Project title: Integration of transcriptome and meabolome analyses in sugarbeet wounding response in comparison to the wild species of *Beta maritima* (Project #621)

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Background

Sugarbeet (*Beta vulgaris* subsp. *vulgaris*) is a cornerstone of U.S. agriculture, accounting for approximately 55-56% of total domestic sugar production (USDA-ERS, 2025). The transition from field to factory involves mechanical harvesting, which necessitates the removal of leafy tops through either mechanical defoliation (flail removal) or scalping (slicing the crown) (Eckhoff et al., 2023). While efficient, these mechanical processes cause significant wounding to the root tissue. This wounding trauma triggers intense metabolic shifts, specifically an increase in glycolysis and fermentation as the plant attempts to manage the stress (Fugate et al., 2025). Historically, postharvest injury has been linked to increased respiration rates and heightened susceptibility to microbial infections, leading to substantial sucrose loss during storage (Halvorson et al., 1978).

Despite these impacts, the wound-healing capabilities of commercial cultivars versus wild relatives remain largely unstudied. This research identifies sea beet (*B. vulgaris* subsp. *maritima*) as a potential "genetic reservoir" to enhance the natural wound-healing capacity and storage life of modern cultivars. Therefore, the aim of this study was to bridge the knowledge gap regarding the genetic mechanisms of wound healing by investigating the transcriptional and metabolomic responses of a commercial sugarbeet cultivar and two wild sea beet lines to harvest-induced injury.

Materials and Methods

The study utilized a comparative framework involving a commercial sugarbeet cultivar: *Beta vulgaris* (BTS), and two wild lines: *Beta maritima* (P334 and P408). A total of 60 sugarbeet samples were analyzed across different injury types (defoliated vs. scalped) and time intervals. Tissue was collected at: day 0: Freshly harvested controls and day 15 and day 30: post-injury (defoliated and scalped). Following wounding, sugarbeet roots were stored at 4 °C and 95% relative humidity to simulate storage conditions in a commercial sugarbeet pile.

Sugarbeet root tissue collected from the wounded site of the root at different time points was snap-frozen in liquid nitrogen and ground into a fine powder. Total RNA was extracted from the root tissue using a RNeasy® Plus Mini Kit (Qiagen GmbH, Hilden, Germany). The quality and quantity of total RNA were analyzed using Bioanalyzer 2100 (Agilent Technologies, CA, United States) and acceptable RIN number for samples >7.0. High quality RNA was used for mRNAseq library preparations. RNA sequencing libraries were prepared according to the Illumina's TruSeq-Stranded-RNA sample preparation protocol, followed by adapter trimming of the raw sequence reads via Trimmomatic (v 0.38). Sequence reads were aligned to the sugarbeet reference genome (EL10.2) using STAR (v2.7.11b) (Dobin et al., 2013) and Samtools (ver. 1.17) was used to convert SAM format to BAM format. The read counts were calculated using featureCounts (ver. 2.0.3). Identification of differentially expressed genes and further downstream analysis were conducted with an integrative RNA-seq analysis platform, iDEP 2.01

(Ge et al., 2018) with the default parameters. Metabolites were extracted in 800 µL of 80% methanol from ~50 mg fresh weight of finely ground sugarbeet root tissues prepared using Geno/Grinder 2010 instrument. A Liquid chromatography-mass spectrometry (LC-MS) (Waters, UPLC; Thermo, Q Exactive) system with an Acquity UPLC HSS T3 (2.1 × 100 mm; 1.8 µm) chromatographic column was used for metabolite analysis. The raw files (.raw) were imported into Compound Discoverer3.1 (CD) to perform spectral processing and database search for qualitative and quantitative analyses of detected metabolites. The biological significance of metabolites was determined through enrichment of metabolites associated with specific metabolic pathways. Metabolites were visualized using the 'pheatmap' package (Kolde and Kolde, 2015) in R (v1.0.12) with default parameters except for scale = "row" (https://CRAN.R-project.org/package=pheatmap).

