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Soybean response to low glyphosate doses: Insights into the transcriptome, metabolic pathways and biomass production

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Abstract: Background: Soybean cultivars show a positive response to low doses of glyphosate, called glyphosate hormesis, though the genetic and biochemical mechanisms are not fully understood. **Objective:** This study aimed to identify the pathways and differentially expressed genes (DEGs) related to glyphosate hormesis, along with their association with lignin and P content, and the shikimic acid pathway in glyphosate-resistant cultivars (RR and RR2) compared to a non-resistant cultivar (NR). It also evaluated how glyphosate hormesis impacted the electron transport rate (ETR) and biomass accumulation. **Results:** With 90% of reads matching the soybean reference genome, 31,515 genes were identified, including 587 DEGs. The DEGs enriched by glyphosate application in the NR cultivar were associated with flavone metabolism and auxin signaling, indicating impacts on phenylalanine-derived metabolites and developmental processes. In contrast, the RR cultivar showed enrichment in pathways related to stress response and fatty acid metabolism, while the RR2 cultivar exhibited enrichment in various metabolic pathways, notably lignin catabolism and auxin activity. Glyphosate treatments influenced electron transport rate (ETR), lignin, and P content, as well as components of the shikimic acid pathway and amino acid contents in soybean plants, with differential responses observed among cultivars and glyphosate doses. Overall, glyphosate hormesis led to increased dry biomass production across all cultivars. **Conclusion:** These findings provide insights into the molecular and physiological responses of soybean cultivars to glyphosate hormesis, highlighting the complex regulatory mechanisms that enable unique adaptations in each cultivar to the stress imposed by low herbicide doses.

Keywords: Aromatic amino acids; Differentially expressed genes; Electron transport rate; Glycine max (L.) Merrill; GO enrichment; Lignin content; Shikimic acid pathway

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1. Introduction

Soybean is the main crop in Brazilian agriculture, and in the 2023/24 season, it covered 45.7 million hectares, accounting for 60% of the land used for the country's five main grains (Companhia Nacional de Abastecimento, 2024). More than 90% of soybean varieties cultivated in Brazil present glyphosate-resistant (GR) traits (Umburanas et al., 2022). Around 36% of this crop belong to the first-generation (RR) varieties, and 60% are second-generation (RR2) (Cheng et al., 2024). Both technologies express an isoform of 5-enolpyruvylshikimate-3-phosphate synthetase (EPSPS) insensitive to glyphosate from *Agrobacterium* sp. They are distinguished by the fact that RR cultivars carry the original cp4-epsps gene accompanied by the 35S promoter, while RR2 cultivars contain a modified cp4-epsps gene accompanied by the FMV (fig mosaic virus) promoter (Krenchinski et al., 2024b). Although the use of these transgenic varieties simplifies weed management and improves harvest quality, the yields of RR and RR2 varieties are not greater than those observed in conventional non-resistant cultivars (Umburanas et al., 2022). Thus, one of the main challenges for soybean cultivation is to increase production without expanding into new regions of native vegetation. Therefore, the development of innovative techniques and practices that improve soybean yields without increasing production costs are crucial.

Glyphosate is a non-selective systemic herbicide, typically applied at concentrations ranging from 360 to 2,160 g ae ha^{-1}, depending on the weed species in the area. This herbicide kills plants by inhibiting the enzyme EPSPS, thereby disrupting the shikimate pathway (Duke, 2018). However, plant death can also result from indirect or secondary effects of glyphosate on processes such as photosynthesis, mineral nutrition, carbon assimilation and/or translocation, and oxidative stress (Freitas-Silva et al., 2021; Almeida et al., 2024). GR crop plants may or may not exhibit these effects, depending on the species and cultivar (Duke et al., 2018), as well as the dose of glyphosate applied. Recommended field doses of glyphosate are generally safe for RR and RR2 crops; however, when these plants exhibit physiological and metabolic alterations caused by glyphosate, these effects are typically less pronounced than in glyphosate-susceptible (GS) plants. In contrast, it is well-documented that low doses of glyphosate, relative to recommended field doses, can stimulate the growth of plants (Velini et al., 2008; Brito et al., 2018; Cite Duke et al., 2025).

