacterial host prior to transformation. Its lack of detection in plant tissues empirically confirms its non-functional status *in planta*.

This result is particularly relevant to the edible portion, as it indicates that dietary exposure to HPT or Kan from food derived from UM-12115T-1 would be negligible. A summary of method performance and the non-detection outcome for HPT and Kan is summarised in Table 7 and sequence identity data relevant to the bridging of published safety information for HPT and Kan is described in detail in *Supplement Report UOM-25-PROT-RPT-02-02*.

Table 7. Method Performance and Peptide Sensitivity

Analyte (Peptide)	Validated On-Column Concentration	Signal-to-Noise (S/N)	Determination Status
OsNAS2 (Labelled Peptide Standard)	725 fg/μL	> 100	Quantifiable (Signal Robust)
HPT and Kan	N/A	N/A	Not Detected (Below assay detection capability)

Additional consideration of other potential newly expressed proteins

In addition to the intended coding sequences, a comprehensive bioinformatic assessment was undertaken for open reading frames associated with the insertion loci (see below Section B.1(d)). This assessment found no biologically meaningful similarity to known allergens, toxins, or celiac disease-associated proteins and did not identify additional proteins of safety concern associated with the insertions (Amnuaycheewa et al., 2022; Goodman et al., 2008, 2016).

As detailed through molecular characterisation of UM-12115T-1, a defined region of vector backbone containing the non-functional *nptIII* gene integrated at Locus 1. Because this sequence lacks functional eukaryotic promoters or terminators, it was not expected to function *in planta*. To empirically verify this lack of function, targeted proteomic analysis was conducted. The NPTIII protein was completely undetectable (below the assay detection capability) in all analysed fresh vegetative leaf tissues and mature grain fractions. This confirms the unintended backbone element is transcriptionally and translationally inert under agronomic conditions.

Conclusion

The new proteins in wheat event UM-12115T-1 are OsNAS2 and HPT. OsNAS2 is the intended trait protein and functions as a nicotianamine synthase within a conserved endogenous plant pathway involved in metal homeostasis. Its expression increases nicotianamine synthesis, consistent with the known biochemical function of the OsNAS2 enzyme, though the compositional characteristics demonstrated for UM-12115T-1 showed no increase in grain micronutrient concentrations. OsNAS2 was detected in the edible flour fraction at low concentration and at higher levels in leaf tissue. HPT is a selectable marker protein used during transformation. Its biochemical function is detoxification of hygromycin B during *in vitro* selection, and it has no intended role in the final food product. Kan is also a selectable marker used for *in vitro* selection of the binary vector in the *Agrobacterium* strain. HPT and Kan were not detected in edible flour or leaf tissue from UM-12115T-1. Collectively, these data demonstrate that the identity, biochemical function, phenotypic effects, expression levels, and sites of accumulation of the new substances in UM-12115T-1 are well characterised.

B.1(b) Information about prior history of human consumption of the new substances, if any, or their similarity to substances previously consumed in food.

The OsNAS2 protein and its enzymatic product, nicotianamine, possess an extensive and well-established history of safe human and animal consumption. Nicotianamine is a naturally occurring non-proteinogenic amino acid present in all higher plants and is consumed routinely as part of a conventional diet. As a central chelating agent for transition metals, NA is required by plants for metal homeostasis, intracellular trafficking, and the long-distance transport of Fe and Zn.

The ubiquity of this substance in the human food supply is well-documented. Staple crops such as soybeans, lentils, and chickpeas are among the foods with the highest NA content, ranging from 31.5 mg/100g to 45 mg/100g (Day, 2020).

Consequently, there is a robust history of likely human and animal dietary exposure to the nicotianamine synthase (NAS) protein class through the consumption of these and many other common plant foods. The biochemical function of OsNAS2 in wheat event UM-12115T-1 is to increase flux through this endogenous and highly conserved metabolic pathway. The introduced enzyme does not create a novel biochemical pathway or produce an unfamiliar class of metabolite; rather, it utilises existing plant chemistry to enhance the mobilisation of micronutrients.

In addition to this historical context, a deterministic dietary exposure assessment was conducted to estimate the anticipated daily intake of the specific introduced OsNAS2 protein by humans and livestock. It is noted that the HPT protein was found to be below the limit of detection, and therefore dietary exposure for humans and animals is considered negligible.

Low Dietary Exposure of OsNAS2 protein to Humans in Australia and New Zealand

Data on the dietary consumption of wheat in Australia and New Zealand vary depending on the source and are difficult to estimate due to the numerous wheat-based products consumed. A comparative analysis of wheat utilisation in 2023 across nine selected countries is presented in Table 8. The dataset details per capita human wheat consumption, the daily protein supply derived from these products, and the total volume of wheat diverted to animal feed.

Table 8. Wheat and Products Consumption for Selected Countries (2023)¹

Region	Wheat Consumption (kg/capita/year)	Protein Supply Quantity (g/capita/day)	Feed (1000t)
Australia	94.5	19.7	6733.0
New Zealand	98.1	23.7	407.0
Argentina	126.8	29.9	56.0
Brazil	54.6	11.7	622.0
China	74.5	22.6	39176.0
Germany	75.2	17.1	8861.0
India	66.5	17.9	6906.0
Indonesia	29.4	6.8	5.0
Pakistan	108.7	31.0	16.0
Russian Federation	136.5	31.7	20076.0
Spain	98.1	20.2	8428.0
Turkey	163.6	37.6	14456.0

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United Kingdom	125.2	24.6	7954.0
United States	88.9	18.1	2903.0

¹ Food and Agriculture Organization of the United Nations Food and Agriculture Organization of the United Nations (FAO), 2026. *FAOSTAT: Food Balances (2010-)*. [online] Rome: FAO. Available at: <https://www.fao.org/faostat/en/#data/FBS> [Accessed 8 Apr. 2026].

The table highlights the Oceania region, demonstrating that Australia records an annual per capita wheat consumption of 94.5 Kg, providing 19.7 g of protein daily, alongside a substantial 6,733.0 thousand tonnes allocated for animal feed. New Zealand exhibits slightly higher human consumption metrics at 98.1 Kg per capita annually and 23.7 g of daily protein but utilises a significantly lower volume for feed at 407.0 thousand tonnes.

Analysing global human consumption patterns within the selected group reveals that the Russian Federation, Argentina, and the United Kingdom lead in both annual per capita intake and daily protein supply, with Russia peaking at 136.5 Kg per person. Conversely, Indonesia and Brazil report the lowest human consumption metrics in the dataset. The allocation of wheat for animal feed varies drastically across jurisdictions, driven by massive utilisation in China at 39,176.0 thousand tonnes and the Russian Federation at 20,076.0 thousand tonnes, while countries such as Indonesia and Argentina report nominal feed usage despite their varying levels of human consumption.

The Australian Bureau of Statistics has estimated the median amount of flour, other cereal, and cereal products as well as cereal-based products and dishes consumed by Australians for various age groups. The largest daily consumption of wheat and wheat-based products are persons and females aged 30-49 (Table 10 and Table 11). In contrast, predominantly more consumption by males was in the 12-17 age range (Table 12). FAO data for New Zealand suggest that consumption of wheat is 3.8 % greater than Australia (Table 8).

A deterministic dietary exposure assessment was conducted to estimate the anticipated daily intake of the introduced OsNAS2 protein by the Australian population following the consumption of UM12115T-1 wheat (Table 9). The assessment utilised minimum and maximum body weights for male and female cohorts derived from the 2022 Australian Bureau of Statistics National Health Survey, specifically targeting the 15–17 and 45–54 age groups to represent the lowest and highest typical adult/adolescent body masses.

To ensure a conservative safety evaluation, exposure was calculated on a per-kilogram body weight basis (g/kg bw/day). This establishes an inverse relationship between body mass and relative consumption, where individuals with lower body weights yield higher relative consumption rates. The concentration of the OsNAS2 protein was established at 0.024 ng/mg of extracted protein. Because natural wheat protein content fluctuates, the final exposure estimates were calculated across an established literature range of 10 to 16 g/100 g total grain protein (OECD, 2003), generating an exposure range for each demographic bracket.

Table 9. Dietary Exposure of Australian People to OsNAS2 from UM-12115T-1 Wheat

Group	Body Weight (Kg) ^{1,2}		Wheat Consumption (g/Kg bw/day) ³		Estimated Exposure to OsNAS2 (ng/Kg bw/day) ⁴	
	Min	Max	Min	Max	Min	Max
Persons	65.4	83.6	3.96	3.10	9.5-15.2	7.4-11.9
Male	70.1	90.9	3.69	2.85	8.9-14.2	6.8-10.9
Female	60.3	76.5	4.29	3.38	10.3-16.5	8.1-13.0

¹Body weights from the Australian Bureau of Statistics. (2022). *National Health Survey*. ABS.

<https://www.abs.gov.au/statistics/health/health-conditions-and-risks/national-health-survey/2022> Accessed 8 April 2026

²Minimum and maximum body weights for males and females from the 15-17- and 45-54-year-old age groups respectively

³Wheat consumption data from Table 8

⁴Estimated exposure to OsNAS2 = (Consumption) x (level of OsNAS2 per gram grain). Level of OsNAS2 based on 0.024ng/mg extracted protein. Values calculated based on literature range for protein content in wheat 10-16g/100g (OECD 2003)

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The data demonstrates that dietary exposure to the OsNAS2 protein across all demographics is extremely low, falling strictly within the low nanogram per kilogram of body weight per day (ng/kg bw/day) range (Table 9). For the general population (Persons), the estimated dietary exposure spans from a minimum of 7.4 ng/kg bw/day to a maximum of 15.2 ng/kg bw/day across all body weight and protein variance scenarios.

In dietary risk assessments, the demographic exhibiting the highest relative exposure represents the worst-case scenario. According to the data, females at the minimum body weight (60.3 kg) represent this cohort. Due to their lower body mass, this group exhibits the highest relative wheat consumption at 4.29 g/kg bw/day. Assuming the upper-bound wheat protein content of 16 %, the maximum potential exposure to OsNAS2 for this demographic is 16.5 ng/kg bw/day. Conversely, the lowest estimated exposure is observed in males at the maximum body weight (90.9 kg), yielding an exposure range of 6.8 to 10.9 ng/kg bw/day due to their lower relative dietary consumption (2.85 g/kg bw/day).

Similarly, a deterministic dietary exposure assessment was conducted to estimate the anticipated daily intake of the introduced OsNAS2 protein by the New Zealand population following the consumption of UM12115T-1 wheat. To capture the demographic diversity of New Zealand, the assessment utilised median, low, and high body weight estimates stratified by gender and key ethnic groups (Māori, Pacific Peoples, Asian, and European). Anthropometric data was derived from the New Zealand Ministry of Health's Annual Data Explorer 2024/25.

The data indicates that dietary exposure to the OsNAS2 protein across all New Zealand demographic groups is exceptionally low, remaining strictly within the low nanogram per kilogram of body weight per day (ng/kg bw/day) range (Table 13). For the general baseline population (Total at median body weight of 82.3 kg), the estimated dietary exposure spans from 7.8 to 12.5 ng/kg bw/day.

In dietary risk characterisation, identifying the demographic with the highest relative exposure establishes the worst-case safety threshold. According to the dataset, Asian females in the Low body weight bracket (62.5 kg) represent this cohort. Due to their lower body mass, this group exhibits the highest relative wheat consumption at 4.3 g/kg bw/day. Assuming the upper-bound wheat protein content of 16 %, the maximum potential exposure to OsNAS2 for this specific demographic peaks at 16.5 ng/kg bw/day.

Conversely, the lowest estimated exposure is observed in Pacific Peoples males in the High body weight bracket (110.0 kg). Their larger body mass results in the lowest relative dietary consumption (2.4 g/kg bw/day), yielding an exposure range of 5.9 to 9.4 ng/kg bw/day. Across all ethnic groups, males generally exhibited lower relative exposures than females due to higher average body weights offsetting their absolute consumption.

The exposure estimates for Australia and New Zealand represent a highly conservative, upper-bound scenario. The model inherently assumes that 100 % of the wheat consumed by the target population is sourced from the UM12115T-1 event and does not account for the likely degradation, denaturation, or loss of the OsNAS2 protein during milling, thermal food processing, or mammalian digestion. Even under these absolute worst-case assumptions, the maximum anticipated dietary exposure of 16.5 ng/kg bw/day (in both Australia and New Zealand) is de minimis. These exposure levels are orders of magnitude below thresholds of toxicological concern, supporting the conclusion that the introduction of the OsNAS2 protein in UM12115T-1 wheat presents a negligible dietary risk to the Australian and New Zealand populations.

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Table 10. Median consumption of wheat-based foods by persons in Australia¹

Category (grams/day)	Age Group (Persons in Years)										
	2-4	5-11	12-17	18-29	30-49	50-64	65-74	75+	2-17	18+	Total 2 years and over
Cereals and cereal products	116.0	140.2	149.2	149.4	152.1	135.3	132.7	124.5	139.3	142.8	142.1
Flours, rice and other cereal grains and starches	26.4	31.2	44.8	64.0	62.0	41.6	29.2	20.0	35.4	50.0	47.1
Regular breads, and bread rolls (plain, unfilled and untopped varieties)	29.2	36.5	35.2	28.4	32.5	37.4	40.9	45.2	34.7	34.9	34.9
English-style muffins, flat breads, and savoury and sweet breads	14.5	17.6	16.5	15.2	17.3	8.9	8.8	6.3	16.7	12.9	13.7
Pasta and pasta products (without sauce)	15.6	25.7	24.2	22.7	16.9	17.2	13.4	7.7	23.4	16.9	18.2
Breakfast cereals, ready to eat	15.4	21.0	23.2	7.7	11.6	15.2	18.4	22.9	20.9	13.5	15.0
Breakfast cereals, hot porridge style	15.1	8.2	*5.3	11.5	11.7	15.1	22.1	22.3	8.3	14.6	13.4
Cereal based products and dishes	149.2	214.6	318.7	287.5	242.6	206.2	163.4	147.3	242.3	225.2	228.6
Sweet biscuits	12.6	14.3	11.6	7.8	10.8	10.0	11.6	16.3	13.0	10.6	11.1
Savoury biscuits	7.6	7.2	5.9	2.9	3.0	3.0	3.8	3.1	6.8	3.1	3.8
Cakes, muffins, scones, cake-type desserts	8.4	13.9	17.0	9.6	10.0	11.7	12.0	13.0	14.1	10.8	11.5
Pastries	*4.9	15.6	30.9	26.1	25.0	28.2	21.9	23.8	19.5	25.5	24.3
Mixed dishes where cereal is the major ingredient	109.8	154.8	243.2	236.2	188.7	149.7	110.2	86.0	180.1	170.6	172.5
Batter-based products	5.9	8.8	10.1	4.9	5.0	3.6	*3.9	5.1	8.8	4.6	5.4

1: Data from Australian Bureau of Statistics (2025)-National Nutrition and Physical Activity Survey: Food and Nutrients, 2023

* estimate has a relative standard error of 25 % to 50 % and should be used with caution.

