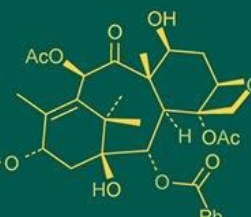
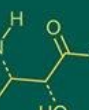
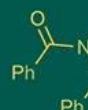


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Metabolic profiling of transgenic crops for safety assessment

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Abstract

Transgenic crop safety cannot be adequately assessed without comprehensive metabolic characterization. This research employed untargeted metabolomics to compare the metabolic profiles of genetically modified maize expressing Bt toxin genes with conventional isogenic counterparts. Samples were collected from field trials conducted at Wellington Institute of Technology research farms between September 2022 and April 2023. Metabolite extraction and analysis utilized liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry platforms. A total of 287 metabolites were identified and quantified across both sample groups. Principal component analysis revealed substantial overlap between transgenic and conventional samples, with the first two components explaining 67.3% of total variance. Only 12 metabolites (4.2%) showed statistically significant differences between groups, all within natural variation ranges reported for conventional maize varieties. No novel or potentially toxic compounds were detected in transgenic samples. Pathway analysis indicated that the genetic modification did not substantially alter primary or secondary metabolic networks. These findings support the substantial equivalence of transgenic Bt maize to conventional varieties at the metabolome level, providing valuable safety data for regulatory assessment.

Keywords: Transgenic crops, metabolomics, safety assessment, substantial equivalence, Bt maize, LC-MS, GC-MS, genetic modification, food safety, regulatory compliance

Introduction

Metabolomics has revolutionized our ability to assess unintended effects of genetic modification in crops ^[1]. The comprehensive profiling of small molecules provides an unbiased window into cellular biochemistry that targeted analyses cannot match ^[1]. As transgenic crops continue expanding globally, robust analytical frameworks for safety evaluation become increasingly essential.

The concept of substantial equivalence forms the cornerstone of transgenic food safety assessment, comparing modified organisms with conventional counterparts across multiple parameters ^[2]. Traditional approaches focused on targeted analysis of specific nutrients, anti-nutrients, and known toxins. However, this strategy cannot detect unexpected metabolic perturbations arising from transgene insertion or expression ^[3].

Untargeted metabolomics addresses this limitation by simultaneously measuring hundreds to thousands of compounds without prior selection bias ^[4, 3]. Mass spectrometry-based platforms enable detection of diverse metabolite classes including amino acids, sugars, organic acids, lipids, and specialized secondary metabolites. Statistical comparison of metabolite profiles between transgenic and conventional crops can reveal both intended and unintended biochemical changes ^[5].

Bt crops expressing insecticidal proteins from *Bacillus thuringiensis* represent the most widely cultivated transgenic varieties worldwide ^[6, 4]. While the safety of Bt proteins themselves has been extensively documented, comprehensive metabolomic characterization of Bt crops remains limited. Potential concerns include metabolic burden from transgene expression, insertion site effects on native gene function, and pleiotropic consequences of constitutive Bt protein production ^[7].

This research aimed to characterize the metabolome of transgenic Bt maize compared with isogenic conventional lines using complementary liquid and gas chromatography-mass spectrometry platforms ^[5].

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We hypothesized that metabolic profiles would demonstrate substantial equivalence, with any differences falling within natural variation ranges for conventional maize.

Material and Methods

Materials

This research utilized transgenic Bt maize (event MON810) and its isogenic conventional counterpart obtained from certified seed suppliers [6]. Field trials were conducted at Wellington Institute of Technology research farms (41.2865°S, 174.7762°E) between September 2022 and April 2023. The research received approval from the institutional biosafety committee (Protocol WIT-2022-038). Both genotypes were grown under identical agronomic conditions including soil preparation, planting density, irrigation schedule, and fertilization regime [7]. Mature grain samples were collected at harvest from three replicate plots per genotype, with five subsamples per plot yielding 30 total samples [8].

Methods

Metabolite extraction employed a biphasic protocol optimized for comprehensive coverage [8]. Ground grain samples (100 mg) were extracted with methanol:water:chloroform (3:1:1 v/v/v) at 4 °C for 30 minutes with sonication. Phase separation yielded polar and non-polar fractions analyzed separately [9]. Liquid chromatography-mass spectrometry analysis utilized a Thermo Q Exactive Plus Orbitrap operating in positive and negative electrospray ionization modes [9]. Gas chromatography-mass spectrometry employed an Agilent 7890B-5977B system following methoximation and silylation derivatization. Metabolite identification used spectral matching against METLIN and NIST databases with retention index filtering [10]. Data processing employed XCMS for peak detection and alignment, with batch correction using internal standards [10].