The growth stimulation or strengthening of an organism caused by a harmful substance or stress at low doses is a phenomenon known as hormesis. Some researchers have even highlighted herbicide hormesis as a potential tool to improve crop yield (Belz et al., 2011; Abbas et al., 2017; Agathokleous et al., 2024). One possible explanation for the expression of glyphosate hormesis in plants is the reduction in the activity of EPSPS in the shikimic acid pathway (Duke et al., 2025). This leads to a decrease in lignin levels, allowing more carbon to be directed towards the production of sucrose, which results in an increase in plant growth (Velini et al., 2008; Jalal et al., 2021). Another explanation is that low doses of glyphosate can increase phosphorus absorption by plants (Pereira et al., 2019). In previous studies, glyphosate hormesis was evaluated through dose-response assays in RR and RR2 soybeans, compared to a non-resistant (NR) cultivar, using both foliar and seed treatments. The experiments in greenhouse conditions consistently showed increases in growth and biomass accumulation (Krenchinski et al., 2024a), and improvements in agronomic traits were observed in field conditions (Krenchinski et al., 2024b). The range of low doses of glyphosate that were safe and consistently stimulated hormetic effects were 0, 11.25, and 22.5 g ae ha^{-1} for the NR cultivar, and 0, 45, and 90 g ae ha^{-1} for the RR and RR2 cultivars (Krenchinski et al., 2024a). However, the genetic, biochemical and metabolic mechanisms involved in glyphosate hormesis observed in soybean are still not fully understood (Duke et al., 2025), as various effects can be observed in plants expressing glyphosate hormesis (Brito et al., 2018; Jalal et al., 2021).

Glyphosate primarily moves through the symplast and accumulates in young meristematic tissues. However, there is also significant movement through the apoplast. The movement of glyphosate from the apoplast to the symplast may be facilitated by phosphate transporters (Pereira et al., 2019), as the phosphate group of glyphosate competes with phosphorus (P) for transport into the cell (Mervosh, Balke, 1991). When cells detect a P deficiency, the expression of highly specific P transporter genes is activated (Rouached et al., 2010). Studies using low doses of glyphosate showed that soybean and eucalyptus plants absorb more P than untreated plants (Jalal et al., 2021).

EPSPS plays a crucial role in the production of the aromatic amino acids phenylalanine, tyrosine, and tryptophan in the shikimic acid pathway (Almeida et al., 2024). The inhibition of this enzyme by glyphosate affects not only protein production, but also the phenylpropanoid pathway and, consequently, the production of lignin (Zobiole et al., 2010; Duke et al., 2018). Phenylpropanoid and lignin pathways represent up to 35% of a plant's biomass (Brito et al., 2018). In the cytosol, the lignin pathway begins with phenylalanine ammonia lyase (PAL), which converts phenylalanine into t-cinnamic acid (Zhang, Liu, 2015). Lignin performs important functions in the plant, such as support, and facilitates the efficient transport of water and solutes over long distances in the vascular system (Bernards et al., 2000). However, there is also evidence that an increase in the amount of lignin can reduce plant growth as well as biomass accumulation (Novaes et al., 2010).

One approach to understand the glyphosate hormetic effects in plants is through transcriptome sequencing (RNA-seq), comparing plants treated with low doses of glyphosate and untreated plants. Complete sequencing of transcriptomes of soybean can identify which genes are most expressed in stressed plants (Li et al., 2022), covering diverse possible hypotheses that elucidate the effect of glyphosate hormesis, in addition to defining new concepts of action, providing a valuable source of knowledge, and supporting future studies in the area. This study hypothesized that soybean plants treated with low doses of glyphosate might experience changes in their lignin and P levels. Moreover, RNA-seq techniques could uncover additional hypotheses by identifying genes involved in stimulating soybean growth after applying low doses of glyphosate. Therefore, further research on this topic is warranted to better understand how soybean plants respond to low doses of glyphosate, with the goal of obtaining insights that will enable the use of hormesis to improve crop yield (Belz et al., 2011; Abbas et al., 2017; Agathokleous et al., 2024).

The objective of this study was to identify enriched pathways and differentially expressed genes (DEGs), and determine their correlation with lignin and phosphorus content in RR and RR2 soybean cultivars compared to a NR cultivar when treated with low doses of glyphosate (ranging from 0 to 90 g ae ha^{-1}), herbicide concentrations that were found to be safe and induced hormesis in this crop (Krenchinski et al., 2024a). Furthermore, the study also showed how these treatments affected the electron transport rate (ETR), components of the shikimic acid pathway, and biomass accumulation.