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Table 11. Median consumption of wheat-based foods by females in Australia¹

Category (grams/day)	Female Age Group (Years)										
	2-4	5-11	12-17	18-29	30-49	50-64	65-74	75+	2-17	18+	Total 2 years and over
Cereals and cereal products	118.8	127.2	117.0	125.9	137.9	120.3	123.2	111.1	121.9	127.1	126.1
Flours, rice and other cereal grains and starches	26.6	25.0	32.7	47.2	55.8	36.9	32.8	20.1	28.2	43.5	40.6
Regular breads, and bread rolls (plain, unfilled and untopped varieties)	29.8	37.1	32.0	25.3	31.0	32.2	34.7	41.9	33.9	31.7	32.1
English-style muffins, flat breads, and savoury and sweet breads	*15.0	17.5	12.8	14.1	14.3	7.8	9.0	7.3	15.3	11.4	12.2
Pasta and pasta products (without sauce)	11.6	22.9	19.7	17.1	15.3	15.4	10.4	*5.5	19.7	14.1	15.2
Breakfast cereals, ready to eat	16.3	16.7	12.5	7.8	10.3	12.1	13.8	19.2	15.1	11.5	12.2
Breakfast cereals, hot porridge style	*19.6	7.9	*7.2	*14.6	11.2	15.8	22.4	17.2	9.7	14.8	13.8
Cereal based products and dishes	123.8	207.5	268.0	234.8	205.0	169.7	147.4	125.1	215.6	187.9	193.2
Sweet biscuits	8.0	11.5	10.5	8.1	11.4	8.0	10.0	15.3	10.5	10.2	10.3
Savoury biscuits	6.4	7.2	4.7	3.3	3.4	3.0	3.5	3.1	6.2	3.3	3.8
Cakes, muffins, scones, cake-type desserts	*5.9	14.9	15.7	8.9	10.9	11.8	10.3	*13.1	13.6	10.9	11.4
Pastries	*5.2	12.7	27.1	19.3	18.6	23.6	19.2	19.6	16.8	20.1	19.4
Mixed dishes where cereal is the major ingredient	93.0	154.1	203.7	190.6	155.4	119.3	101.6	68.0	162.1	138.9	143.3
Batter-based products	*5.3	7.1	*6.3	*4.6	5.2	4.1	2.8	5.8	6.4	4.6	4.9

1: Data from Australian Bureau of Statistics (2025)-National Nutrition and Physical Activity Survey: Food and Nutrients, 2023

* estimate has a relative standard error of 25 % to 50 % and should be used with caution.

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Table 12. Median consumption of wheat-based foods by males in Australia¹

Category (grams/day)	Male Age Group (Years)										
	2-4	5-11	12-17	18-29	30-49	50-64	65-74	75+	2-17	18+	Total 2 years and over
Cereals and cereal products	113.4	152.4	180.1	171.6	165.8	151.1	143.0	140.0	155.9	158.8	158.2
Flours, rice and other cereal grains and starches	26.2	36.9	56.3	79.9	68.1	46.6	25.3	19.9	42.3	56.5	53.6
Regular breads, and bread rolls (plain, unfilled and untopped varieties)	28.6	35.9	38.2	31.3	34.1	42.8	47.5	49.2	35.5	38.4	37.8
English-style muffins, flat breads, and savoury and sweet breads	14.0	17.7	20.1	16.3	20.6	10.1	*8.6	5.2	18.0	14.5	15.2
Pasta and pasta products (without sauce)	19.3	28.4	28.5	28.0	18.6	19.0	*16.6	*10.3	26.8	19.7	21.2
Breakfast cereals, ready to eat	14.5	25.0	33.4	7.5	13.1	18.4	23.3	27.2	26.3	15.5	17.8
Breakfast cereals, hot porridge style	*10.8	8.4	*3.6	*8.6	11.3	14.3	21.8	28.3	7.0	14.1	12.6
Cereal based products and dishes	173.1	221.4	367.2	337.3	282.2	244.5	180.6	173.2	267.5	264.0	264.7
Sweet biscuits	*16.8	17.0	12.7	7.4	10.3	12.1	13.3	17.4	15.3	11.1	12.0
Savoury biscuits	8.6	7.2	*7.1	*2.6	2.6	3.0	4.2	3.1	7.4	2.9	3.8
Cakes, muffins, scones, cake-type desserts	10.8	12.9	18.3	*10.2	9.1	11.6	13.9	12.8	14.6	10.8	11.6
Pastries	*4.6	18.3	34.6	32.5	31.8	33.0	24.9	28.7	22.0	31.2	29.3
Mixed dishes where cereal is the major ingredient	125.7	155.4	280.8	279.3	223.5	181.7	119.4	106.9	197.2	203.5	202.2
Batter-based products	*6.5	10.5	*13.7	*5.2	4.9	*3.1	*5.1	*4.4	11.0	4.5	5.9

1: Data from Australian Bureau of Statistics (2025)-National Nutrition and Physical Activity Survey: Food and Nutrients, 2023

* estimate has a relative standard error of 25 % to 50 % and should be used with caution.

Table 13. Dietary Exposure of New Zealand people to OsNAS2 from UM-12115T-1 wheat

Group	Body Weights (Kg) ¹			Wheat Consumption (g/Kg bw/day) ²			Estimated Exposure to OsNAS2 (ng/Kg bw/day) ³		
	Median	Low	High	Median	Low	High	Median	Low	High
Total	82.3	81.7	82.8	3.3	3.3	3.2	7.8-12.5	7.9-12.6	7.8-12.5
Males									
Total	88.3	87.4	89.1	3.0	3.1	3.0	7.3-11.7	7.4-11.8	7.2-11.6
Māori	94	91.6	96.3	2.9	2.9	2.8	6.9-11.0	7.0-11.3	6.7-10.7
Pacific Peoples	105.1	100.1	110	2.6	2.7	2.4	6.1-9.8	6.4-10.3	5.9-9.4
Asian	77.3	75.4	79.3	3.5	3.6	3.4	8.3-13.4	8.6-13.7	8.1-13.0
European	89.2	88.2	90.1	3.0	3.0	3.0	7.2-11.6	7.3-11.7	7.2-11.5
Females									
Total	76.4	75.7	77.2	3.5	3.6	3.5	8.4-13.5	8.5-13.6	8.4-13.4
Māori	85	83.1	86.9	3.2	3.2	3.1	7.6-12.1	7.8-12.4	7.4-11.9
Pacific Peoples	99.1	95.1	103	2.7	2.8	2.6	6.5-10.4	6.8-10.9	6.3-10.0
Asian	64.2	62.5	65.8	4.2	4.3	4.1	10.0-16.1	10.3-16.5	9.8-15.7
European	76.3	75.5	77.1	3.5	3.6	3.5	8.5-13.5	8.5-13.7	8.4-13.4

¹Groups and body weights from Ministry of Health. 2025. Annual Data Explorer 2024/25: New Zealand Health Survey [Data File]. URL: <https://minhealthnz.shinyapps.io/nz-health-survey-2024-25-annual-data-explorer/> Accessed 8 April 2026

²Wheat consumption data from Table 8

⁴Estimated exposure to OsNAS2 = (Consumption) x (level of OsNAS2 per gram grain). Level of OsNAS2 based on 0.024ng/mg extracted protein. Values calculated based on literature range for protein content in wheat 10-16g/100g (OECD 2003)

Dietary Exposure of Ruminant Livestock to OsNAS2

Grain, including wheat grain, is commonly used as an energy-dense and cost-effective supplement in cattle diets in Australia. Because wheat starch is rapidly fermented in the rumen, wheat grain should be introduced gradually to reduce the risk of ruminal pH depression and acidosis. In an Australian study of late-lactation dairy cows, wheat was progressively substituted for forage during the adaptation period until wheat reached approximately 8 kg dry matter (DM) per cow per day, and the study confirmed that wheat adaptation strategy influenced ruminal responses associated with acidosis risk (Russo et al., 2018). In Australian Holstein–Friesian dairy cows, feeding 6 kg DM/day of crushed wheat grain has been reported in cows with a mean bodyweight of 558 ± 37 kg, which is consistent with the use of about 6 kg/day in lactating cows weighing approximately 550–600 kg (Garner et al., 2022). Higher wheat grain intakes have also been used experimentally in Australian grazing dairy systems; for example, a partial mixed ration containing 10.5 kg DM wheat grain per cow per day has been reported, although this feeding level was noted to be greater than would typically be used commercially (Auld et al., 2014). For the present dietary exposure assessment, a crude protein concentration of 15 % on a DM basis was assumed for cattle feed consisting solely of wheat grain from UM12115T-1, together with an average cattle bodyweight of 580 kg. Published Australian data from a comparable dairy feeding study reported crude protein in wheat grain of 136 g/kg DM, equivalent to 13.6 % DM, indicating that the 15 % assumption is slightly conservative relative to at least one peer-reviewed Australian dataset (Garner et al., 2022).

Peer-reviewed Australia/New Zealand literature directly reporting cattle consumption of fresh wheat forage is more limited than the literature for wheat grain. However, Australian work on the nutritive value of cereal forages indicates that wheat forage could meet the metabolizable protein requirements of high-producing dairy cows if intake reached approximately 11 kg DM/cow/day, providing a useful upper reference point for forage intake potential under favourable conditions (Fulkerson et al., 2008). In New Zealand, whole-crop wheat silage has been proposed as a practical forage supplement at 5 kg DM/cow/day in pasture-based dairy systems, although this value comes from a review-based feeding strategy rather than a direct intake trial of wheat forage alone (Wilkinson and Waldron, 2017). Additional New Zealand field data on whole-crop cereal silages, which included cereal silages such as wheat, barley, or triticale, showed supplementation rates ranging from 3.0 to 6.6 kg DM/cow/day, supporting the practical use of cereal forage supplements in dairy systems, albeit not as wheat-only intake measurements (Stevens et al., 2004). Taken together, these data

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support the view that wheat may contribute materially to both grain- and forage-based cattle diets, but they also indicate that the evidence base is stronger for wheat grain feeding rates than for direct measurements of wheat forage intake.

Estimated dietary exposure of cattle to OsNAS2 following consumption of feed derived from wheat event UM-12115T-1 is presented in Table 14. The assessment was based on an assumed average cattle body weight of 580 kg and considered two representative feed sources: wheat grain for lactating dairy cows and fresh wheat forage for high-producing lactating dairy cows. Wheat consumption was assumed to be 6.0 kg/day for grain and 11.0 kg/day for fresh forage. Using crude protein concentrations of 15 % dry matter for wheat grain and 11 % dry matter for fresh wheat forage (Heuzé et al., 2015), the corresponding daily protein intakes were estimated to be 1.55 and 2.09 g/kg body weight/day, respectively. Based on measured OsNAS2 concentrations of 24 ng/g in wheat grain and 319 ng/g in fresh wheat forage, estimated dietary exposure to OsNAS2 was 0.248 µg/kg body weight/day for grain and 6.05 µg/kg body weight/day for fresh forage. When expressed relative to total daily protein intake, OsNAS2 represented an extremely small proportion of protein consumed, accounting for 0.000016 % of daily protein intake for wheat grain and 0.00029 % for fresh wheat forage. Collectively, these data indicate that dietary exposure of cattle to OsNAS2 from feed derived from UM-12115T-1 would be very low under the assessed feeding scenarios.

Table 14. Dietary Exposure Assessment of OsNAS2 for cattle

Protein Source	Production Stage	Average weight (kg)	Wheat Consumption (kg/d)	Daily Protein Consumption (g/kg bw/d) ^{ab}	Exposure to OsNAS2 (µg/Kg bw/d) ^c	Percentage of Daily Protein Consumed that is OsNAS2 ^d
Wheat grain	Lactating dairy cow	580	6.0	1.55	0.248	0.000016%
Wheat forage (fresh)	High-producing lactating dairy cow	580	11.0	2.09	6.05	0.00029%

^aDaily protein consumption (g/kg bw/day) was calculated as: wheat consumption (kg/day) × crude protein concentration × 1000 / body weight (kg).

^bA crude protein concentration of 15 % dry matter was assumed for wheat grain and a crude protein concentration of 11 % dry matter was assumed for fresh wheat forage.

^cExposure to OsNAS2 (µg/kg bw/day) was calculated as: OsNAS2 concentration in feed (ng/g) × feed consumption (g/day) / 1000 / body weight (kg). OsNAS2 concentrations of 24 ng/g in wheat grain and 319 ng/g in fresh wheat forage were used.

^dPercentage of daily protein consumed that is OsNAS2 was calculated as: daily OsNAS2 intake / daily total protein intake × 100, after conversion to a common mass basis.

Dietary Exposure of Monogastric Livestock to OsNAS2

Screening-level estimates of dietary exposure to OsNAS2 in pigs following consumption of wheat grain derived from wheat event UM-12115T-1 is presented in Table 15. Wheat forage was not included for the conventional pig production stages assessed because pigs are generally fed grain-based rations, although pasture or forage may contribute in some outdoor or heritage systems (NSW Government, 2025). Representative body weights of 8 kg for weaner piglets, 87.5 kg for grower/finisher pigs, 250 kg for dry sows, and 225 kg for lactating sows were used to reflect the production-stage ranges identified in the source guidance (Australian Pork Limited, 2017; Moore, 2020; NRM, 2023).

Corresponding wheat grain consumption values of 0.276, 2.6, 2.5, and 7.0 kg/day were selected as midpoint or representative intake values from the cited feeding guidance for each class of pig (Australian Pork Limited, 2017; Moore, 2020; NRM, 2023; NSW Government, 2025). Using an assumed crude protein concentration of 15 % dry matter for wheat grain and an OsNAS2 concentration of 24 ng/g in grain, estimated daily protein consumption ranged from 1.50 to 5.17 g/kg body weight/day, and estimated dietary exposure to OsNAS2 ranged from 0.240 to 0.827 µg/kg body weight/day across the pig classes assessed. When expressed relative to total daily protein intake, OsNAS2 represented an extremely small proportion of protein consumed, at

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approximately 0.000016 % in each case. Collectively, these calculations indicate that dietary exposure of pigs to OsNAS2 from wheat grain derived from UM-12115T-1 would be very low under the assessed feeding scenarios.

Table 15. Dietary Exposure Assessment of OsNAS2 from Wheat grain for Swine

Production Stage	Average weight (kg)	Wheat Consumption (kg/d)	Daily Protein Consumption (g/kg bw/d) ^{ab}	Exposure to OsNAS2 (µg/kg bw/d) ^c	Percentage of Daily Protein Consumed that is OsNAS2 ^d
Weaner piglet	8.0	0.276	5.17	0.827	0.000016 %
Growing/finisher pig	87.5	2.6	4.46	0.713	0.000016 %
Dry sow (gestation)	250	2.5	1.50	0.240	0.000016 %
Lactating sow	225	7.0	4.67	0.747	0.000016 %

^aDaily protein consumption (g/kg bw/day) was calculated as: wheat consumption (kg/day) × crude protein concentration × 1000 / body weight (kg).

^bA crude protein concentration of 15 % dry matter was assumed for wheat grain

^cExposure to OsNAS2 (µg/kg bw/day) was calculated as: OsNAS2 concentration in feed (ng/g) × feed consumption (g/day) / 1000 / body weight (kg). OsNAS2 concentrations of 24 ng/g in wheat grain and 319 ng/g in fresh wheat forage were used.

^dPercentage of daily protein consumed that is OsNAS2 was calculated as: daily OsNAS2 intake / daily total protein intake × 100, after conversion to a common mass basis.

Dietary Exposure of Poultry to OsNAS2

Screening-level estimates of dietary exposure to OsNAS2 in poultry following consumption of wheat grain derived from wheat event UM-12115T-1 is presented in Table 16. Wheat forage was not included for the poultry production stages assessed because poultry are generally fed complete grain-based rations rather than forage-based diets. Representative body weights of 0.40 kg for broiler chickens at 11–14 days, 3.30 kg for broiler chickens at 35–42 days, 0.615 kg for growing pullets, and 1.75 kg for adult laying hens were used to reflect the live-weight ranges identified in the source guidance. Corresponding wheat grain consumption values of 0.0725, 0.1865, 0.056, and 0.110 kg/day were selected as midpoint or representative intake values from the cited feeding information for each class of poultry (Moss 2022; Poultry Hub Australia, n.d.). Using an assumed crude protein concentration of 15 % dry matter for wheat grain and an OsNAS2 concentration of 24 ng/g in grain, estimated daily protein consumption ranged from 8.48 to 27.19 g/kg body weight/day, and estimated dietary exposure to OsNAS2 ranged from 1.36 to 4.35 µg/kg body weight/day across the poultry classes assessed. When expressed relative to total daily protein intake, OsNAS2 represented an extremely small proportion of protein consumed, at approximately 0.000016 % in each case. Collectively, these calculations indicate that dietary exposure of poultry to OsNAS2 from wheat grain derived from UM-12115T-1 would be very low under the assessed feeding scenarios.