Results and discussion

The study identified 29,299 unique genes across 60 samples from a commercial sugarbeet cultivar (BTS) and two wild sea beet lines (P334 and P408). Significant transcriptional divergence was observed between wild lines and a commercial cultivar regarding their response to injury as shown in the PCoA plot (Figure 1). The findings presented in this report are based on observations made from scalped beets on day 15. The P334 line (scalped at day 15) exhibited 3,801 upregulated genes and over 15 enriched pathways (Figure 2). The most prominent mechanism was Phenylpropanoid biosynthesis (44 genes), which is essential for structural fortification in wounded tissues. Key genes included Cationic peroxidase 1 (gene ID: *LOC104898205*) and Phenylalanine ammonia-lyase (gene ID: *LOC104898673*) (Data not shown). Phenylalanine ammonia-lyase (PAL) is regarded as the "gatekeeper" gene and it converts phenylalanine into cinnamic acid, the first step in creating phenolic compounds (Vogt, 2010). Its high expression in wild lines suggests a rapid commitment to secondary metabolism following mechanical injury. While PAL generates the necessary building blocks (precursors), peroxidase facilitates the final polymerization of monolignols into lignin, establishing a recalcitrant physical barrier at the wound surface (Dixon and Paiva, 1995). Furthermore, Shikimate O-hydroxycinnamoyltransferase (gene ID: *LOC104884151*) was significantly differentially expressed. This enzyme is critical for redirecting carbon flow toward the synthesis of lignin precursors over soluble phenolics to ensure that the plant's energy is prioritized for physical repair (Hoffmann et al., 2004). Additional enrichment in P334 included the Zeatin biosynthesis (11 genes) which involved in hormonal regulation of cell division via adenylate isopentenyltransferase (gene ID: *LOC104896430*). Tryptophan metabolism (19 genes) was the third most enriched pathway in line P334. This pathway links secondary metabolism to defense signaling through enzymes such as tryptophan decarboxylase (TDC2) and tyrosine decarboxylase (Höglund et al., 2010).

Meanwhile, line P408 (scalped at day 15) exhibited 3,592 upregulated genes across 12 pathways (Figure 2), with a distinct emphasis on oxidative stress management via Glutathione metabolism (31 genes) (Figure 3). High expression of L-ascorbate peroxidase 3 and 5 (gene ID: *LOC104895166*, and gene ID: *LOC104895169*, respectively) (Data not shown) suggests a superior capacity to neutralize reactive oxygen species (ROS) at the wound site. These genes protect the cell from hydrogen peroxide (H_2O_2) accumulation (Caverzan et al., 2012). By keeping oxidative stress in check, the wild line prevents "runaway" programmed cell death, allowing healthy cells adjacent to the wound to divide and heal (Caverzan et al., 2012). In addition to phenylpropanoid biosynthesis (31 genes), P408 showed enrichment in Flavonoid biosynthesis

(16 genes) (Figure 4). This pathway features Chalcone synthase (gene ID: *LOC104905367*) and Flavanone 3-hydroxylase (gene ID: *LOC104905155*), which were highly active in scalped roots by day 15. Chalcone synthase is the rate-limiting enzyme for flavonoid production; in the wounded tissue, it facilitates the synthesis of phytoalexins, natural antimicrobial compounds that inhibit microbial during storage (Dao et al., 2011).

From the observation above, the superior wound-healing capacity of wild sea beets is driven by three primary biological pillars: physical sealing, chemical defense and metabolic efficiency. Physical sealing: the Phenylpropanoid pathway supplies precursors that Cationic peroxidase 1 polymerizes into lignin, creating a physical seal that prevents moisture loss and pathogen entry. Chemical defense: upregulation of Chalcone synthase facilitates the production of chrysophanic acid and rhein observed in line P334 (Figure 5). These anthraquinone derivatives protect the wound while promoting cell proliferation to close the injury site. Compounds, particularly methoxylated phenolics like veratrole (linked to caffeic acid 3-O-methyltransferase genes, gene ID: *LOC104887972*) act as chemical deterrents against microbial rot which is important in minimizing sucrose loss during sugarbeet storage. Metabolic Efficiency: minimizing sucrose loss currently caused by high respiration in slow-healing commercial cultivar like BTS (Fugate et al., 2016).