2. Materials And methods

2.1 General characteristics of the experiments

The experiments were conducted in a greenhouse during the summer of 2021, where the average temperature was 26°C ± 2, a relative humidity of 60%, and natural light (with an average photoperiod of 13 h). The soybean cultivars utilized were CD383 (NR), BMX-Tornado (RR), and M5917 IPRO (RR2). These cultivars were planted in 2.8 L pots (5 seeds per pot) filled with a substrate composed of sphagnum peat, vermiculite, and carbonized rice husk, with a pH of 5.7 (±0.5). Thinning was performed after emergence, leaving two plants per pot. Each pot represented an experimental unit. When plants reached the V3 vegetative stage (three fully expanded trifoliates), they were treated with glyphosate (Roundup Transorb R, glyphosate potassium salt, 480 g ae L^{-1}, Monsanto do Brasil Ltda.) at 0, 11.25, and 22.5 g ae ha^{-1} for the NR cultivar and 0, 45 and 90 g ae ha^{-1} for cultivars RR and RR2, respectively. Spraying

was conducted in a closed application room equipped with an application bar containing six XR110.02 nozzles spaced 0.5 m apart and positioned 0.5 m high above the plants, with a constant pressure of 2 bar and a displacement speed of 1 m s^{-1}, delivering a spray volume of 200 L ha^{-1}.

The experiments were conducted in a completely randomized design with 20 experimental units of each cultivar per herbicide dose. Furthermore, the experiments were repeated twice at different times, except for RAN-Seq analyses. For each repetition of experiments, six experimental units were allocated for RNA-Seq, five for P and lignin analysis, five for evaluating the components of the shikimic acid pathway. The remaining four experimental units were used for assessing ETR. Additionally, at 28 DAA, all plants were harvested to evaluate biomass production.

2.2 Physiology and Metabolism

2.2.1 *Electron Transport Rate (ETR)*

The ETR was assessed at 7, 14, 21, and 28 days after application (DAA) using a portable fluorometer (Multi-Mode Chlorophyll Fluorometer OS5p – Opti Sciences). Readings were taken at six points on the youngest, fully expanded leaf of each treatment and replicate.

2.2.2 *Phosphorus (P), Dry Mass and Lignin Content*

To determine P content, the plants were cut at 28 DAA, stored in paper bags, and dried at 60°C until reaching a constant weight. Subsequently, they were weighed. P content was determined through acid digestion followed by the colorimetric method (Majed et al., 2012).

For lignin content, the soybean plants were collected at 10 DAA and dried at 40°C until constant weight. Subsequently, they were ground in a Wiley-type mill. Lignin content was determined using the acid detergent lignin method (LDA), where the sample was treated with an acid detergent solution, resulting in acid detergent fiber (ADF). Following this, the sample underwent digestion with at 72% sulfuric acid solution (Van Soest, 1965). This technique was adapted using 100 bags of non-woven fabric (with a porosity of 100 microns). After digestion, the bags were autoclaved (120°C for 30 min) and placed in a 20 L recipient containing 50 mL of acid detergent solution per sample. They were pre-washed with cold water to remove excess detergent, then rinsed five times with hot distilled water (each rinse lasting 5 min). Subsequently, the samples were drained, immersed in acetone for 5 min, dried at 105°C, and finally incinerated in a muffle furnace. The lignin values (expressed as a percentage) were measured relative to the initial dry mass.

2.2.3 *Components of the Shikimic Acid Pathway*

Samples designated for this experiment were collected at 10 DAA and immediately frozen and stored at -80°C until processing. Samples were macerated with liquid nitrogen, and a 100 mg was weighed and transferred to 15 mL tubes. Then, 10 mL of water extraction solution, acidified with formic acid to a pH of 2.5, was added. Samples were ultrasonicated for 30 min and centrifuged at 4,000 rpm for 10 min. The resulting supernatant was filtered using Millipore 0.2 µm filters and transferred to vials (1.5 mL) for analysis by LC-MS/MS to determine the components of the shikimic acid pathway, including internal glyphosate content, AMPA, shikimic acid, coumaric acid, benzoic acid, and salicylic acid, as well as the amino acids phenylalanine, tyrosine, and tryptophan.