Summary of Dietary Estimates for OsNAS2 in Animal Feed

Overall, the dietary exposure assessment indicates that exposure of animals to OsNAS2 from feed derived from wheat event UM-12115T-1 would be very low under the representative feeding scenarios assessed for cattle, pigs and poultry. Across grain-based diets, estimated exposure ranged from 0.240 to 4.35 µg/kg body weight/day, and OsNAS2 represented only 0.000016 % of total daily protein intake. In cattle consuming fresh wheat forage, estimated exposure was higher at 6.05 µg/kg body weight/day because of the higher measured OsNAS2 concentration in forage and the lower assumed crude protein concentration relative to grain; however, even in this scenario OsNAS2 accounted for only 0.00029 % of daily protein intake. Taken together, these data demonstrate that OsNAS2 would contribute only a negligible proportion of total dietary protein intake in animals consuming feed derived from UM-12115T-1.

Table 16. Dietary Exposure Assessment of OsNAS2 from Wheat grain for Poultry

Production Stage	Average weight (kg)	Wheat Consumption (kg/d)	Daily Protein Consumption (g/kg bw/d) ^{ab}	Exposure to OsNAS2 (µg/kg bw/d) ^c	Percentage of Daily Protein Consumed that is OsNAS2 ^d
Broiler chicken (starter, 11–14 days)	0.40	0.0725	27.19	4.35	0.000016 %
Broiler chicken (finisher, 35–42 days)	3.30	0.1865	8.48	1.36	0.000016 %
Laying hen (growing pullet)	0.615	0.056	13.66	2.19	0.000016 %
Laying hen (adult/peak lay)	1.75	0.110	9.43	1.51	0.000016 %

^aDaily protein consumption (g/kg bw/day) was calculated as: wheat consumption (kg/day) × crude protein concentration × 1000 / body weight (kg).

^bA crude protein concentration of 15 % dry matter was assumed for wheat grain

^cExposure to OsNAS2 (µg/kg bw/day) was calculated as: OsNAS2 concentration in feed (ng/g) × feed consumption (g/day) / 1000 / body weight (kg). OsNAS2 concentrations of 24 ng/g in wheat grain and 319 ng/g in fresh wheat forage were used.

^dPercentage of daily protein consumed that is OsNAS2 was calculated as: daily OsNAS2 intake / daily total protein intake × 100, after conversion to a common mass basis.

B.1(c) information on whether any new protein has undergone any unexpected post-translational modification in the new host

The subcellular localisation and potential for post-translational modification of the OsNAS2 protein were evaluated using a suite of six independent bioinformatic algorithms. The database outputs are provided in *Supplement Report UOM-25-PROT-RPT-02-01* (summarised in Figure 24).

For the proteins sequences introduced in UM-12115T-1, the analyses supported the absence of secretory pathway targeting and indicated localisation within the intracellular soluble compartment. Although isolated theoretical glycosylation motifs were identified by sequence-based prediction, these motifs are not considered biologically relevant *in vivo* because the proteins are not predicted to enter the endoplasmic reticulum or Golgi apparatus, where the major plant glycosylation processes occur. Accordingly, OsNAS2, HPT or the non-functional Kan are expected to undergo unexpected post-translational modification in the wheat host.

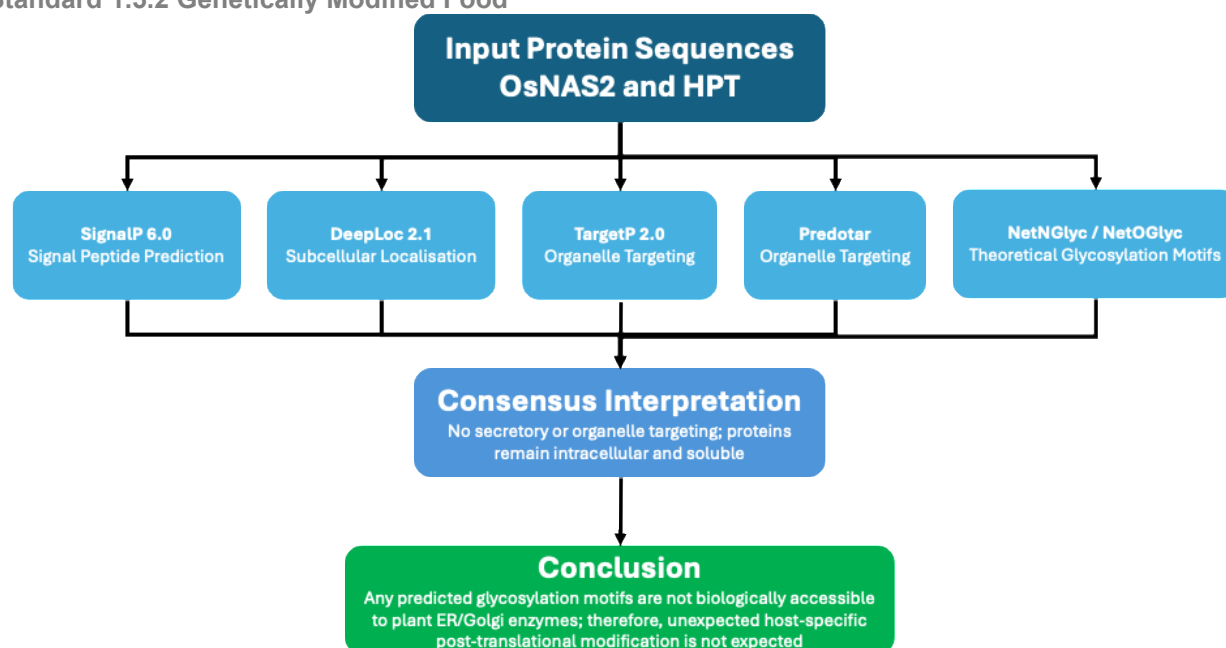


Figure 24. Weight-of-evidence workflow used to assess whether OsNAS2, HPT undergo unexpected post-translational modification in wheat.

This figure outlines the bioinformatic framework used to assess the potential for host-specific post-translational modification of OsNAS2, HPT in wheat. The assessment integrated prediction of signal peptides, subcellular localisation, organelle-targeting signals, and theoretical glycosylation motifs using multiple independent algorithms. The combined evidence supported a consistent conclusion that both proteins lack trafficking signals required for entry into the secretory pathway or import into organelles and therefore are not expected to be exposed to the cellular machinery responsible for glycosylation or other compartment-dependent post-translational modifications in wheat.

B.1(d) where any ORFs have been identified (in subparagraph A.3(c)(v) of this Guideline (3.5.1)), bioinformatics analyses to indicate the potential for allergenicity and toxicity of the ORFs

A comprehensive bioinformatic assessment was undertaken to evaluate whether any putative open reading frames (ORFs) associated with event UM-12115T-1 encode peptides or proteins with similarity to known allergens or toxins (see *Supplement Report UOM-25-PROT-RPT-01-02*). Two genomic integration sites were identified in the event, and ORF analysis was conducted across all six reading frames spanning each insertion and its flanking genomic regions using a minimum threshold of 30 amino acids from the first ATG codon to the next in-frame stop codon. This analysis identified 94 unique ORFs in total, comprising 53 ORFs associated with insertion site 1 on chromosome 1D and 41 ORFs associated with insertion site 2 on chromosome 4D. The location, orientation and distribution of these ORFs at the two integration loci are shown in Figure 25 and Figure 26.

The predicted amino acid sequences of all 94 ORFs were assessed using a weight-of-evidence approach that incorporated NCBI BLASTP searches, full-length allergen homology assessment, 80-amino-acid sliding window analysis, 8-amino-acid exact match analysis, machine learning-based allergenicity prediction, and BLASTP analysis against a curated toxin database. The allergenicity assessment used AllergenOnline version 24 and a full-length FASTA36 allergen reference set, while toxin similarity was assessed using a curated local BLAST database built from canonical UniProtKB entries annotated with the keyword 'Toxin (KW-0800)'. These approaches were selected to provide both sensitivity and specificity for detecting biologically meaningful similarity to proteins of toxicological or allergenic concern.

No significant full-length sequence homology to known allergens was identified for any ORF. Limited local matches were observed in the 80-amino-acid sliding window analysis for one ORF at insertion site 1 and the identical ORF at insertion site 2, with alignments to collagens from fish and cattle and to some plant storage proteins, including Arah1. However, these alignments were confined to short regions and the corresponding

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full-length alignments showed only 25–29 % identity with moderate to weak E-values, indicating that they did not represent biologically meaningful structural or immunological homology. Likewise, isolated 8-amino-acid exact matches were detected, including a single GLAGARGA motif match to bovine collagen and several low-complexity acidic tract matches at insertion site 2, but these were not supported by full-length or 80-mer evidence and were therefore interpreted as chance or artefactual matches rather than indicators of allergenic cross-reactivity. A summary of the allergenicity assessment is provided in (Table 17)

Machine learning-based allergenicity prediction using NetAllergen-1.0 was also applied to all ORFs (Li et al., 2023). Although the physicochemical module flagged a subset of ORFs as potential allergens, the homology screening component classified all ORFs as non-allergens. The report concluded that these positive calls were computational false positives because they lacked supporting sequence homology, were associated with only borderline prediction scores, and included erroneous classification of the well-characterised *nptII* protein as a putative allergen. On this basis, the NetAllergen outputs did not alter the conclusion drawn from the sequence-based analyses that the identified ORFs do not indicate allergenic risk.

For toxicity assessment, all 94 ORFs were translated *in silico* and queried against the curated UniProt toxin database using BLASTP with standard parameters, including the BLOSUM62 substitution matrix, an E-value threshold of 1×10^{-5} , and low-complexity filtering. No statistically significant sequence similarities were identified between any ORF and any toxin sequence in the curated database, and no alignments indicative of toxin-like motifs were observed. These findings indicate that the ORFs associated with the two genomic insertions do not encode proteins with similarity to known toxins and therefore do not raise toxicological concern.

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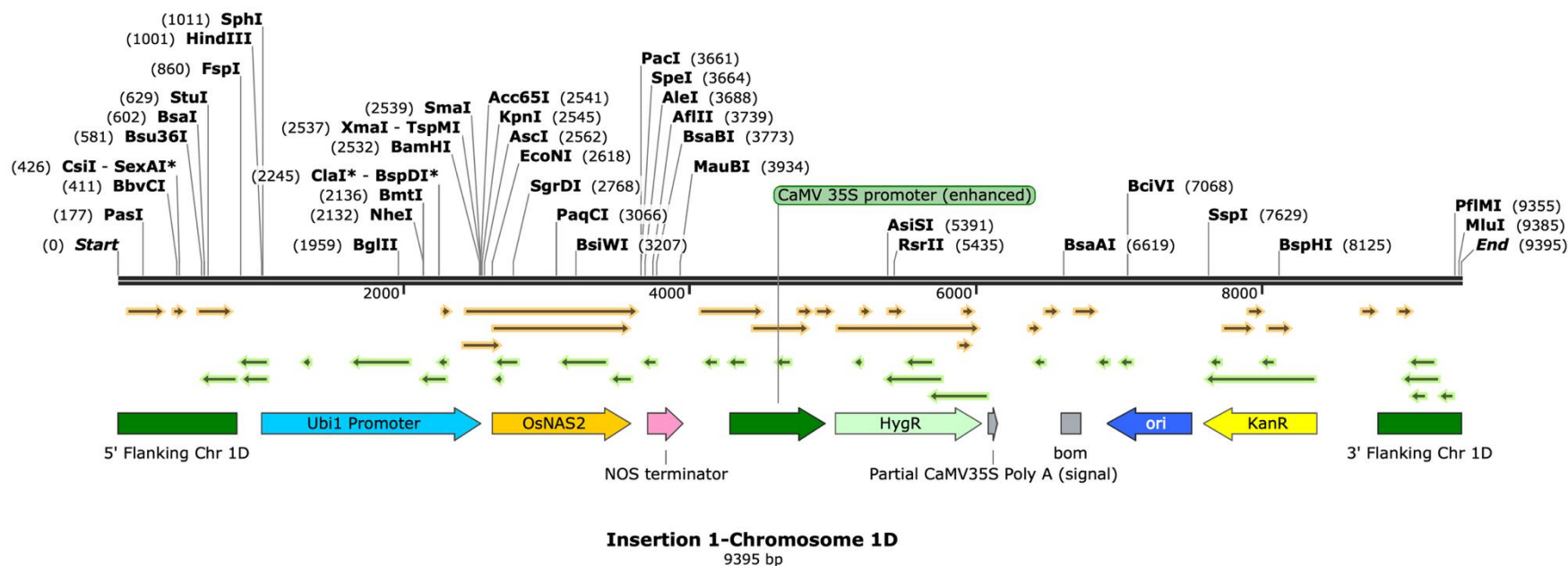


Figure 25. ORF analysis of Site 1.

Open reading frame analysis of the genomic region surrounding Site 1 on chromosome 1D. The analysis identifies all potential ORFs (6 reading frames), including their orientation and position, to assess any unintended insertional effects or potential for novel protein expression.

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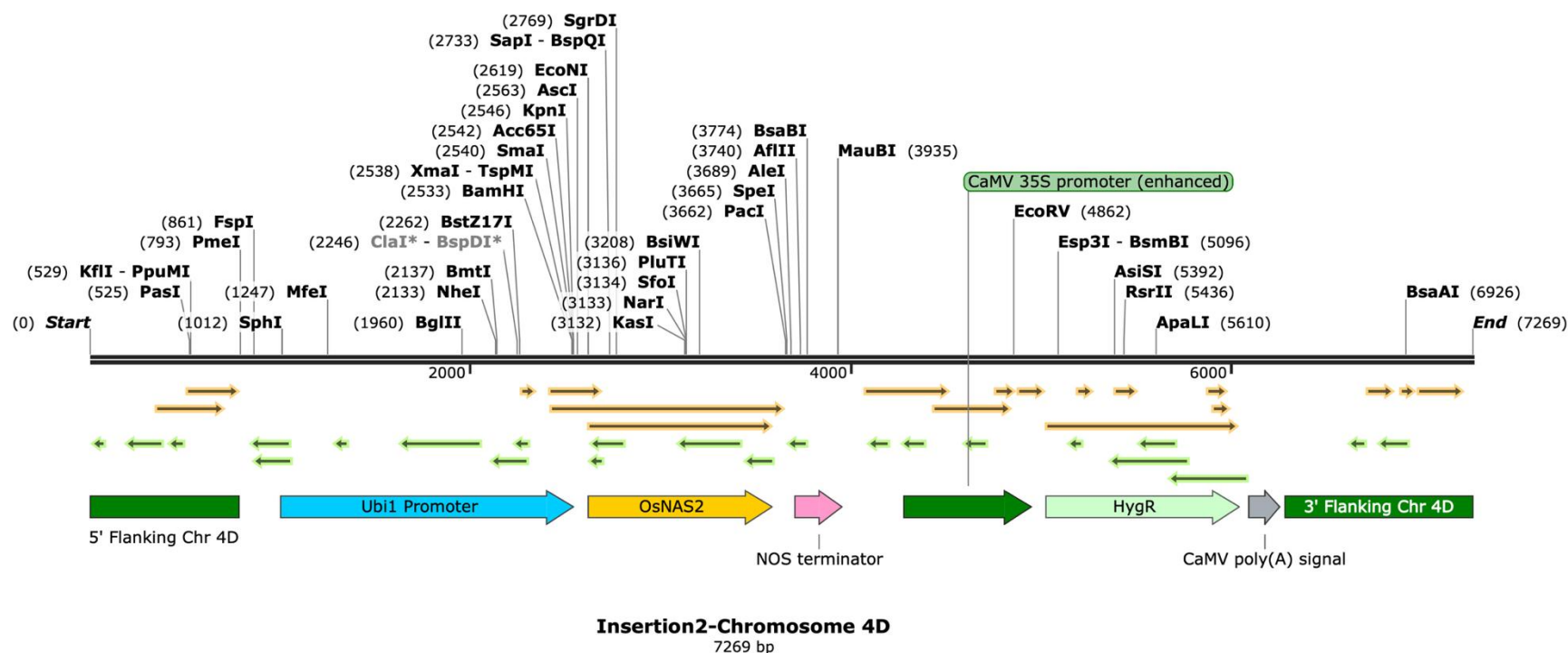


Figure 26. ORF analysis of Site 2.