From a breeding perspective, genes like *LOC104898205* (involved in lignification) are prime candidates for Marker-Assisted Selection (MAS) to improve healing from wound and mechanical injury stress. Introgressing glutathione metabolism traits from P408 could improve cellular integrity in storage piles (Fugate et al., 2016), directly increasing extractable sucrose yields. Modern genomic tools, such as CRISPR-Cas9, offer the potential to upregulate these phenylpropanoid pathways in commercial cultivars, bypassing the "yield drag" often associated with traditional wide-crossing.

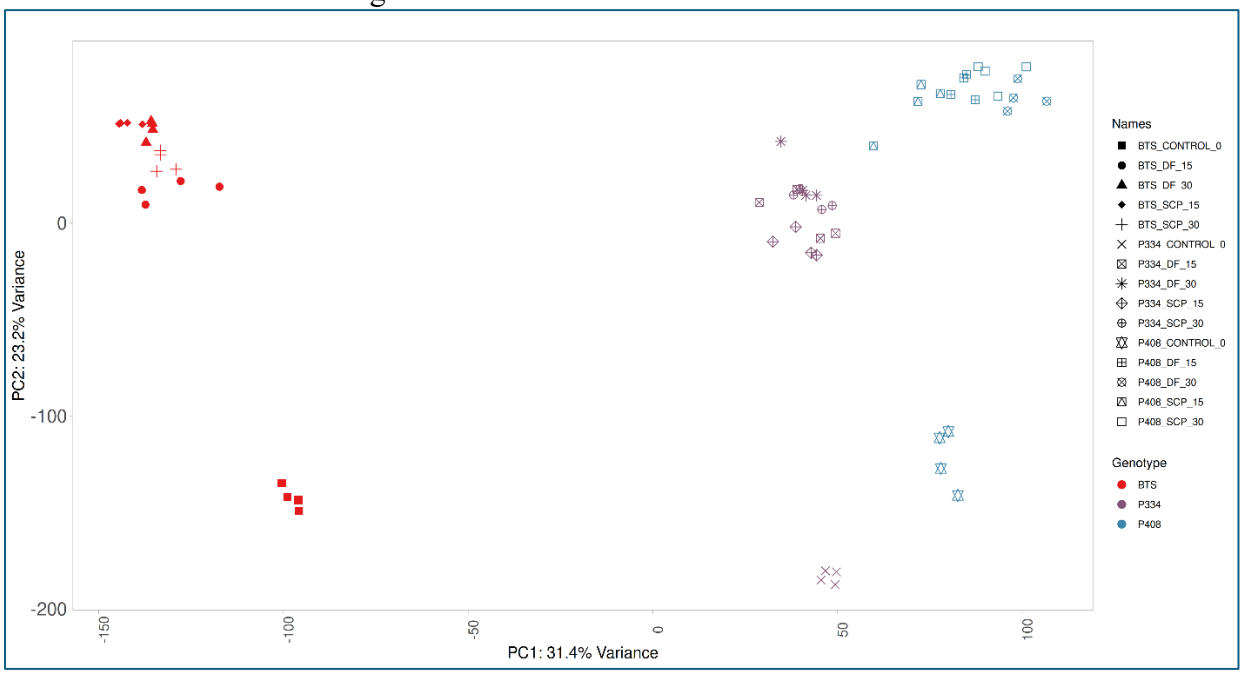

Figure 1: Principal coordinates analysis (PCoA) of RNA-seq transcriptomes from three sugarbeet genotypes (BTS, P334, and P408) subjected to defoliation (DF) or scalping (SCP) treatments. Data represents samples at harvest (Day 0) and following 15 or 30 days of storage at

4 °C. Legend abbreviations: CONTROL_0 (harvest), DF_15/30 (defoliated for 15/30 days), and SCP_15/30 (scalped for 15/30 days).