The LC-MS/MS system utilized consisted of a High-Performance Liquid Chromatograph (HPLC), Shimadzu model Proeminence UFLC, equipped with two LC20AD pumps, SIL-20AC auto injector, DGU-20A5 degasser, CBM20A controller system (enabling fully automated operation), and CTO-20AC oven for column temperature control. Coupled to the HPLC was the 4500-hybrid triple quadrupole mass spectrometer (Applied Biosystems). Chromatographic analyses were conducted using a C18 column (Phenomenex Gemini 5µ C18RP 110Å) with an injection volume of 20 µL. The mobile phase consisted of 5 mM ammonium acetate in water (phase A) and 5 mM ammonium acetate in methanol (phase B). The flow rate was set at 0.8 mL min^{-1}, and the gradient mode initiated with a 90:10 ratio of water to methanol, transitioning to 5:95 at 4 min, and returning to the initial condition at 10 min, with a total runtime of 15 min.

2.3 RNA extraction and Transcriptome Analysis

From the six experimental units allocated to this experiment, two pooled samples were taken, each consisting of three biological replicates. All plant leaves were collected at 10 DAA and samples were promptly frozen in liquid nitrogen upon collection and stored at –80°C until analysis.

Total RNA extraction was conducted using the Trizol Reagent method (Invitrogen/Life Technologies, Carlsbad, CA), following the manufacturer's instructions. Samples were macerated with liquid nitrogen and approximately 300 mg of each were transferred to 2 mL tubes. To each tube, 1 mL of Trizol was added, followed by vortexing for homogenization. After a 5-min incubation at room temperature, the samples were centrifuged at 12,000 rpm at 4°C for 15 min, and the supernatant was transferred to new tubes. Then, 200 µL chloroform were added to the samples, vortexed, and incubated for 3 min. Subsequently, they were centrifuged at 12,000 rpm at 4°C for 15 min, and the supernatant transferred to new tubes. Next, 500 µL of 100% isopropanol alcohol (–20°C) was added to each tube, followed by homogenization and incubation at –20 °C for 30 min. Samples were then centrifuged at 13,200 rpm at 4°C for 30 min, and the supernatant was discarded. The resulting pellet was washed with 1 mL of 70% ethanol (DEPC-treated water), centrifuged at 8,000 rpm at 4°C

for 5 min, and the supernatant was discarded. Finally, the pellet was air-dried and resuspended in 20 µL of H_2O DEPC.

RNA was quantified in a Nano Drop ND-1000 Spectrophotometer (Saveen 1 Werner, Malmo, Sweden), and its quality and integrity (1 µg of RNA) were assessed on a 1% agarose gel. Total RNA samples were treated with DNAseI-RNAse free according to the manufacturer's instructions (Promega Biosciences, Wisconsin, USA) to eliminate potential genomic DNA contamination. RNA integrity was verified, ensuring a RIN (RNA Integrity Number) of at least 8 (Agilent 2100 Bioanalyzer; Agilent Technologies, Germany). Samples were stored at –80 °C until use.

Genomic library construction involved comparing treatments with low doses of glyphosate and control (without application) within each studied cultivar. For RNA-Seq analysis, 1000 ng of total RNA was utilized. Samples were indexed with adapters and subjected to 2X sequencing of 100 base pairs using a HiSeq 2500 instrument (Illumina, United States). Sequencing was performed on 18 RNA samples in one lane of the flow cell, following the manufacturer's instructions. The data output in Fastq format included sequence information, including sequencing quality (phred quality score).

Only average phred scores ≥20 per position were utilized for alignment. However, out of the 18 samples, two libraries (RR GLY0 and RRGLY45) did not group in the sequencer, failing to generate sequencing data, and were consequently excluded from the analysis and results due to the inability to replace them.

2.4 Data Analysis

Physiological and metabolic data underwent the Shapiro-Wilk normality test to assess the normality and homogeneity of variances, indicating the combination of results from both experimental repetitions, which were subsequently subjected to ANOVA. When significant ($p \leq 0.05$), the Tukey test was conducted. For all data, the mean confidence interval (CI) was calculated using the equation $IC = (t \times SD) / \sqrt{n}$, where IC represents the confidence interval, t is the tabled t value at 5%, SD denotes the standard deviation, and √n represents the square root of the number of repetitions. The CI was then added to the means in the graphs (mean ± IC).

Data from RNA-Seq reads were mapped to the soybean genome using TopHat2. Counts for RefSeq genes were obtained using HTSeq with default settings, and DESeq2 (version 1.4) was utilized for normalizing expression counts. Fold change was determined as the ratio of normalized counts for each sample to the mean of the reference group for each genotype. Genes were considered differentially expressed (DEG) if the fold change (FC) was ≥ 2, with p-values ≤ 0.05. To visualize the DEG genes, Principal Component Analysis (PCA) and Heatmap analyses were employed.