Open reading frame analysis of the genomic region surrounding Site 2 on chromosome 4D. The analysis identifies all potential ORFs (6 reading frames), including their orientation and position, to assess any unintended insertional effects or potential for novel protein expression.

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Table 17. Summary of bioinformatic allergenicity assessment for Insertion Sites 1 and 2

Criterion	Database/Method	Result	Interpretation
Full-length sequence homology	FASTA 36 allergen reference set	No significant alignments detected	Confirms absence of meaningful sequence homology to known allergens
80-amino acid sliding window (>35% identity)	AllergenOnline v24 (accessed 20 Apr 2026)	Local alignments >35 % to collagens (fish, cattle) and plant storage proteins (<i>Ara h 1</i> , <i>Oryza sativa</i> , <i>Lupinus angustifolius</i>). Full-length identity only 25–29 % with moderate E-values (0.0002–9.6).	Threshold formally triggered, but alignments represent weak similarity over limited regions, not biologically relevant cross-reactivity
8-amino acid exact match	AllergenOnline v24 (accessed 20 Apr 2026)	One 8-mer match (GLAGARGA) between ORF7 (insert 1) and ORF6 (insert 2) and collagen alpha-2(I) chain precursor (<i>Bos taurus</i>). Occurred once in 319 8-mers tested. Seven 8-mers (ORF2, insert 2) matching conglutin β (<i>Lupinus</i>) and glycinin-like (<i>Glycine max</i>), all motifs either EEEEEEEE or EEEEEED.	Collagen is a ubiquitous structural protein and not a recognised food allergen. The isolated glycine-rich motif match is consistent with chance similarity and not indicative of allergenic potential. Poly-glutamate motifs are low-complexity tracts found widely in proteins; no supporting 80-mer or full-length homology; part of host genome; interpreted as artefactual.
Machine Learning Prediction	NetAllergen-1.0 (Random Forest + MHC-II propensity)	Physicochemical flags (Class_60F = 1) observed for 34 ORFs (e.g., ORFs 2, 15, 29). However, homology screening consistently returned 'Non-Allergen' (Class_BLAST = 0). Critically, the tool erroneously flagged the known safe protein <i>nptII</i> (ORF 29) as a Class 1 allergen.	The 'Class 1' flags are confirmed computational false positives due to model over-sensitivity. The lack of epitope homology and the erroneous flagging of a safe control protein confirm no allergenic risk.
Celiac-toxic motifs	AllergenOnline Celiac Disease FASTA3 tool (accessed 20 Apr 2026)	No significant alignments detected	Confirms absence of meaningful sequence homology to known celiac toxic motifs

Overall, the bioinformatic assessment provides no evidence that any of the ORFs identified in event UM-12115T-1 encode proteins with biologically relevant similarity to known allergens or toxins. The isolated local similarities observed in some allergen screening outputs were weak, restricted to short regions, unsupported by full-length homology, and consistent with chance or low-complexity matches. No toxin-like sequence similarity was detected under the defined search criteria. Taken together, these data support the conclusion that the identified ORFs do not present a plausible allergenicity or toxicity concern relevant to food safety assessment.

B.2. New Proteins**B.2 (a) Information on potential toxicity**

Details of the potential toxicity of the protein OsNAS2 protein as well as other putative ORFs are presented in *Supplement Report UOM-25-PROT-RPT-01-02* and the following Sections:

- Section A.2(a)(i) and
- Section B.1(d)

The bio-informatic analysis demonstrated no relevant similarity between the putative peptides and known toxins.

B.2(a)(ii) information on the stability of the protein to proteolysis in appropriate gastrointestinal model systems

The susceptibility of the novel proteins associated with wheat event UM-12115T-1 to proteolysis was assessed using a weight-of-evidence approach based on *in vitro* gastrointestinal model systems relevant to gastric digestion. For OsNAS2, direct experimental evidence was generated in a simulated gastric fluid (SGF) assay using purified recombinant protein (Thomas et al., 2004; Oliva 2019). In this study, OsNAS2 was incubated at 37 °C in SGF at pH 1.2 containing pepsin, and aliquots were collected over a 60-minute time course for analysis by SDS-PAGE and western immunoblotting. The study design is consistent with established *in vitro* pepsin digestibility approaches used in the safety assessment of novel proteins.

OsNAS2 was shown to be rapidly degraded under these conditions. No intact OsNAS2 protein, with an apparent molecular weight of approximately 37.1 kDa, was detectable after 30 seconds of incubation in SGF containing pepsin, which was the earliest sampled post-exposure time point (Figure 27). This finding was demonstrated by both Coomassie-stained SDS-PAGE and western immunoblot analysis, indicating that the full-length protein is highly unstable in the gastric model system used.

The assay included appropriate controls to confirm system performance. Bovine serum albumin, used as a digestion-labile control, was no longer detectable as intact protein after 1 minute of incubation in SGF containing pepsin. In contrast, β -lactoglobulin, included as a digestion-stable control, remained detectable after 60 minutes under the same conditions. These control results confirm that the assay conditions were suitable to distinguish proteins with differing susceptibilities to pepsin digestion and support the validity of the conclusion reached for OsNAS2.

For HPT, a bridging approach was applied. The protein safety report notes that HPT is a well-characterised selectable marker protein for which supporting evidence indicates similarly rapid degradation under digestive conditions. The report states that HPT is rapidly degraded in simulated gastric fluid, with full-length protein becoming undetectable within approximately 30–40 seconds of pepsin exposure and concludes that HPT is not expected to persist as an intact protein during gastrointestinal transit.

Although analytical data confirms the Kan protein is not expressed in UM-12115T-1, a highly conservative safety assessment was nonetheless conducted. The susceptibility of the Kan protein to proteolytic degradation was also assessed via a bridging approach, leveraging the extensive body of existing safety data for this widely utilised bacterial selectable marker. Rather than conducting *de novo* experiments for this specific event, the assessment relies on peer-reviewed studies (e.g., Woegerbauer 2007).

An *in-silico* analysis was performed to identify potential protease cleavage sites. The full amino acid sequences of the Nicotianamine Synthase 2 (OsNAS2) and Hygromycin Phosphotransferase (HPT) and the Neomycin-kanamycin phosphotransferase proteins were analysed using the PeptideCutter tool available on the ExPASy Proteomics Server (Gasteiger et al., 2005).

The analysis screened for cleavage sites specific to major gastrointestinal enzymes, including pepsin (at pH 1.3 and pH >2), trypsin, chymotrypsin (high and low specificity), proteinase K, and general endopeptidases.

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The frequency and distribution of these theoretical cleavage sites were evaluated to predict the likelihood of rapid degradation relative to dietary proteins.

The *in-silico* analysis predicted numerous potential cleavage sites for both OsNAS2 and HPT across a range of gastrointestinal proteases, indicating a high theoretical susceptibility to digestion. Although analytical data confirms the Kan protein is not expressed in UM-12115T-1, a highly conservative safety assessment was nonetheless conducted and therefore potential cleavage sites for the Kan protein were also assessed.

- **OsNAS2:** The sequence analysis identified 66 potential cleavage sites for pepsin at pH 1.3, increasing to 78 sites at pH >2. Furthermore, the protein contained abundant sites for intestinal proteases, including 30 sites for trypsin, 21 high-specificity sites for chymotrypsin (68 low-specificity), and 81 sites for endopeptidases.
- **HPT:** Similarly, the HPT sequence exhibited a high density of potential cleavage sites, with 64 predicted sites for pepsin at pH 1.3 (89 sites at pH >2). The analysis also identified 28 sites for trypsin, 35 high-specificity sites for chymotrypsin (70 low-specificity), and 99 sites for endopeptidases.
- **Kan:** The Kan sequence exhibits a high density of potential cleavage sites, with 65 predicted sites for pepsin at pH 1.3 (86 sites at pH >2). The analysis also identified 32 sites for trypsin, 30 high-specificity sites for chymotrypsin (70 low-specificity), and 120 sites for endopeptidases.

The high frequency of predicted cleavage sites for both acid-active (pepsin) and neutral-active (trypsin, chymotrypsin) enzymes suggests that the OsNAS2, HPT and Kan possess unique structural features that would confer resistance to proteolysis. These results support the expectation that both proteins will be readily degraded during gastric and intestinal transit, comparable to standard dietary proteins.

Overall, the available evidence demonstrates that the proteins relevant to event UM-12115T-1 are not stable to proteolysis in gastrointestinal model systems. OsNAS2 was directly shown to be rapidly and completely degraded in SGF containing pepsin, with no intact protein detectable at the earliest measured time point of 30 seconds. The supporting bridging evidence for HPT indicates a comparable pattern of rapid degradation. These data support the conclusion that neither OsNAS2 nor HPT is expected to persist in intact form following oral consumption, and therefore no food safety concern is identified based on resistance to gastrointestinal proteolysis.

[Redacted – Confidential Commercial Information (CCI)]

Figure 27. Pepsin digestion of recombinant OsNAS2 in simulated gastric fluid.

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B.2(a)(iii) an animal toxicity study if the bioinformatic comparison and biochemical studies indicate either a relationship with known protein toxins/anti-nutrients or resistance to proteolysis.

The bioinformatic analyses did not indicate any relationships with known protein toxins/anti-nutrients and did not show any resistance to proteolysis. However, two nutritional assessments were undertaken to compare UM12115T-1 wheat with the wild type control on poultry performance (see *Supplement Reports UOM-25-FEED-RPT-01-01 Poultry Trial 2025* and *UOM-26-FEED-RPT-01-01 Poultry Trial 2026* and Section D).

B.2(b) information on the potential allergenicity of any new proteins, including:

Details of the potential allergenicity of the OsNAS2 protein as well as other putative ORFs are presented in *Supplement Report UOM-25-PROT-RPT-01-02* and the following Sections:

- Section A.2(a)(i) and
- Section B.1(d)

The bio-informatic analysis demonstrated no relevant similarity between the putative peptides and known allergens. The OsNAS2 protein is from rice and shares homology with numerous proteins found in food plants and therefore has a history of safe use.

Additional information is provided below.

B.2(b)(iii) source of the new protein the new protein's structural properties, including, but not limited to, its susceptibility to enzymatic degradation (e.g. proteolysis), heat and/or acid stability

The primary new protein expressed in wheat event UM-12115T-1 is OsNAS2, a nicotianamine synthase derived from *Oryza sativa* (rice). OsNAS2 is an enzyme involved in the biosynthesis of nicotianamine from S-adenosyl-methionine and therefore functions within a well-characterised plant metabolic pathway associated with metal chelation and transport. The selectable marker protein HPT is hygromycin phosphotransferase, which was used during plant transformation but was not detected in the analysed leaf or seed tissues of UM-12115T-1.

The structural and functional properties of OsNAS2 relevant to food safety were assessed using a weight-of-evidence approach that considered its biochemical identity together with its behaviour under conditions relevant to digestion and food processing. OsNAS2 is an enzymatically active protein when purified under non-denaturing conditions, and its thermal stability was evaluated directly by monitoring enzymatic production of nicotianamine from S-adenosyl-methionine following pre-incubation of the purified protein at increasing temperatures (see *Supplement Report UOM-25-PROT-RPT-02-02* and Tako et al., 2022). In this study, purified microbial-expressed OsNAS2 was heated for 15 minutes over a temperature range of approximately 25–65 °C, after which residual enzymatic activity was quantified by LC-MS.

OsNAS2 was shown to be thermolabile. The additional thermostability study reported that the enzyme lost approximately 50 % of its activity following pre-incubation at around 40 °C and was completely inactivated following pre-incubation at 60 °C for 15 minutes. The study concluded that the temperatures required to inactivate OsNAS2 are substantially lower than those normally encountered during cooking or food processing, and therefore dietary exposure to functional OsNAS2 would be expected to be negligible (Figure 28).

In addition to heat lability, OsNAS2 was previously shown to be highly susceptible to proteolytic degradation under gastric conditions. In simulated gastric fluid at pH 1.2 containing pepsin, no intact OsNAS2 protein was detectable after 30 seconds of incubation, the earliest sampled time point, as assessed by SDS-PAGE and western immunoblotting (Figure 27). This demonstrates that OsNAS2 is not stable under strongly acidic gastric conditions and is rapidly degraded in an appropriate *in vitro* gastrointestinal model system. Taken together, the pepsin digestibility data and thermostability data indicate that OsNAS2 does not persist as an intact, functional protein under conditions relevant to oral consumption and food preparation.

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For HPT, a bridging approach was used. HPT is a well-characterised selectable marker protein with an established history of prior safety assessment. HPT is rapidly degraded under digestive conditions and is also heat sensitive, such that it would not be expected to persist as an intact or functional protein during gastrointestinal transit or following typical food processing. This conclusion is further supported in the context of UM-12115T-1 by the absence of detectable HPT in the analysed edible tissues.

Overall, the available evidence supports the conclusion that the new protein OsNAS2, derived from rice, has structural and functional properties that do not raise a food safety concern. It is an enzyme with a defined role in a normal plant metabolic pathway, but it is highly susceptible to proteolysis under acidic gastric conditions and readily loses activity following modest heat treatment. HPT, which served only as a selectable marker during transformation, is likewise not considered stable to digestion or heat and was not detected in the analysed UM-12115T-1 tissues. On this basis, the new proteins associated with event UM-12115T-1 are not expected to persist in intact or functional form following consumption or conventional processing.

[Redacted – Confidential Commercial Information (CCI)]

Figure 28. Heat stability of OsNAS2 enzyme.

B.2(b)(iv) specific serum screening where a new protein is derived from a source known to be allergenic or has sequence homology with a known allergen

Not applicable. The OsNAS2 protein is not from a source known to be allergenic nor does it display sequence homology with known allergens.

B.2(b)(v) information on whether the new protein(s) have a role in the elicitation of gluten-sensitive enteropathy, in cases where the introduced genetic material is obtained from wheat, rye, barley, oats, or related cereal grains.

Where the new protein has been produced from an alternative source (e.g. microbial expression system) in order to obtain sufficient quantities for analysis, information **must** be provided to demonstrate that the protein tested is biochemically, structurally and functionally equivalent to that expressed in the food produced using gene technology.

Identity and Equivalence of OsNAS2 Test Substance

Typically, the concentrations of newly expressed proteins in genetically modified plants are too low to allow for the direct extraction of the gram quantities required for robust safety characterisation. In wheat event UM-12115T-1, the OsNAS2 protein is expressed at very low levels in the grain, with a mean concentration of only 0.024 ng/mg of extractable protein. To address this, a heterologous bacterial expression system was utilised to produce the necessary quantities of purified test protein, followed by a weight-of-evidence assessment to demonstrate functional and biochemical equivalence to the plant-expressed form.

The production and characterisation of the OsNAS2 test protein are described in detail in the study by Oliva (2023). The protein was produced in an *Escherichia coli* over-expression system for use in pepsin digestibility and heat stability studies. The form of the protein expressed in recombinant *E. coli* consisted of a full-length version of the OsNAS2 protein with a 'GSG' linker sequence and a C-terminal myc-(His)8-tag, which was purified from clarified lysate by batch metal affinity chromatography and size exclusion chromatography. The resulting test substance was approximately 86 % pure by reverse-phase HPLC and migrated as a single species with an apparent molecular weight of ca. 37.1 kDa by SDS-PAGE. This expected mass difference from the native 34.5 kDa plant-expressed form is solely attributable to the 2.6 kDa purification tag and does not alter the core functional domains of the enzyme. Identity was confirmed via Edman N-terminal sequencing (MEAQNQEVAA) and mass spectral analysis of trypsin-digested peptide fragments, which provided 100 % sequence coverage (Figure 29).