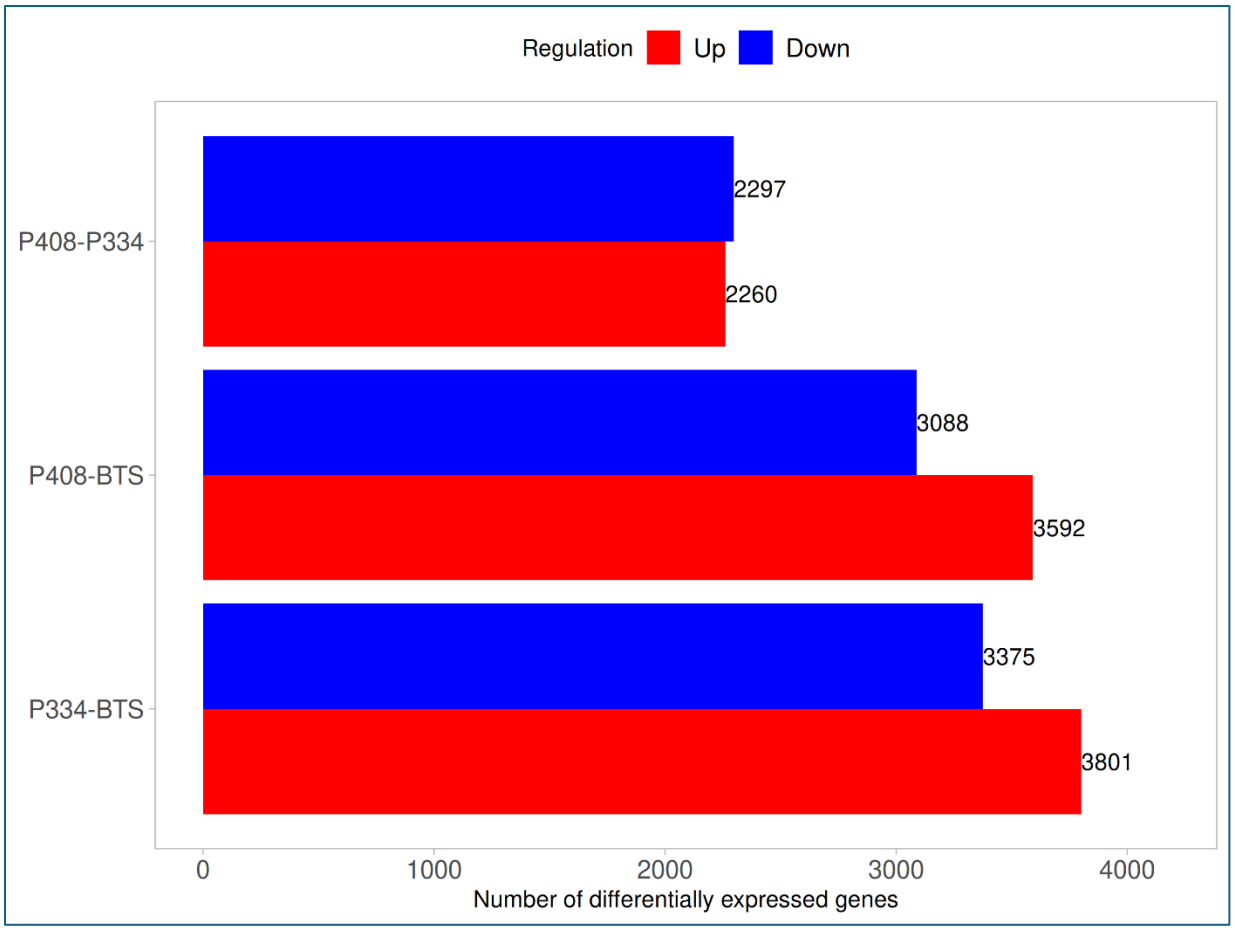

Figure 2: Number of differentially expressed genes in wild sea beet lines (P334 and P408) compared to a commercial line (BTS) and each other after 15 days of storage at 4 °C.