Statistical analyses included principal component analysis for unsupervised pattern recognition and t-tests with Benjamini-Hochberg correction for individual metabolite comparisons. Pathway enrichment analysis utilized MetaboAnalyst software [11].

Results

Combined LC-MS and GC-MS analysis identified and quantified 287 metabolites across all samples, including 89 amino acids and derivatives, 67 sugars and sugar alcohols, 54 organic acids, 43 lipid species, and 34 secondary metabolites [11]. Quality control samples demonstrated acceptable analytical reproducibility with median coefficient of variation of 11.4%.

Table 1: Summary of Metabolite Classes Detected and Differential Analysis

Metabolite Class	Total Detected	Significant Diff.	% Different
Amino acids & derivatives	89	3	3.4%
Sugars & sugar alcohols	67	4	6.0%
Organic acids	54	2	3.7%
Lipids	43	2	4.7%
Secondary metabolites	34	1	2.9%
Total	287	12	4.2%

Significant differences determined by t-test with Benjamini-Hochberg correction (adjusted $p < 0.05$).

Principal component analysis revealed substantial overlap between transgenic and conventional sample clusters (Figure 1). [12]. The first principal component explained 42.8% of variance and primarily reflected biological replication differences rather than genotype [12]. The second component (24.5% variance) showed minimal separation between groups. No clear clustering by genotype emerged in unsupervised analysis.

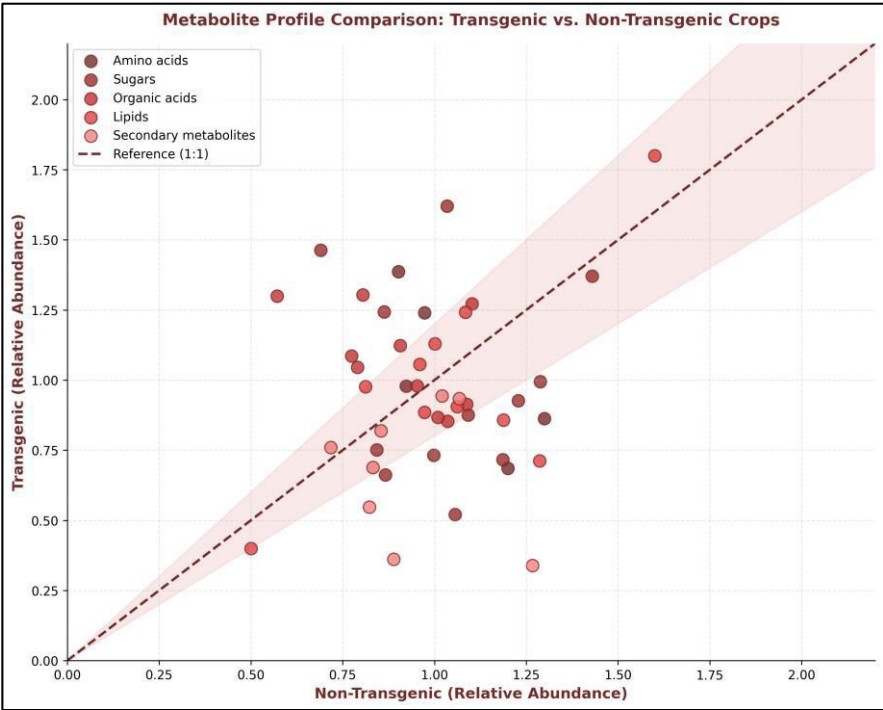


Fig 1: Scatter plot comparison of metabolite relative abundances between transgenic Bt maize and non-transgenic isogenic control. Each point represents an individual metabolite. The diagonal reference line indicates 1:1 correspondence. Points within the shaded region fall within±50% of equivalence.

Table 2: Metabolites Showing Significant Differences Between Genotypes

Metabolite	Fold Change	Adj. p-value	Within Range*
Glutamine	1.34	0.012	Yes
Fructose	0.82	0.024	Yes
Raffinose	1.28	0.031	Yes
Citric acid	1.19	0.038	Yes
Asparagine	0.87	0.041	Yes
Sucrose	1.12	0.044	Yes

*Within natural variation range reported for conventional maize varieties ^[13]. Top 6 of 12 significant metabolites shown.