[Redacted – Confidential Commercial Information (CCI)]

Figure 29. Amino acid sequence of the microbial expressed OsNAS2

Furthermore, the microbial surrogate displayed the expected enzymatic activity in catalysing the condensation of S-adenosyl-L-methionine (SAM) into nicotianamine, confirming its functional integrity. To bridge these results to the plant-expressed protein, a weight-of-evidence assessment confirmed that both forms share an identical non-glycosylated status. Bioinformatic analysis using multiple algorithms demonstrated that OsNAS2 lacks an N-terminal signal peptide and is localised to the cytosol, ensuring it is physically sequestered from the plant's glycosylation machinery. These findings confirm that the microbially-produced OsNAS2 is biochemically and functionally equivalent to the protein expressed in wheat event UM-12115T-1, justifying its use as a suitable surrogate for safety assessments.

B.3. Other (non-protein) new substances

If other (non-protein) substances are produced as a result of the introduced DNA, information must be provided on the following:

B.3(a) the identity and biological function of the substance

B.3(b) whether the substance has previously been safely consumed in food

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B.3(c) potential dietary exposure to the substance

Only two proteins are added wheat event UM12115T-1, OsNAS2 and HPT.

B.3(d)(i) where RNA interference has been used: the role of any endogenous target gene and any changes to the food as a result of silencing that gene

Not applicable to this submission.

B.3(d)(ii) where RNA interference has been used: the expression levels of the RNA transcript

Not applicable to this submission.

B.3(d)(iii) where RNA interference has been used: the specificity of the RNA interference

Not applicable to this submission.

B.4. Novel herbicide metabolites in GM herbicide tolerant plants

The identity and levels of herbicide and any novel metabolites that may be present in the food produced using gene technology.

If novel metabolites are present then the application should address the following, where appropriate:

- (a) toxicokinetics and metabolism
- (b) acute toxicity
- (c) short-term toxicity
- (d) long-term toxicity and carcinogenicity
- (e) reproductive and developmental toxicity
- (f) genotoxicity.

Not applicable to this submission

B.5 Compositional analyses of the food produced using gene technology**B.5(a) Levels of relevant key nutrients, toxicants, and anti-nutrients in the food produced using gene technology compared with the levels in an appropriate comparator.**

To address the levels of relevant key nutrients, toxicants, and anti-nutrients, a comprehensive assessment of the genetically modified wheat event UM-12115T-1 was conducted on both forage and grain tissues to establish compositional equivalence against a near-isogenic wild type control and standard commercial reference varieties (see *Supplement Report UOM-26-COMP-RPT-01-01*). The primary statistical framework utilised a Linear Mixed-Effects Model for combined-site analyses across four multi-location field trials cultivated in 2025 at Moama, Leeton 1, Leeton 2, and Binya. In this model, plant genotype was evaluated as a fixed effect, while trial location and replicates were modelled as random effects. Pairwise comparisons against the wild type control were conducted using Dunnett's test at a 0.05 significance level.

Assessment of UM-12115T-1 Forage Composition

For the forage assessment, which evaluates above-ground biomass critical for some livestock diets, the combined-site analysis demonstrated that UM-12115T-1 is strictly equivalent to the non-GM wild type Control. As detailed in Table 1, No statistically significant differences ($p > 0.05$) were observed across any of the measured proximate analytes, including crude protein, acid detergent fibre, neutral detergent fibre, digestibility, and estimated metabolizable energy (Table 18). Similarly, the combined-site mineral profile of the forage revealed no statistically significant differences between the varieties for any of the tested elements, confirming absolute environmental stability across standard growing environments (Table 21).

Assessment of UM-12115T-1 Grain Composition

The combined-site analysis demonstrated that the grain from UM-12115T-1 exhibits a comparable macronutrient and energy profile to its conventional counterparts, as detailed in Table 20. Total dietary fibre and carbohydrates showed no significant variance between varieties ($p > 0.05$). While a statistically significant difference was observed in protein content ($p < 0.001$), the mean value for UM-12115T-1 (13.1 %) aligned closely with the wild type control (13.9 %) and remained well within the biological range established by the commercial checks (Table 20). Similarly, the combined across-site amino acid composition (Table 21) and fatty acid composition (Table 22) remained highly conserved, with structural lipids maintaining expected biological proportionality without relevant alterations.

Regarding anti-nutrients, phytic acid showed no notable statistical divergence ($p = 0.1235$) in the combined site analysis, confirming that the genetic modification does not elevate anti-nutritional profiles or compromise the bioavailability of dietary minerals (Table 23). While minor site-specific deviations in phytate were observed solely at the Leeton 2 site, the GM event accumulated significantly less phytate than the commercial check Ironbark (see *Supplement Report UOM-26-COMP-RPT-01-01*). Analysis of trace heavy metals acting as potential toxicants, including arsenic, cadmium, lead, and nickel, demonstrated strict equivalence. No statistical differences were observed between UM-12115T-1 and the control lines across the pooled data, confirming that the genetic insertion introduces no novel environmental accumulation profile or toxicity risk (Table 24).

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Table 18. Proximates, in UM-12115T-1 and Control Wheat Forage-Combined Sites

Variable ¹	Variety ²	Mean	P-Value ³	STDV ⁴	N	Range		Combined Literature Range ⁵	
						Min	Max	Min	Max
Crude Protein	UM-12115T-1	22.80	0.633	4.26	16	14.30	25.90	7.3	24.4
	Wild Type	22.48		4.48	16	13.10	27.00		
	Check	23.29		4.12	16	15.50	27.70		
Moisture	UM-12115T-1	79.26	0.901	5.29	16	69.80	84.60	–	–
	Wild Type	79.11		4.97	16	70.10	84.30		
	Check	80.86		5.33	16	70.40	86.30		
Ash	UM-12115T-1	9.55	0.993	1.46	16	6.50	11.0	5.5	12.9
	Wild Type	9.54		1.50	16	6.30	11.50		
	Check	9.68		1.37	16	7.20	11.40		
Dry Matter (%)	UM-12115T-1	20.74	0.901	5.29	16	15.40	30.20	19.1	39.2
	Wild Type	20.89		4.97	16	15.70	29.90		
	Check	19.14		5.33	16	13.70	29.60		
Fat	UM-12115T-1	5.60	0.081	0.11	16	5.40	5.80	–	–
	Wild Type	5.53		0.11	16	5.40	5.70		
	Check	5.59		0.13	16	5.30	5.70		
DCAD ((Na+K)-(Cl+S)) mequiv/Kg Dry Matter	UM-12115T-1	395.81	0.135	123.16	16	222	708	–	–
	Wild Type	475.94		193.16	16	146	858		
	Check	460.56		168.60	16	164	749		
Acid Detergent Fibre	UM-12115T-1	19.50	0.455	2.14	16	15.80	23.00	19.9	52.0
	Wild Type	20.04		2.34	16	16.50	22.90		
	Check	20.24		2.46	16	14.90	24.40		
Neutral Detergent Fibre	UM-12115T-1	45.04	0.536	2.90	16	39.30	49.80	37.7	81.6
	Wild Type	45.66		3.33	16	40.60	50.10		
	Check	45.14		3.28	16	38.60	49.70		
Digestibility	UM-12115T-1	76.63	0.481	2.01	16	72.70	81.20	–	–
	Wild Type	75.98		2.58	16	72.50	79.30		
	Check	77.16		2.79	16	72.60	83.40		
Digestibility (DOMD)	UM-12115T-1	71.72	0.495	1.76	16	68.40	75.60	–	–
	Wild Type	71.19		2.18	16	68.20	74.00		
	Check	72.19		2.36	16	68.30	77.50		
Estimated Metabolizable Energy (MJ/Kg DM)	UM-12115T-1	11.57	0.457	0.35	16	10.90	12.30	–	–
	Wild Type	11.46		0.42	16	10.90	12.00		
	Check	11.66		0.48	16	10.90	12.70		

¹Units are in % Dry Matter unless otherwise stated.

²The check variety was Sunmaster

³P-values indicating significant differences * p<0.05; ** p<0.01; *** p<0.001 are underlined and in bold. P-values identified with a \$ were obtained from analysis of the data using a Welch's ANOVA

⁴Standard Deviation

⁵Combined literature ranges are ranges are from Obert et al., 2004 and Heuzé et al., 2015

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Table 19. Minerals, in UM-12115T-1 and Control Wheat Forage-Combined Sites

Variable ¹	Variety ²	Mean	P-Value ³	STDV ⁴	N	Range		Combined Literature Range ⁵	
						Min	Max	Min	Max
Aluminum	UM-12115T-1	238.75	0.765	64.17	16	170	390		
	Wild Type	250.00		92.66	16	120	490		
	Check	231.25		78.56	16	170	490		
Boron	UM-12115T-1	3.42	0.583	0.79	16	2.50	5.20		
	Wild Type	3.68		1.25	16	2.50	6.50		
	Check	4.24		1.55	16	2.60	7.90		
Calcium	UM-12115T-1	3193.75	0.984	543.41	16	2300	3800	3000	4800
	Wild Type	3212.50		750.89	16	2000	4300		
	Check	3512.50		777.93	16	2400	5000		
Copper	UM-12115T-1	6.14	0.189	1.21	16	4.10	7.80		
	Wild Type	6.57		1.62	16	3.70	8.90		
	Check	5.93		1.31	16	3.40	7.30		
Iron	UM-12115T-1	166.88	0.865	40.62	16	120	260		
	Wild Type	171.88		54.68	16	100	320		
	Check	166.88		50.03	16	120	320		
Potassium	UM-12115T-1	37062.50	0.131	6697.95	16	27000	49000		
	Wild Type	40812.50		10857.99	16	21000	57000		
	Check	41875.00		8655.44	16	28000	53000		
Magnesium	UM-12115T-1	1596.25	0.117	462.86	16	970	2400		
	Wild Type	1725.63		530.01	16	910	2700		
	Check	1750.00		461.88	16	1000	2300		
Manganese	UM-12115T-1	47.88	0.303	9.13	16	34.00	71.00		
	Wild Type	51.44		10.25	16	35.00	69.00		
	Check	58.94		9.05	16	46.00	76.00		
Sodium	UM-12115T-1	301.56	0.458	305.30	16	51.00	950		
	Wild Type	370.19		422.01	16	52.00	1600		
	Check	405.81		383.23	16	89.00	1300		
Phosphorus	UM-12115T-1	3193.75	0.524	1121.59	16	1800	5000	2000	34000
	Wild Type	3387.50		1285.76	16	1400	6400		
	Check	3706.25		1698.03	16	1900	6700		
Sulphur	UM-12115T-1	3368.75	0.768	760.46	16	1900	4400		
	Wild Type	3456.25		871.76	16	1700	4600		
	Check	3375.00		976.73	16	2000	5000		
Zinc	UM-12115T-1	29.88	0.538	8.35	16	19.00	54.00		
	Wild Type	27.88		7.97	16	16.00	39.00		
	Check	23.38		5.61	16	15.00	35.00		
Chloride (% DM)	UM-12115T-1	1.27	0.642	0.32	16	0.84	1.89		
	Wild Type	1.32		0.32	16	0.78	1.92		
	Check	1.49		0.30	16	0.99	1.98		

¹Units are in mg/Kg Dry Matter unless otherwise stated.

²The check variety was Sunmaster

³P-values indicating significant differences * p<0.05; ** p<0.01; *** p<0.001 are underlined and in bold. P-values identified with a \$ were obtained from analysis of the data using a Welch's ANOVA

⁴Standard Deviation

⁵Combined literature ranges are ranges are from Obert et al., 2004 and Heuzé et al., 2015

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Table 20. Combined Across-Site Proximate and Fibre Composition of Wheat Grain

Variable ¹	Variety	Mean ²	P-value (Overall)	STDEV	N	Range (Min - Max)	Combined Literature Range
Energy (Calories/100g)	Ironbark	347 ab	<u>0.015*</u>	4.23	28	340 - 355	329.11-342.02
	Wild Type	348 a		2.22	28	343 - 352	
	Sunmaster	345 bc		4.14	28	338 - 353	
	UM12115T-1	348 a		3.77	28	339 - 355	
Carbohydrates	Ironbark	62.5	0.175	2.76	28	57 - 67.6	65.4-78.0
	Wild Type	60.8		2.53	28	54.9 - 64.2	
	Sunmaster	61.5		3.24	28	56.1 - 66.2	
	UM12115T-1	61.5		2.92	28	54.7 - 65.4	
Protein (N x 5.7)	Ironbark	12.2 bc	<u><0.001***</u>	1.22	28	9.4 - 14.8	10.0-16.0
	Wild Type	13.9 a		1.48	28	11.6 - 17.4	
	Sunmaster	12.2 b		1.43	28	10.5 - 15.2	
	UM12115T-1	13.1 a		1.47	28	11.2 - 16.7	
Moisture	Ironbark	9.05	0.54	0.44	28	8.5 - 9.9	8.0-18.0
	Wild Type	9.2		0.50	28	8.5 - 10.1	
	Sunmaster	9.2		0.42	28	8.7 - 10	
	UM12115T-1	9.09		0.53	28	8.4 - 10	
Ash	Ironbark	1.46	0.97	0.24	28	1.07 - 1.88	1.2-3.0
	Wild Type	1.48		0.22	28	1.12 - 1.97	
	Sunmaster	1.46		0.17	28	1.07 - 1.73	
	UM12115T-1	1.46		0.21	28	1.13 - 1.88	
Total Free Sugars	Ironbark	0.647 c	<u><0.001***</u>	0.22	28	0.23 - 0.97	2.1-2.6
	Wild Type	0.928 a		0.30	28	0.22 - 1.4	
	Sunmaster	0.646 c		0.17	28	0.32 - 0.94	
	UM12115T-1	0.827 a		0.25	28	0.24 - 1.2	
Total Dietary Fibre	Ironbark	12.6	0.13	1.85	28	10.4 - 15.5	11.0-14.6
	Wild Type	12.2		1.36	28	10.1 - 15.1	
	Sunmaster	13.4		2.09	28	10.7 - 16.8	
	UM12115T-1	12.6		2.05	28	10 - 16.9	
Fat	Ironbark	2.23 bc	<u>0.01**</u>	0.19	28	2 - 2.8	1.5-2.0
	Wild Type	2.41 a		0.25	28	2 - 2.8	
	Sunmaster	2.26 b		0.15	28	2 - 2.5	
	UM12115T-1	2.31 a		0.21	28	2 - 2.8	
Gluten (mg/Kg)	Ironbark	9.24 x 10 ⁴ bc	<u>0.01**</u>	40142.54	28	4.4 x 10 ⁴ –2.3 x 10 ⁵	20->160,000
	Wild Type	1.19 x 10 ⁵ a		50867.54	28	5.4 x 10 ⁴ –3 x 10 ⁵	
	Sunmaster	1.1 x 10 ⁵ ab		49403.14	28	5.1 x 10 ⁴ –2.8 x 10 ⁵	
	UM12115T-1	1.4 x 10 ⁵ a		73875.23	28	5.6 x 10 ⁴ –3.6 x 10 ⁵	

¹Units in g/100g dry weight unless otherwise stated.

²Means with the same letter are not significantly different p<0.05.

³P-values indicating significant differences * p<0.05; ** p<0.01; *** p<0.001 are underlined and in bold.