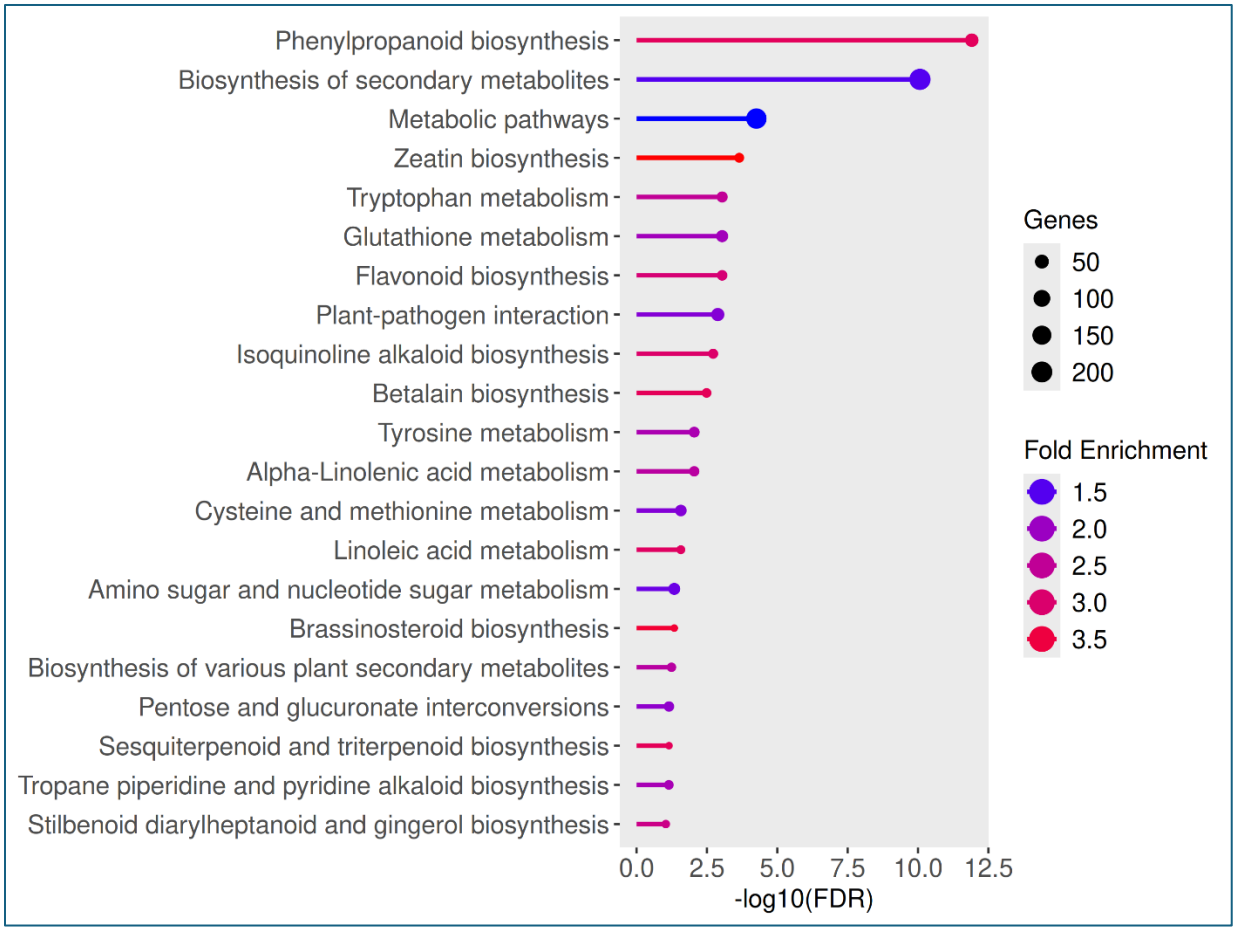

Figure 3: Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of target genes. This analysis identifies sugarbeet gene targets regulated by differentially expressed (DE) RNAs in scalped roots (P334) following 15 days of storage at 4 °C.