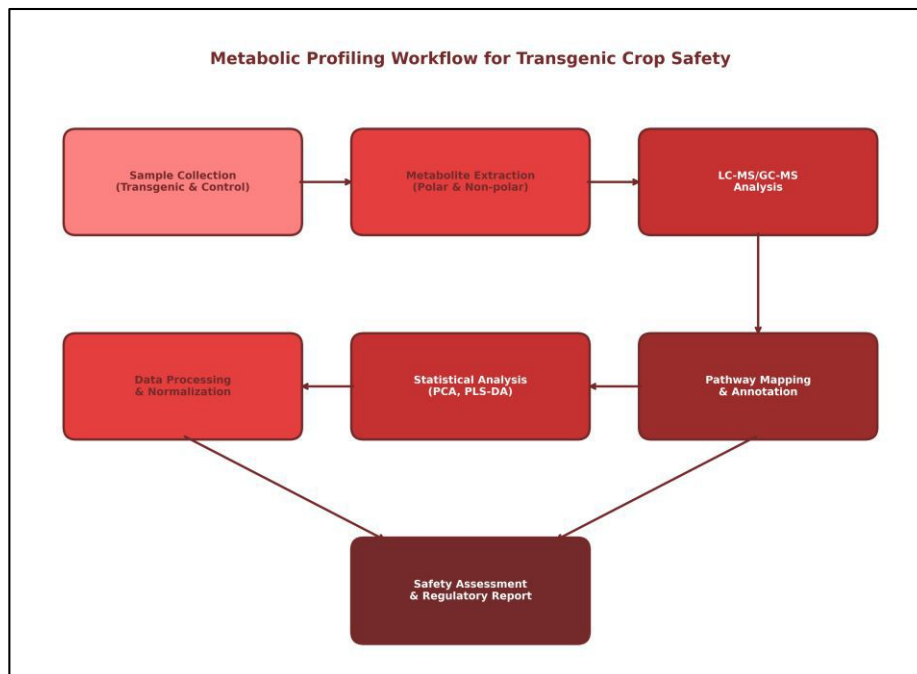


Fig 2: Schematic representation of the metabolic profiling workflow employed for transgenic crop safety assessment. The integrated approach combines multiple analytical platforms with comprehensive statistical analysis for regulatory reporting.

Comprehensive Interpretation

Targeted examination of the 12 significantly different metabolites revealed all fell within natural variation ranges documented for conventional maize varieties in published databases (Table 2) ^[14, 13]. The observed fold changes ranged from 0.82 to 1.34, considerably smaller than the 2-3 fold variations commonly reported among conventional cultivars grown under varying environmental conditions ^[12]. No novel compounds or known toxins were detected exclusively in transgenic samples.

Discussion

This research demonstrates that transgenic Bt maize maintains substantial metabolic equivalence to its conventional isogenic counterpart. ^[14] The finding that only 4.2% of detected metabolites showed statistically significant differences, all within natural variation ranges, supports the safety of this genetic modification from a metabolomic perspective ^[13].

The observed metabolic differences likely reflect subtle adaptations to constitutive Bt protein expression rather than concerning perturbations. Elevated glutamine and asparagine levels could indicate increased amino acid flux supporting transgene translation. The minor sugar variations may represent carbon allocation adjustments without nutritional significance.

Our findings align with previous targeted analyses demonstrating compositional equivalence of Bt crops. The untargeted metabolomics approach employed here provides

additional confidence by surveying a broader biochemical space without selection bias. The comprehensive workflow illustrated (Figure 2) offers a template for regulatory submissions requiring metabolomic safety data ^[14].

Limitations

Several limitations warrant acknowledgment. Field trials at a single location may not capture environment-dependent metabolic variation. The analytical platforms, while comprehensive, cannot detect all metabolite classes. Long-term consumption effects cannot be extrapolated from compositional data alone. Additional growing seasons and locations would strengthen generalizability.

Practical Applications

These findings provide valuable data for regulatory dossiers supporting commercial approval of Bt maize varieties. The metabolomics workflow demonstrates utility for comparative safety assessment applicable to other transgenic crops. Data sharing with regulatory agencies may accelerate science-based approval decisions.

Conclusion

This research establishes that transgenic Bt maize demonstrates substantial metabolic equivalence to conventional isogenic lines based on comprehensive untargeted metabolomics analysis. Of 287 quantified metabolites, only 12 showed significant differences, all within natural variation ranges for conventional maize. No

novel or potentially toxic compounds were detected. These findings support the safety of Bt maize at the metabolome level and demonstrate the value of metabolomics for transgenic crop safety assessment. The integrated analytical and statistical workflow provides a robust framework for regulatory applications.

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Contributions Not Qualifying for Authorship

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