⁴Combined literature ranges are ranges are from OECD (2023), Gluten content, minimum gluten free- (CODEX STAN 118-1979), maximum from Garcia-Molina et al., (2019)

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Table 21. Combined Across-Site Amino Acid Composition of Wheat Grain

Variable ¹	Variety	Mean ²	P-value ³	STDEV	N	Range (Min - Max)	Combine Literature Ranges ⁴
Aspartic Acid	Ironbark	5490 b	0.01**	649.05	28	3800 - 6500	4800-8960
	Wild Type	5880 a		663.91	28	4600 - 7300	
	Sunmaster	5360 bc		504.31	28	4100 - 6400	
	UM12115T-1	5620 a		413.70	28	4900 - 6500	
Serine	Ironbark	6340 ab	0.03*	636.21	28	5400 - 7800	4300-9120
	Wild Type	6920 a		625.76	28	6000 - 8700	
	Sunmaster	6310 abc		791.96	28	4900 - 8000	
	UM12115T-1	6350 a		1324.55	28	800 - 8200	
Glutamic Acid	Ironbark	40600 ab	0.01**	6344.60	28	27000 - 53000	29,900-55,680
	Wild Type	44400 a		5743.53	28	31000 - 57000	
	Sunmaster	39400 bc		5729.81	28	28000 - 51000	
	UM12115T-1	41700 a		5637.53	28	34000 - 57000	
Glycine	Ironbark	4840 c	0.001***	1188.97	28	2200 - 6400	3800-9760
	Wild Type	5990 a		566.81	28	4900 - 7500	
	Sunmaster	4810 c		1169.97	28	2400 - 6800	
	UM12115T-1	5560 a		758.86	28	3500 - 7600	
Histidine	Ironbark	2890 a	0.001***	330.78	28	2200 - 3400	2000-4480
	Wild Type	2870 a		341.95	28	2100 - 3800	
	Sunmaster	2700 ab		445.12	28	1900 - 3700	
	UM12115T-1	2560 c		415.82	28	1600 - 3700	
Arginine	Ironbark	5380 ab	0.01**	503.78	28	4300 - 6500	4000-9120
	Wild Type	5750 a		804.84	28	4500 - 7100	
	Sunmaster	5190 bc		475.26	28	4200 - 6200	
	UM12115T-1	5440 a		502.01	28	4700 - 7000	
Threonine	Ironbark	3980	0.89	665.35	28	3300 - 5900	2400-5120
	Wild Type	4030		568.98	28	3300 - 5800	
	Sunmaster	3920		490.17	28	3300 - 4800	
	UM12115T-1	4010		535.21	28	3400 - 5300	
Alanine	Ironbark	4490	0.62	489.68	28	3400 - 5400	3400-5920
	Wild Type	4630		520.58	28	3800 - 5900	
	Sunmaster	4550		508.80	28	3700 - 5800	
	UM12115T-1	4490		329.66	28	3900 - 5200	
Proline	Ironbark	12500 a	0.02*	1319.37	28	11000 - 16000	9800-18,560
	Wild Type	13500 a		1562.06	28	8900 - 16000	
	Sunmaster	12200 bc		2051.53	28	8600 - 16000	
	UM12115T-1	12400 ab		1870.12	28	9000 - 17000	
Tyrosine	Ironbark	2720 a	0.02*	445.24	28	1600 - 3600	1800-5920
	Wild Type	2950 a		410.49	28	2100 - 3700	
	Sunmaster	2590 bc		516.35	28	1900 - 3600	
	UM12115T-1	2660 ab		420.07	28	1900 - 3600	
Valine	Ironbark	5140	0.11	510.87	28	4400 - 6700	4400-7680
	Wild Type	5280		611.39	28	4200 - 6500	
	Sunmaster	4990		314.28	28	4400 - 5600	
	UM12115T-1	5020		450.56	28	4300 - 5900	
Lysine	Ironbark	3770 ab	0.04*	800.49	28	1700 - 4700	2200-4800
	Wild Type	4250 a		732.58	28	2600 - 5700	
	Sunmaster	3720 bc		662.97	28	2200 - 4800	
	UM12115T-1	3910 a		734.01	28	2300 - 5100	
Isoleucine	Ironbark	4090 a	0.02*	994.32	28	3200 - 8800	3000-6880
	Wild Type	4060 a		530.15	28	3000 - 5100	
	Sunmaster	3650 abc		362.59	28	2800 - 4100	
	UM12115T-1	3730 ab		522.85	28	2900 - 4700	
Leucine	Ironbark	8300 ab	0.01**	754.74	28	7000 - 10000	5000-11,680
	Wild Type	8890 a		815.46	28	7100 - 11000	
	Sunmaster	8080 bc		848.11	28	6400 - 9800	
	UM12115T-1	8390 a		982.94	28	7000 - 11000	
Phenylalanine	Ironbark	5600 a	0.001***	642.33	28	4300 - 6900	3500-8640
	Wild Type	5990 a		615.07	28	4300 - 7200	
	Sunmaster	5250 bc		810.34	28	4000 - 8000	
	UM12115T-1	5470 b		834.02	28	4100 - 7600	
Methionine	Ironbark	1410 b	0.001***	149.91	28	1100 - 1700	1300-2720
	Wild Type	1560 a		196.90	28	1000 - 1900	
	Sunmaster	1360 bc		229.87	28	860 - 1800	

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Variable ¹	Variety	Mean ²	P-value ³	STDEV	N	Range (Min - Max)	Combine Literature Ranges ⁴
	UM12115T-1	1440 a		164.27	28	1100 - 1700	
Hydroxyproline	Ironbark	162	0.67	112.01	28	50 - 430	–
	Wild Type	155		100.84	28	50 - 360	
	Sunmaster	184		112.95	28	50 - 450	
	UM12115T-1	150		114.70	28	50 - 480	
Taurine	Ironbark	50	1.00	0.00	28	50 - 50	–
	Wild Type	50		0.00	28	50 - 50	
	Sunmaster	50		0.00	28	50 - 50	
	UM12115T-1	50		0.00	28	50 - 50	

¹Units in mg/1Kg dry weight unless otherwise stated.

²Means with the same letter are not significantly different p<0.05.

³P-values indicating significant differences * p<0.05; ** p<0.01; *** p<0.001 are underlined and in bold.

⁴Combined literature ranges are ranges are from OECD (2023). OECD values converted from % total protein. Values expressed in mg/kg represent the absolute theoretical minimum and maximum concentrations. To account for maximum natural variance, the absolute minimum applies the lowest reported amino acid % to the lowest total protein level (10 %), while the absolute maximum applies the highest amino acid % to the highest total protein level (16 %). Calculations utilise the conversion: mg/kg = [Amino Acid (% of protein)] × [Total Protein (% of sample)] × 100.

Application to FSANZ for the Inclusion of wheat event UM-12115T-1 with increased Nicotianamine in
Standard 1.5.2 Genetically Modified Food

Table 22. Combined Across-Site Fatty Acid Composition of Wheat Grain

Variable	Variety	Mean	P-value	STDEV	N	Range (Min - Max)	Combined Literature Ranges
C14_0_ Myristic_acid	Ironbark	0.1	0.40	0.00	28	0.1 - 0.1	0
	Wild Type	0.1		0.00	28	0.1 - 0.1	
	Sunmaster	0.1		0.00	28	0.1 - 0.1	
	UM12115T-1	0.104		0.02	28	0.1 - 0.2	
C16_0_ Palmitic_acid	Ironbark	17.9 a	<0.001***	0.39	28	17.2 - 18.6	11-32
	Wild Type	17 c		0.24	28	16.6 - 17.5	
	Sunmaster	18 a		0.22	28	17.7 - 18.4	
	UM12115T-1	17 c		0.31	28	16.4 - 17.5	
C16_1cis_ Palmitoleic_acid	Ironbark	0.104 d	<0.001***	0.02	28	0.1 - 0.2	0
	Wild Type	0.168 ab		0.05	28	0.1 - 0.2	
	Sunmaster	0.189 a		0.03	28	0.1 - 0.2	
	UM12115T-1	0.189 a		0.03	28	0.1 - 0.2	
C17_0_ Heptadecanoic_acid	Ironbark	0.1	0.40	0.00	28	0.1 - 0.1	0.1
	Wild Type	0.1		0.00	28	0.1 - 0.1	
	Sunmaster	0.1		0.00	28	0.1 - 0.1	
	UM12115T-1	0.104		0.02	28	0.1 - 0.2	
C18_0_ Stearic_acid	Ironbark	1.13	0.25	0.13	28	0.9 - 1.4	0-4.6
	Wild Type	1.17		0.08	28	1.1 - 1.3	
	Sunmaster	1.12		0.07	28	1 - 1.3	
	UM12115T-1	1.12		0.13	28	1 - 1.5	
C18_1cis_ Oleic_acid	Ironbark	17.3 bc	<0.001***	0.80	28	15.9 - 19.2	11-29
	Wild Type	17.4 b		0.81	28	16.3 - 18.9	
	Sunmaster	18.7 a		0.80	28	17.7 - 20.9	
	UM12115T-1	17.4 b		1.10	28	16.2 - 20	
C18_2cis_ Linoleic_acid	Ironbark	58.2 c	<0.001***	0.83	28	56 - 59.2	44-74
	Wild Type	59.4 a		0.77	28	57.7 - 60.3	
	Sunmaster	56.5 d		0.82	28	54.3 - 57.7	
	UM12115T-1	59.2 a		0.95	28	56.9 - 60.1	
C18_3_ alpha_Linolenic_acid_ALA	Ironbark	3.9 b	<0.001***	0.20	28	3.5 - 4.3	0.7-4.4
	Wild Type	3.6 c		0.17	28	3.2 - 3.8	
	Sunmaster	4.18 a		0.20	28	3.9 - 4.5	
	UM12115T-1	3.59 c		0.18	28	3.2 - 3.9	
C20_0_ Arachidic_acid	Ironbark	0.146	0.25	0.05	28	0.1 - 0.2	0.1
	Wild Type	0.157		0.05	28	0.1 - 0.2	
	Sunmaster	0.171		0.05	28	0.1 - 0.2	
	UM12115T-1	0.168		0.05	28	0.1 - 0.3	
C20_1_ Eicosonic_acid	Ironbark	0.961 a	<0.001***	0.05	28	0.9 - 1	0
	Wild Type	0.814 b		0.04	28	0.7 - 0.9	
	Sunmaster	0.771 d		0.06	28	0.7 - 0.9	
	UM12115T-1	0.825 b		0.04	28	0.8 - 0.9	
C20_2_cis_ Eicosadienoic_acid	Ironbark	0.107	0.11	0.03	28	0.1 - 0.2	0
	Wild Type	0.1		0.00	28	0.1 - 0.1	
	Sunmaster	0.1		0.00	28	0.1 - 0.1	
	UM12115T-1	0.1		0.00	28	0.1 - 0.1	
C22_0_	Ironbark	0.136	0.10	0.05	28	0.1 - 0.2	0

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Variable	Variety	Mean	P-value	STDEV	N	Range (Min - Max)	Combined Literature Ranges
Behenic_acid	Wild Type	0.111		0.03	28	0.1 - 0.2	
	Sunmaster	0.125		0.04	28	0.1 - 0.2	
	UM12115T-1	0.114		0.04	28	0.1 - 0.2	
C22_1_Erucic_acid	Ironbark	0.111	0.27	0.03	28	0.1 - 0.2	0
	Wild Type	0.104		0.02	28	0.1 - 0.2	
	Sunmaster	0.1		0.00	28	0.1 - 0.1	
	UM12115T-1	0.104		0.02	28	0.1 - 0.2	
C24_0_Lignoceric_acid	Ironbark	0.139 a	<0.001***	0.06	28	0.1 - 0.3	0
	Wild Type	0.125 b		0.04	28	0.1 - 0.2	
	Sunmaster	0.171 a		0.05	28	0.1 - 0.2	
	UM12115T-1	0.118 bc		0.04	28	0.1 - 0.2	
Omega_3	Ironbark	3.9 b	<0.001***	0.20	28	3.5 - 4.3	
	Wild Type	3.6 c		0.17	28	3.2 - 3.8	
	Sunmaster	4.18 a		0.19	28	3.9 - 4.5	
	UM12115T-1	3.59 c		0.19	28	3.2 - 3.9	
Omega_6	Ironbark	58.3 c	<0.001***	0.82	28	56.1 - 59.2	
	Wild Type	59.4 a		0.76	28	57.8 - 60.3	
	Sunmaster	56.5 d		0.80	28	54.4 - 57.7	
	UM12115T-1	59.3 a		0.94	28	57 - 60.3	
Omega_9	Ironbark	17.4 bc	<0.001***	0.87	28	16.1 - 19.4	
	Wild Type	17.5 b		0.82	28	16.4 - 19.1	
	Sunmaster	18.7 a		0.84	28	17.7 - 21	
	UM12115T-1	17.5 b		1.11	28	16.3 - 20.2	
ALA_Alpha_Linolenic_acid (mg/100g)	Ironbark	86.5 b	<0.001***	8.49	28	72 - 110	
	Wild Type	87 b		10.09	28	70 - 109	
	Sunmaster	94.4 a		7.19	28	83 - 108	
	UM12115T-1	82.6 bc		6.17	28	71 - 98	
EPA_Eicosapentaenoic_acid (mg/100g)	Ironbark	<2	1.00	0.00	28	2 - 2	
	Wild Type	<2		0.00	28	2 - 2	
	Sunmaster	<2		0.00	28	2 - 2	
	UM12115T-1	<2		0.00	28	2 - 2	
DHA_Docosaeic_acid (mg/100g)	Ironbark	2	1.00	0.00	28	2 - 2	
	Wild Type	2		0.00	28	2 - 2	
	Sunmaster	2		0.00	28	2 - 2	
	UM12115T-1	2		0.00	28	2 - 2	
Saturated	Ironbark	19.4 b	<0.001***	0.33	28	18.6 - 20.1	20.2
	Wild Type	18.6 c		0.29	28	18 - 19.1	
	Sunmaster	19.6 a		0.23	28	19.2 - 19.9	
	UM12115T-1	18.6 c		0.26	28	18 - 19	
Mono_unsaturated	Ironbark	18.4 b	<0.001***	0.92	28	17.1 - 20.5	13.8
	Wild Type	18.4 bc		0.85	28	17.4 - 20.1	
	Sunmaster	19.7 a		0.89	28	18.5 - 22	
	UM12115T-1	18.6 b		1.15	28	17.1 - 21.3	
Poly_unsaturated	Ironbark	62.2 c	<0.001***	0.95	28	59.7 - 63.3	64.1
	Wild Type	63 a		0.87	28	61 - 63.8	
	Sunmaster	60.7 d		0.96	28	58.3 - 62.1	

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Variable	Variety	Mean	P-value	STDEV	N	Range (Min - Max)	Combined Literature Ranges
	UM12115T-1	62.9 a		1.07	28	60.2 - 64	
Trans	Ironbark	0.1		0.00	28	0.1 - 0.1	0.1
	Wild Type	0.1		0.00	28	0.1 - 0.1	
	Sunmaster	0.1		0.00	28	0.1 - 0.1	
	UM12115T-1	0.1		0.00	28	0.1 - 0.1	

¹Units in % dry weight unless otherwise stated.

²Means with the same letter are not significantly different $p < 0.05$.

³P-values indicating significant differences * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ are underlined and in bold.

⁴Combined literature ranges are ranges are from OECD (2023) and the Australian Food Composition Database Release 3.0

Application to FSANZ for the Inclusion of wheat event UM-12115T-1 with increased Nicotianamine in
Standard 1.5.2 Genetically Modified Food

Table 23. Combined Across-Site Vitamins and Anti-nutrient Composition of Wheat Grain

Variable ¹	Variety	Mean ²	P-value ³	STDEV	N	Range (Min - Max)	Combined Literature Range ⁴
Thiamine B1	Ironbark	0.579 b	<u><0.001***</u>	0.20	28	0.21 - 0.97	0.13-0.99
	Wild Type	0.725 a		0.19	28	0.34 - 1.1	
	Sunmaster	0.68 a		0.20	28	0.26 - 0.97	
	UM12115T-1	0.523 c		0.16	28	0.31 - 0.83	
Riboflavin B2	Ironbark	0.127	0.39	0.05	28	0.05 - 0.21	0.06-0.31
	Wild Type	0.143		0.06	28	0.05 - 0.22	
	Sunmaster	0.138		0.05	28	0.066 - 0.23	
	UM12115T-1	0.122		0.05	28	0.062 - 0.24	
Niacin B3	Ironbark	5.01 bc	<u><0.001***</u>	0.45	28	4.2 - 5.9	2.20-11.10
	Wild Type	5.54 a		0.59	28	4.2 - 6.7	
	Sunmaster	5.06 b		0.61	28	3.8 - 6.4	
	UM12115T-1	5.31 a		0.46	28	4 - 6.4	
Pyridoxine (Vitamin B6)	Ironbark	0.243	0.69	0.06	28	0.2 - 0.4	0.09-0.79
	Wild Type	0.258		0.06	28	0.2 - 0.36	
	Sunmaster	0.254		0.06	28	0.2 - 0.4	
	UM12115T-1	0.244		0.05	28	0.19 - 0.35	
Folate (µg/g)	Ironbark	0.892 a	<u><0.001***</u>	0.14	28	0.67 - 1.13	0.227-1.118
	Wild Type	0.783 c		0.11	28	0.516 - 0.964	
	Sunmaster	0.889 a		0.09	28	0.692 - 1.05	
	UM12115T-1	0.767 c		0.15	28	0.509 - 1.04	
Phytate (mg/g)	Ironbark	6.29	0.12	2.60	28	2.23 - 10.9	2.82-40.71
	Wild Type	5.33		1.62	28	3 - 8.25	
	Sunmaster	5.2		1.99	28	2.19 - 10.1	
	UM12115T-1	6.25		2.45	28	1.7 - 11	

¹Units in mg/100g dry weight unless otherwise stated.