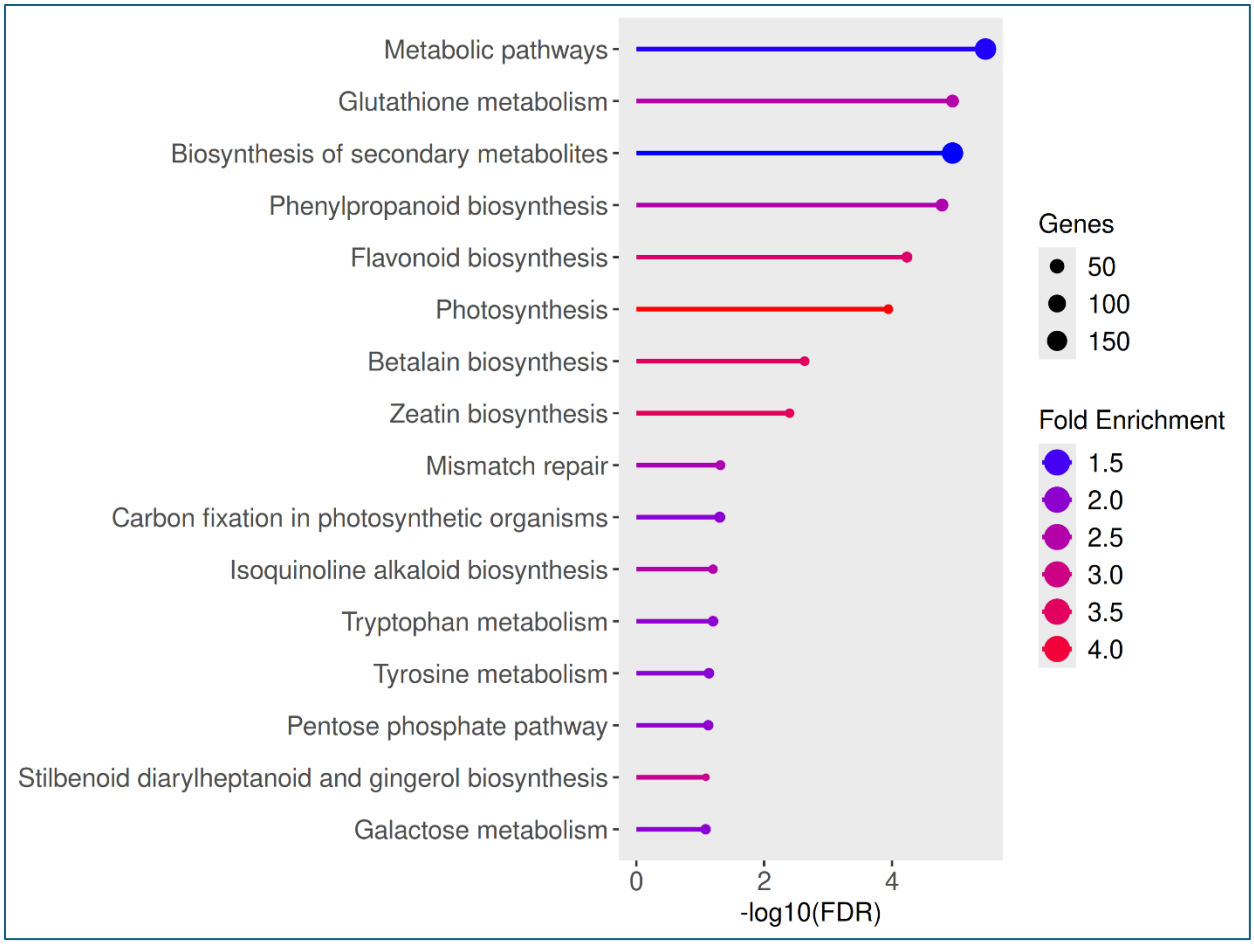

Figure 4: Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of target genes. This analysis identifies sugarbeet gene targets regulated by differentially expressed (DE) RNAs in scalped roots (P408) following 15 days of storage at 4 °C.