²Means with the same letter are not significantly different $p < 0.05$.

³P-values indicating significant differences * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ are underlined and in bold.

⁴Combined literature ranges are ranges are from OECD 2003 except folate (Zheng et al., 2022).

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Table 24. Combined Across-Site Minerals Composition of Wheat Grain

Variable ¹	Variety	Mean ²	P-value ³	STDEV	N	Range (Min - Max)	Combined Literature Range ⁴
Calcium	Ironbark	45.4 a	<0.001***	8.15	28	35 - 63	25.0-53.8
	Wild Type	41.9 a		6.01	28	31 - 56	
	Sunmaster	37.5 bc		6.88	28	28 - 52	
	UM12115T-1	41.5 ab		7.94	28	31 - 65	
Copper	Ironbark	0.371	0.37	0.08	28	0.27 - 0.55	0.4-2.0
	Wild Type	0.397		0.07	28	0.29 - 0.52	
	Sunmaster	0.368		0.06	28	0.28 - 0.5	
	UM12115T-1	0.389		0.08	28	0.28 - 0.56	
Iron	Ironbark	2.69 c	<0.001***	0.33	28	2.1 - 3.4	2.38-7.63
	Wild Type	3.24 a		0.34	28	2.7 - 3.9	
	Sunmaster	2.92 c		0.36	28	2.1 - 3.5	
	UM12115T-1	3.33 a		0.44	28	2.7 - 4.2	
Magnesium	Ironbark	118	0.31	14.69	28	95 - 150	124.0-180.2
	Wild Type	117		12.22	28	96 - 140	
	Sunmaster	112		13.93	28	92 - 150	
	UM12115T-1	114		14.80	28	93 - 160	
Manganese	Ironbark	3.67	0.70	0.50	28	2.8 - 4.9	3.8-6.32
	Wild Type	3.66		0.50	28	3 - 5.2	
	Sunmaster	3.56		0.44	28	2.7 - 4.5	
	UM12115T-1	3.54		0.61	28	2.3 - 4.7	
Phosphorus	Ironbark	299	0.59	57.34	28	210 - 400	332.0-516.0
	Wild Type	308		64.71	28	200 - 440	
	Sunmaster	287		59.26	28	200 - 430	
	UM12115T-1	291		61.75	28	200 - 430	
Sodium	Ironbark	1.15	0.11	0.38	28	0.68 - 2.3	0.0-20.0
	Wild Type	1.03		0.33	28	0.53 - 1.9	
	Sunmaster	1.22		0.45	28	0.71 - 2.2	
	UM12115T-1	1.01		0.30	28	0.56 - 1.5	
Zinc	Ironbark	2.25 c	<0.001***	0.42	28	1.6 - 3.4	0.8-8.82
	Wild Type	2.64 a		0.36	28	2.1 - 3.5	
	Sunmaster	2.1 c		0.29	28	1.5 - 2.7	
	UM12115T-1	2.73 a		0.54	28	1.9 - 3.7	
Arsenic	Ironbark	0.0134	0.17	0.01	28	0.01 - 0.044	0.005-0.208
	Wild Type	0.0107		0.00	28	0.01 - 0.019	
	Sunmaster	0.0118		0.00	28	0.01 - 0.024	
	UM12115T-1	0.0112		0.00	28	0.01 - 0.022	
Cadmium	Ironbark	0.0104	0.94	0.00	28	0.01 - 0.016	0.003-0.025
	Wild Type	0.0103		0.00	28	0.01 - 0.014	
	Sunmaster	0.0103		0.00	28	0.01 - 0.013	
	UM12115T-1	0.0103		0.00	28	0.01 - 0.014	
Lead	Ironbark	0.01	0.40	0.00	28	0.01 - 0.01	0.005-0.089
	Wild Type	0.01		0.00	28	0.01 - 0.01	
	Sunmaster	0.01		0.00	28	0.01 - 0.01	
	UM12115T-1	0.0101		0.00	28	0.01 - 0.012	
Nickel	Ironbark	0.302	0.38	0.26	28	0.088 - 0.83	0.05-0.169

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Variable ¹	Variety	Mean ²	P-value ³	STDEV	N	Range (Min - Max)	Combined Literature Range ⁴
	Wild Type	0.257		0.15	28	0.096 - 0.65	
	Sunmaster	0.22		0.19	28	0.072 - 0.94	
	UM12115T-1	0.299		0.19	28	0.081 - 0.81	

¹Units in mg/100g dry weight unless otherwise stated.

²Means with the same letter are not significantly different $p < 0.05$.

³P-values indicating significant differences * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ are underlined and in bold.

⁴Combined literature ranges are ranges are from Obert et al., (2004) adjusted from ppm; Zinc content from Wang et al., (2020); Iron content from Cheung et al., (2024); Copper and Sodium from Heuzé et al., (2015); heavy metals from Liu et al., (2025)

B.5(b) Information on the range of natural variation for each constituent measured to allow for assessment of biological significance should any statistically significant differences be identified.

To accurately assess the biological significance of any statistically significant differences detected by the mixed-effects models, all data were contextualised against natural biological baselines. These baselines were established by the two commercial reference varieties grown concurrently, as well as extensive literature ranges sourced from, for example, OECD 2003, Obert et al. (2004), Heuzé et al. (2015) and the Australian Food Composition Database.

In the forage analysis, while the overall combined model proved equivalence, localised environmental variances were observed at individual sites. For example, highly elevated levels of aluminium and iron were detected across all genotypes at specific trial locations (See *Supplement Report UOM-25-COMP-RPT-01-01*). However, a Pearson correlation analysis demonstrated a near-perfect linear relationship between these two elements, confirming that the elevated levels were definitively attributed to environmental contamination via soil splash on the exterior forage surface rather than physiological uptake by the plant, thus ruling out any genetic or metabolic effect of the modification.

For grain proximates and minerals, while statistical models identified minor fractional differences in certain analytes such as protein, calcium, iron, and zinc, all recorded values for UM-12115T-1 securely nested within the natural physiological baselines established by the reference materials (Table 20 and Table 24). For example, while calcium levels in UM-12115T-1 (41.5 mg/kg) differed statistically from the controls, they were safely bound within the variance observed in the commercial checks, which ranged from 37.5 mg/kg to 45.4 mg/kg (Table 24).

Furthermore, significant differences in essential vitamins, such as thiamine and folate, were fractionally small and aligned safely with the natural variation profiles characteristic of traditional Australian wheat varieties, presenting no risk of nutritional deficiency (Table 23). For amino acids, absolute minimum and maximum baseline concentrations were mathematically derived to account for maximum natural variance based on total protein fluctuations. In historical instances where amino acids showed statistical variation, concentrations for the GM event consistently fell well within these absolute theoretical literature ranges (Table 25 and Table 26).

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Table 25. Amino Acids, in UM-12115T-1 and Wild Type Control Wheat Seed-2022

Variable ¹	Variety	Mean	P-Value ¹	STDV ³	N	Range		Combined Literature Range ⁴	
						Min	Max	Min	Max
Histidine	UM-12115T-1	2.80	<u><0.001***</u>	0.00	5	2.80	2.80	1.78	5.38
	Wild Type	2.98		0.045	5	2.90	3.0		
Serine	UM-12115T-1	5.20	<u><0.001***</u>	0.00	5	5.20	5.20	3.65	9.79
	Wild Type	5.44		0.06	5	5.40	5.50		
Arginine	UM-12115T-1	5.66	<u><0.001***</u>	0.06	5	5.60	5.70	3.56	10.37
	Wild Type	5.96		0.06	5	5.90	6.0		
Glycine	UM-12115T-1	4.50	<u><0.001***</u>	0.00	5	4.50	4.50	3.12	8.26
	Wild Type	4.74		0.06	5	4.70	4.80		
Aspartic Acid + Asparagine	UM-12115T-1	6.00	<u><0.001***</u>	0.00	5	6.00	6.00	3.92	11.33
	Wild Type	6.24		0.06	5	6.20	6.30		
Glutamic Acid + Glutamine	UM-12115T-1	34.56	<u><0.001***</u>	0.31	5	34.10	34.90	21.81	60.48
	Wild Type	37.18		0.13	5	37.00	37.30		
Threonine	UM-12115T-1	3.30	<u><0.001***</u>	0.00	5	3.30	3.30	2.31	6.14
	Wild Type	3.48		0.05	5	3.40	3.50		
Alanine	UM-12115T-1	3.70	<u><0.001***</u>	0.00	5	3.70	3.70	2.76	8.06
	Wild Type	3.84		0.06	5	3.80	3.90		
Proline	UM-12115T-1	11.44	<u><0.001***</u>	0.09	5	11.30	11.50	7.48	22.27
	Wild Type	12.28		0.05	5	12.20	12.30		
Lysine	UM-12115T-1	3.30	<u><0.001***</u>	0.00	5	13.40	13.70	2.22	6.34
	Wild Type	3.42		0.05	5	14.50	14.60		
Tyrosine	UM-12115T-1	2.94	<u>0.004**</u>	0.09	5	2.80	3.00	1.87	6.34
	Wild Type	3.12		0.05	5	3.10	3.20		
Methionine	UM-12115T-1	1.56	0.724	0.09	5	1.50	1.70	1.25	3.65
	Wild Type	1.58		0.08	5	1.50	1.70		
Valine	UM-12115T-1	4.90	<u><0.001***</u>	0.00	5	4.90	4.90	3.29	9.41
	Wild Type	5.16		0.06	5	5.10	5.20		
Leucine	UM-12115T-1	7.78	<u><0.001***</u>	0.05	5	7.70	7.80	5.25	13.44
	Wild Type	8.20		0.00	5	8.20	8.20		
Isoleucine	UM-12115T-1	4.10	<u><0.001***</u>	0.00	5	4.10	4.10	2.76	7.30
	Wild Type	4.36		0.06	5	4.30	4.40		
Phenylalanine	UM-12115T-1	5.66	<u><0.001***</u>	0.06	5	5.60	5.70	3.56	9.60
	Wild Type	6.00		0.00	5	6.00	6.00		
Tryptophan	UM-12115T-1	1.08	0.583	0.19	5	0.90	1.40	0.8	2.69
	Wild Type	1.14		0.13	5	1.00	1.30		

¹Units are in mg/g unless otherwise stated

²P-values indicating significant differences * p<0.05; ** p<0.01; *** p<0.001 are underlined and in bold.

³Standard Deviation

⁴Combined literature ranges are calculated from Heuzé et al., 2015. Values expressed in mg/g represent the absolute theoretical minimum and maximum concentrations. To account for maximum natural variance, the absolute minimum applies the lowest reported amino acid % to the lowest total protein level (8.9%), while the absolute maximum applies the highest amino acid % to the highest total protein level (19.2%). Calculations utilise the conversion: mg/g = [Amino Acid (% of protein)] × [Total Protein (% of sample)] × 0.1.

Table 26. Amino Acids, in UM-12115T-1 and Wild Type Control Wheat Seed-2023

Variable ¹	Variety	Mean	P-Value ¹	STDV ³	N	Range		Combined Literature Range ⁴	
						Min	Max	Min	Max
Histidine	UM-12115T-1	2.74	0.084	0.09	5	2.60	2.80	1.78	5.38
	Wild Type	2.90		0.16	5	2.70	3.10		
Serine	UM-12115T-1	4.94	0.120	0.15	5	4.70	5.10	3.65	9.79
	Wild Type	5.18		0.27	5	4.90	5.50		
Arginine	UM-12115T-1	5.34	0.228	0.17	5	5.10	5.50	3.56	10.37
	Wild Type	5.52		0.26	5	5.20	5.80		
Glycine	UM-12115T-1	4.20	0.084	0.12	5	4.00	4.30	3.12	8.26
	Wild Type	4.42		0.22	5	4.20	4.70		
Aspartic Acid + Asparagine	UM-12115T-1	5.68	0.213	0.19	5	5.40	5.90	3.92	11.33
	Wild Type	5.90		0.31	5	5.60	6.30		
Glutamic Acid + Glutamine	UM-12115T-1	32.94	<u>0.043*</u>	1.11	5	31.30	34.30	21.81	60.48
	Wild Type	35.18		1.76	5	33.30	37.20		
Threonine	UM-12115T-1	3.20	0.073	0.07	5	3.10	3.30	2.31	6.14
	Wild Type	3.34		0.13	5	3.20	3.50		
Alanine	UM-12115T-1	3.52	0.133	0.08	5	3.40	3.60	2.76	8.06
	Wild Type	3.66		0.17	5	3.50	3.90		
Proline	UM-12115T-1	10.82	<u>0.040*</u>	0.34	5	10.30	11.20	7.48	22.27
	Wild Type	11.56		0.59	5	10.90	12.20		
Lysine	UM-12115T-1	3.22	0.347	0.08	5	3.10	3.30	2.22	6.34
	Wild Type	3.30		0.16	5	3.10	3.50		
Tyrosine	UM-12115T-1	2.78	0.614	0.11	5	2.60	2.90	1.87	6.34
	Wild Type	2.82		0.13	5	2.70	3.00		
Methionine	UM-12115T-1	1.50	0.608	0.07	5	1.40	1.60	1.25	3.65
	Wild Type	1.48		0.05	5	1.40	1.50		
Valine	UM-12115T-1	4.74	0.094	0.15	5	4.50	4.90	3.29	9.41
	Wild Type	4.98		0.24	5	4.70	5.30		
Leucine	UM-12115T-1	7.44	0.084	0.22	5	7.10	7.70	5.25	13.44
	Wild Type	7.82		0.37	5	7.40	8.30		
Isoleucine	UM-12115T-1	3.98	0.066	0.11	5	3.80	4.10	2.76	7.30
	Wild Type	4.18		0.18	5	4.00	4.40		
Phenylalanine	UM-12115T-1	5.40	0.058	0.19	5	5.10	5.60	3.56	9.60
	Wild Type	5.74		0.29	5	5.40	6.10		
Tryptophan	UM-12115T-1	1.12	0.260	0.08	5	1.00	1.20	0.8	2.69
	Wild Type	1.22		0.16	5	1.00	1.40		

¹Units are in mg/g unless otherwise stated²P-values indicating significant differences * p<0.05; ** p<0.01; *** p<0.001 are underlined and in bold.³Standard Deviation⁴Combined literature ranges are calculated from Heuzé et al., 2015. Values expressed in mg/g represent the absolute theoretical minimum and maximum concentrations. To account for maximum natural variance, the absolute minimum applies the lowest reported amino acid % to the lowest total protein level (8.9 %), while the absolute maximum applies the highest amino acid % to the highest total protein level (19.2 %). Calculations utilise the conversion: mg/g = [Amino Acid (% of protein)] × [Total Protein (% of sample)] × 0.1.

B.5(c) Levels of any other constituents that may potentially be influenced by the genetic modification, as a result of downstream metabolic effects, compared with an appropriate comparator and natural variation.

To evaluate the potential for downstream metabolic effects resulting from the genetic modification in UM-12115T-1, a dual-analytical approach was employed to assess both intended and unintended compositional changes. This assessment incorporates the targeted quantification of NA, the primary metabolic product of the introduced trait, alongside broad-spectrum screening via Near-Infrared Reflectance (NIR) spectroscopy to detect any broader alterations in the grain's nutritional profile. By comparing the GM event against its near-isogenic wild type control and conventional reference varieties, these data provide a comprehensive

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evaluation of metabolic equivalence and ensure that the modification does not disrupt fundamental biosynthetic pathways.

Targeted Quantification of Nicotianamine in Grain

To quantify the primary downstream metabolic effect of OsNAS2 overexpression, the levels of nicotianamine were measured in grain from UM-12115T-1, the near-isogenic wild type control, and commercial reference varieties across multiple sites. This analysis confirms a targeted increase in NA flux, consistent with the intended biofortification trait, while ensuring the metabolic profile remains comparable to conventional wheat.

Multi-Year Environmental Stability of Nicotianamine Expression-Single Site

To evaluate the stability and consistency of the NA trait under varying environmental conditions, NA concentrations were measured in the grain of the UM-12115T-1 line and its corresponding wild type across three consecutive harvest years at the Dookie site (2021, 2022, and 2023). The distributions of these concentrations, along with the individual biological replicates, are presented in Figure 30.

A Two-Way ANOVA was conducted to assess the main effects of the harvest year (environment) and genetic entry (UM-12115T-1 vs. Wild Type), as well as their interaction, on grain NA concentrations.

The analysis revealed a statistically significant main effect of Year ($F=26.93_{[2,36]}$, $P < 0.001$), indicating that the distinct baseline environmental and agronomic factors present in each growing season significantly influenced the overall accumulation of NA across both wheat lines. For example, 2022 yielded higher baseline NA concentrations in the Wild Type compared to 2021 and 2023.

Importantly, the ANOVA demonstrated a highly significant main effect of Entry ($F=362.48_{[1,36]}$, $P < 0.001$). This confirms that, independent of the environmental variation between harvest years, the genetic modification in the UM-12115T-1 line results in robustly elevated levels of NA compared to the unmodified Wild type.

Furthermore, a significant interaction effect between Year and Entry was observed ($F=53.48_{[2,36]}$, $P < 0.001$). This interaction indicates that the environment actively influences the magnitude of the NA difference between the transgenic line and the wild type from year to year. However, as demonstrated visually in Figure 30, this interaction acts only on the scale of the increase rather than the overall outcome: the UM-12115T-1 line maintained consistently and significantly higher absolute NA concentrations than the wild type control across every evaluated harvest year.

The multi-year field analysis confirms that while environmental variation naturally impacts absolute NA baseline levels in wheat grain, the UM-12115T-1 line exhibits a stable, reliable trait phenotype. Across diverse growing seasons, the transgenic line consistently delivers significantly elevated NA levels relative to its conventional counterpart.

[Redacted – Confidential Commercial Information (CCI)]

Figure 30. Multi-year comparison of Nicotianamine concentrations in wheat line UM-12115T-1 and Wild Type.

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Multi-environment analysis of nicotianamine trait stability

To assess the stability of the elevated NA phenotype in wheat event UM-12115T-1 across a range of agronomic conditions, NA concentration data were pooled from four field trials representing different seasons and locations: Dookie 2023, Dookie 2024, Leeton 2025, and Moama 2025. The analysis was designed to determine whether the modified line consistently expressed elevated NA relative to its corresponding wild type and to place the observed values within the range of natural variation represented by conventional reference varieties.

For statistical analysis, technical replicates were first averaged within each independent biological replicate so that the analysis reflected biological rather than assay-level variation and avoided pseudoreplication. Comparator materials that did not have replicated biological field data within this environmental dataset, including Soybean Defatted Flour and Industry Standard ASW1, were excluded from the hypothesis test. The pooled biological replicate dataset across all environments was then analysed using a one-way Welch ANOVA at a significance level of 0.05 to compare NA concentrations among the defined wheat groups. Use of the Welch correction was appropriate for this pooled multi-environment dataset because it provides a more robust test in the presence of unequal variances and unequal sample sizes among comparison groups. Following detection of an overall treatment effect, post-hoc multiple comparisons were undertaken using Tukey's HSD procedure to identify statistically equivalent and distinct groups.

The Welch-corrected ANOVA identified a statistically significant difference in mean NA concentration among the wheat groups tested ($F_{[6, 4.909]}=29.99$, $p=0.001$). This result indicates that differences in NA expression among the wheat lines were greater than would be expected from environmental and residual variation alone, despite the intentional pooling of samples from multiple sites, seasons, and agronomic conditions. As shown in Table 27, UM-12115T-1 consistently exhibited NA concentrations above those of the wild type and the lower-NA conventional reference lines, while remaining within the broader range observed across conventional wheat germplasm.

[Redacted – Confidential Commercial Information (CCI)]

Table 27. Pooled multi-environment Nicotianamine concentrations across conventional, wild type, and transgenic wheat lines.

Across the pooled dataset, UM-12115T-1 had a mean NA concentration of [CCI] and remained clearly elevated relative to the corresponding wild type, supporting the conclusion that the introduced trait was stably expressed under diverse field conditions. The graphical presentation of the pooled data further shows that the distribution of UM-12115T-1 values clusters with the higher-NA conventional comparators rather than with the wild type or low-NA reference lines (Table 27). This is consistent with stable phenotypic expression of the intended trait across the environments examined.

Comparison with the conventional reference set further showed that the NA concentration in UM-12115T-1 fell within the range of natural variation observed among conventional wheats. In particular, UM-12115T-1 grouped with conventional high-NA reference lines, including Conventional 3 and Conventional 4, indicating that the elevated NA phenotype in the modified line is comparable to naturally occurring levels already present in conventional germplasm. Although numerical differences were observed among some conventional reference lines, not all these differences were statistically distinct following multiple-comparison testing. This outcome is consistent with the overlapping and quantitative nature of NA expression in wheat, together with the broader variance expected in a pooled multi-environment dataset where biological replicate numbers differed among reference entries.

Importantly, the pooled multi-environment dataset also showed that UM-12115T-1 did not exceed the upper end of naturally occurring NA concentrations in conventional wheat. Conventional 2 exhibited NA

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concentrations higher than those observed in UM-12115T-1, demonstrating that the modified line remains within the bounds of natural compositional variation. Taken together, the pooled field data and the statistical analysis support the conclusion that UM-12115T-1 expresses a stable elevated NA phenotype across multiple environments, while remaining compositionally comparable to conventional wheat varieties already present in the food supply.

Broad-Spectrum Compositional Screening via Near-Infrared Reflectance Spectroscopy

To evaluate the levels of constituents potentially influenced by downstream metabolic effects, a multi-year historical analysis (2022 and 2023) alongside advanced Near-Infrared Reflectance Spectroscopy assessments was evaluated. Analysis of grain cultivated in the 2022 and 2023 seasons confirmed that any minor compositional differences identified between UM-12115T-1 and the wild type Control reflected normal seasonal fluctuations and standard genotype-by-environment interactions, rather than a fundamental disruption of metabolic networks (See *Supplement Report UOM-26-COMP-RPT-01-01*).

To specifically evaluate downstream digestibility and bioavailability as a metabolic output, predictive analysis was used to assess the total amino acid profile on an "as is" basis (Table 28) and the species-specific digestible amino acid profiles for swine and broiler chickens (Table 29). The digestive bioavailability of key limiting amino acids, such as lysine, methionine, and threonine, in UM-12115T-1 aligned closely with the commercial references across both evaluated species (Table 29). Ultimately, the pooled compositional data for both forage and grain, trace mineral evaluations, and downstream digestibility profiles collectively confirm that the inserted event does not disrupt fundamental nitrogen metabolism, normal mineral ion transport, or amino acid biosynthetic pathways, demonstrating total metabolic equivalence to conventional wheat.

Conclusion of the Compositional Analysis for UM-12115T-1

The comprehensive compositional analysis of the genetically modified wheat event UM-12115T-1 provides robust evidence of its compositional and nutritional equivalence to conventional wheat varieties. By evaluating both forage and grain tissues across multiple geographically diverse environments and across multiple growing seasons, the assessment framework successfully isolated the specific genetic effect of the inserted trait from background environmental noise and natural genotype-by-environment interactions.

The pooled multi-environment models and individual site analyses confirm that the genetic modification does not induce any systemic or biologically hazardous alterations to the fundamental metabolic pathways of the plant. Across all critical nutritional domains, including proximates, dietary fibre, and estimated metabolizable energy, UM-12115T-1 proved statistically and biologically comparable to the near-isogenic wild type Control and the commercial reference varieties. The data demonstrate that fundamental nitrogen metabolism, amino acid biosynthetic pathways, and macroscopic lipid categorisations remain entirely uncompromised. Furthermore, the anti-nutritional profile, evaluated primarily through phytic acid accumulation, remained completely undisturbed, ensuring that the bioavailability of essential dietary nutrients is consistent with that of traditional wheat.

Regarding toxicological and environmental safety, UM-12115T-1 demonstrated no novel accumulation of heavy metals, nor did it exhibit a depletion of essential trace dietary minerals outside of established natural baselines. Any minor statistical deviations observed across the field trials were fractional and consistently tracked within the natural agronomic variance expected in standard commercial wheat cultivation. Ultimately, the analytical data conclusively demonstrate that the genetic insertion introduces no unintended nutritional, toxicological, or environmental hazards, confirming that UM-12115T-1 is biologically equivalent to conventional wheat and poses no novel risks to human food safety or animal feed supply chains.

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Table 28. Total Amino Acid Profile ("As Is" Basis)

[Redacted – Confidential Commercial Information (CCI)]

Table 29. Digestible Essential Amino Acids (Livestock Specific)

[Redacted – Confidential Commercial Information (CCI)]

C. Information related to the nutritional impact of the genetically modified food

Based on the comprehensive compositional analysis of both the forage and grain of UM-12115T-1, no biologically significant changes to the levels of key nutrients, toxicants, or anti-nutrients were identified when compared to the near-isogenic non-GM counterpart and standard commercial reference varieties. Furthermore, the genetic modification in UM-12115T-1 does not introduce an intended nutritional change. Because the event has been robustly demonstrated to be compositionally and metabolically equivalent to conventional wheat, the specific requirements to provide anticipated dietary intake data, altered nutrient bioavailability information, or clinical trial data are not applicable to this application. The established equivalence confirms that UM-12115T-1 will interact with the overall human diet and animal feed supply chains in a manner fundamentally identical to traditional commercial wheat varieties.

D. Other Information

Summary of *In Vivo* Poultry Feed Evaluations for UM-12115T-1 Wheat

Two independent *in vivo* broiler trials were conducted by Poultry Hub Australia at the University of New England and the South Australian Research and Development Institute (SARDI) to evaluate the nutritional efficacy of wheat event UM-12115T-1 (*Supplement Reports UOM-25-FEED-RPT-01-01 Poultry Trial 2025 and UOM-26-FEED-RPT-01-01 Poultry Trial 2026*). Both trials evaluated the GM wheat, against a conventional Australian Standard White (ASW1) control and a non-GM near-isoline comparator, the wild type control. Importantly, the basal feed formulations in both trials were standardised across all dietary treatments, ensuring that essential components such as vitamin and mineral premixes were included at equivalent baseline levels to accurately isolate the specific physiological effects of the wheat varieties.

[Quantitative performance and mortality metrics have been removed from this section as they constitute Confidential Commercial Information (CCI)]. Data from these trials indicate that incorporating UM-12115T-1 wheat into broiler diets provides a consistent advantage in growth and feed utilisation. In the University of New England trial, unchallenged birds fed UM-12115T-1 wheat demonstrated a significant improvement in their feed conversion ratio (FCR) over a 42-day period compared to the ASW1 control. The SARDI trial observed a similar numerical advantage, with UM-12115T-1 wheat producing the lowest numerical FCR across a 43-day period. Regarding weight gain, the SARDI trial demonstrated that sex-adjusted individual end-weights at Day 43 were significantly heavier for birds on the UM-12115T-1 wheat diet compared to the ASW1 control. Additionally, birds fed UM-12115T-1 wheat in the SARDI trial met or slightly exceeded the Ross 308 Australia/Pacific performance objectives for both males and females. The University of New England trial corroborated these growth benefits, noting a numerical increase in final bird weight in unchallenged birds compared to the ASW1 control.

Carcass evaluations at market age suggest that UM-12115T-1 wheat supports favourable partitioning of nutrients into lean muscle mass rather than adipose tissue. The SARDI trial reported numerically higher total meat yields in the UM-12115T-1 wheat cohort compared to the ASW1 control. Concurrently, UM-12115T-1 wheat-fed birds exhibited a trend toward reduced abdominal fat pads, with this reduction approaching statistical significance in male birds compared to the ASW1 control. The University of New England trial supported this trend, also noting numerical reductions in fat pad content for birds fed the UM-12115T-1 wheat.

The trials also assessed the impact of the wheat on animal health, welfare, and mineral bioavailability under both standard and pathogen-challenged conditions. In the SARDI trial, the adjusted mortality rate, which excluded planned standardisation removals, was lower for the UM-12115T-1 wheat cohort compared to the ASW1 cohort and the wild type. During the University of New England trial, the flock experienced an unintended *E. coli* infection from the hatchery, which compounded a planned mild coccidiosis challenge. While higher protein diets can lead to undigested protein in the hindgut that acts as a substrate for harmful bacterial growth, birds fed UM-12115T-1 wheat achieved the most efficient FCR despite the diet containing

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a higher crude protein level than the ASW1 control. This indicates that the UM-12115T-1 wheat likely imparted a protective effect on gut health and functionality that overcame the negative impacts typically associated with excess dietary protein during an enteric challenge. Furthermore, despite the potential for enhanced bioavailability of iron and zinc in UM-12115T-1 wheat, the University of New England trial found no significant influence of the UM-12115T-1 wheat on iron concentrations in the liver or breast muscle at Day 42. This suggests that intestinal regulatory mechanisms for iron absorption effectively prevent excess mineral accumulation in edible tissues.

Conclusion: Food, Animal, and Environmental Safety of UM-12115T-1 Wheat

[Quantitative performance and mortality metrics have been removed from this section as they constitute Confidential Commercial Information (CCI)]. Based on the combined findings of the University of New England and SARDI trials, the incorporation of UM-12115T-1 wheat into broiler diets presents a safe, effective, and sustainable nutritional strategy with positive implications across the production chain.

Regarding food safety and product quality, the evaluation of carcass composition indicates that UM-12115T-1 wheat supports the favourable development of lean muscle without compromising tissue integrity. Importantly, despite the potential for enhanced iron and zinc bioavailability in the UM-12115T-1 wheat, there was no significant increase in iron accumulation within the liver or breast muscle tissues of the birds at market age. This demonstrates that the birds' natural intestinal regulatory mechanisms tightly and effectively manage mineral absorption, ensuring the resulting meat products remain safe for human consumption and do not accumulate excess heavy minerals.

In terms of animal safety and welfare, the trials demonstrated that UM-12115T-1 wheat is well-tolerated and may impart physiological benefits that enhance overall flock health. The SARDI trial reported a lower adjusted mortality rate for the UM-12115T-1 wheat cohort compared to the conventional and non-GM control diets, highlighting a potential welfare advantage. Furthermore, the University of New England trial provided evidence of enhanced gastrointestinal resilience; despite the introduction of a mild coccidiosis challenge compounded by an unintended, hatchery-derived *E. coli* infection, birds fed the UM-12115T-1 wheat diet maintained superior feed conversion efficiency. This indicates that the biofortified grain likely supports robust gut health and immune competence, potentially mitigating the negative impacts of enteric diseases and the stress of excess dietary protein.

Finally, the use of UM-12115T-1 wheat offers notable environmental safety and sustainability benefits. By naturally enhancing the delivery of essential micronutrients, the widespread adoption of this GM biofortified wheat could substantially reduce the poultry industry's reliance on synthetic mineral supplements and antimicrobial interventions. Additionally, the higher inherent crude protein content of UM-12115T-1 wheat presents an opportunity to decrease the inclusion rates of external protein sources, such as soybean meal, which could lower the overall environmental footprint associated with feed formulation and agricultural transport. Coupled with the significant improvements in feed conversion efficiency, which allows farmers to feed more chickens and produce greater meat yields for the exact same amount of feed input, UM-12115T-1 wheat represents a highly sustainable alternative to conventional feed ingredients.

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