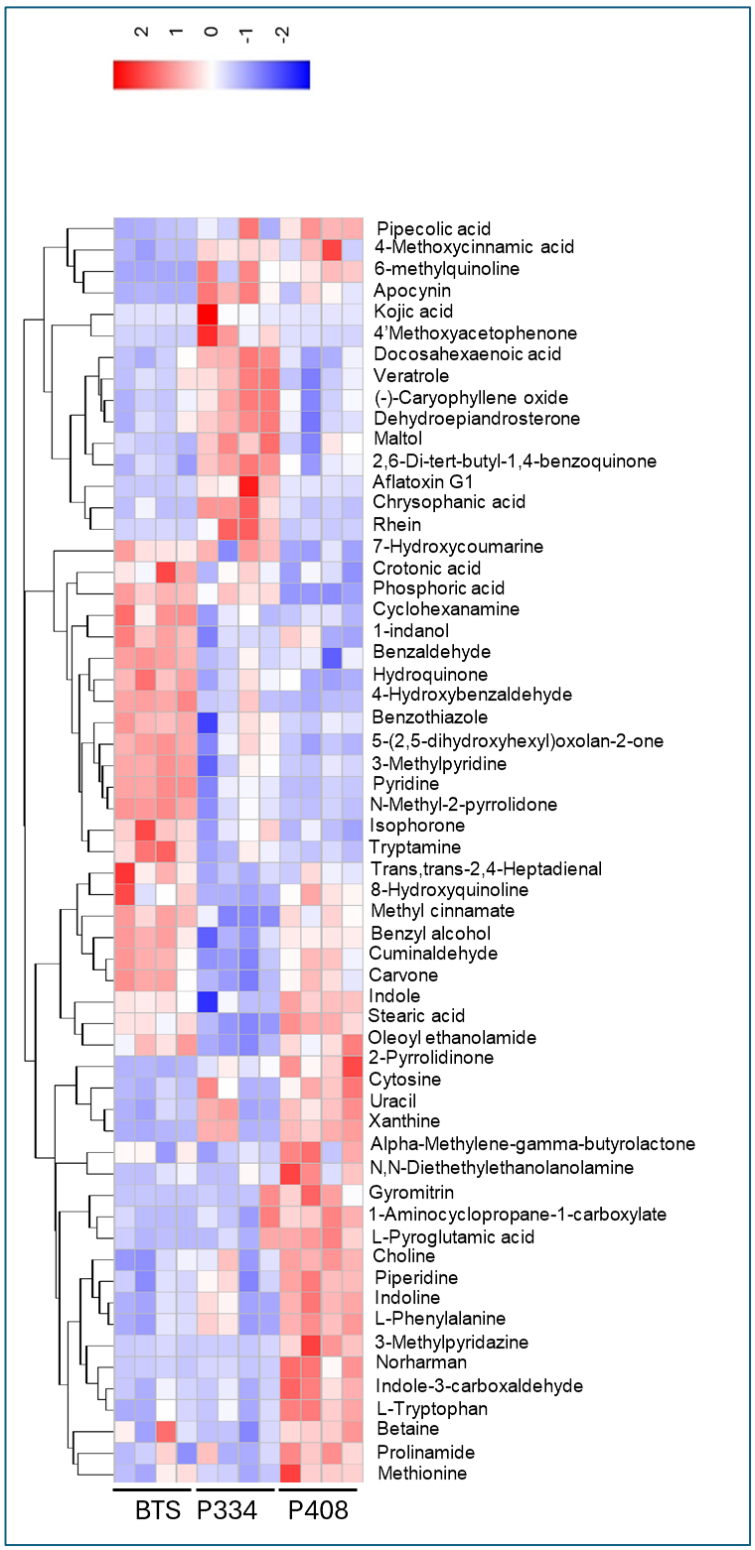

Figure 5: Untargeted metabolome analysis (+ve ion mode) of sugarbeet roots showed distinct metabolic patterns with the genotype at D15 scalped roots.

Conclusion

Wound healing is a complex physiological process involving the synchronized activation of diverse metabolic pathways to rebuild structural barriers and synthesize defense-related compounds. This research highlights that the transition from a wounded state to a stabilized root requires a coordinated genetic response, demonstrating that the wild lines P334 and P408 possess specialized genetic "toolkits" for rapid wound recovery that have likely been diluted in commercial cultivars. By identifying genes with sustained upregulation, particularly within the phenylpropanoid, glutathione, and flavonoid biosynthetic pathways, this study provides a clear framework for marker-assisted breeding. Leveraging these specific genes offers a roadmap for breeding sugarbeets with significantly improved storage characteristics, as enhancing these pathways in commercial lines can drastically improve postharvest outcomes by accelerating the formation of protective suberin and lignin layers while protecting the tissue from microbial invasion and oxidative damage. This fortification directly mitigates the respiration and microbial decay that leads to significant sucrose loss. Moving forward, the focus of this research will shift toward assessing the persistence of these upregulated genes in 30-day post-injury and defoliated samples to ensure that these protective mechanisms remain active throughout the entire storage lifecycle.

Acknowledgements

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