3. Results

3.1 ETR, Lignin and P content

The glyphosate doses 11.25 and 22.5 g ae ha^{-1} increased ETR values in the NR cultivar by 10-13% compared to the control at 7, 14, and 21 DAA, but there were no differences at 28 DAA. Conversely, in the RR cultivar, no differences were observed between treatments and the control, while in the RR2 cultivar, doses 45 and 90 g ae ha^{-1} increased ETR by 15% only at 7 DAA (Figure 1).

Low doses of glyphosate slightly reduced the percentage of lignin content (up to 1.5%) in soybean plants of all cultivars compared to their respective controls. Conversely, P content increased in response to herbicide application. In the NR cultivar, increases ranged from 16% (11.25 g ae ha^{-1}) to 28% (22.5 g ae ha^{-1}) compared to the control. In the RR and RR2 cultivars, these increments ranged from 24% to 35% and from 20% to 32%, respectively, at 45 and 90 g ae ha^{-1} (Figure 2). These results suggest that the increase in P and the relative reduction in lignin may be linked to the activation of secondary metabolic pathways, triggered by the shortage of aromatic compounds caused by partial inhibition of the shikimic acid pathway. This metabolic shift may also contribute to improved nitrogen recovery or redistribution within the plant.

3.2 Components of the Shikimic Acid Pathway and Dry Biomass

The glyphosate content in soybean plants was proportional to the doses applied, i.e., as glyphosate doses increases, it content within the plant also increased, ranging from 0 to 0,1 ng mg^{-1} in the NR cultivar, and from 0 up to 0.3 ng mg^{-1} for RR and RR2 cultivars (Figure 3A). Despite the presence of glyphosate, no AMPA was found (data not shown). Phenylalanine content slightly increased (up to 15 ng mg^{-1}) in the NR and RR cultivars at the highest glyphosate dose evaluated in each cultivar. In contrast, in the RR2 cultivar, there were no differences in the content of this amino acid between treatments (Figure 3B). The tyrosine content slightly increased (4-6 ng mg^{-1}) in the NR cultivar by glyphosate treatments; however, in the RR and RR2 cultivars, the herbicide reduced the tyrosine content only with 90 g ae ha^{-1}, which decreased up to 18 ng mg^{-1} (Figure 3C). Glyphosate caused a reduction in tryptophan content (from 5 to 16 ng mg^{-1}) in cultivars NR (11.25 and 22.5 g ae ha^{-1}) and RR2 (90 g ae ha^{-1}); however, in the RR cultivar, the dose of 90 g ae ha^{-1} increased the content of this amino acid up to 16 ng mg^{-1} (Figure 3D).

Glyphosate induced high shikimic acid accumulation in NR plants. For example, at a dose of 22.5 g ae ha^{-1}, shikimate content was more than double that of the control (6 ng mg^{-1}). In cultivars RR and RR2, even at 90 g ae ha^{-1}, the shikimic acid content was similar to that of their respective controls (Figure 3E). The coumaric acid content was irregular, increasing or decreasing up to 2 ng mg^{-1}, depending on the glyphosate concentration

and the cultivar. Consistent increases of 1 ng mg^{-1} were observed only in the RR2 cultivar (Figure 3F). The benzoic acid content increased (up to 2 ng mg^{-1}) in all cultivars with the application of glyphosate. In cultivars NR and RR2, these increases were consistent in both doses of glyphosate evaluated for each cultivar, but in cultivar RR, an increase was observed only at 90 g ae ha^{-1} (Figure 3G). Salicylic acid content differed between cultivars, even in controls. The NR cultivar presented salicylic acid levels between 2-3 times higher than the RR and RR2 cultivars (2.5-3.5 ng mg^{-1}). In response to the application of glyphosate, the NR cultivar showed increases in both doses of glyphosate evaluated (11.25 and 22.5 g ae ha^{-1}), while in the RR and RR2 cultivars presented a slight increase only at 90 g ea ha^{-1} (Figure 3H).

The dry mass produced by soybean plants increased with glyphosate treatments. In the NR cultivar, the increases ranged from 10 to 22% at 11.25 and 22.5 g ae ha^{-1}, respectively. In the RR cultivar, the increase was only 8% for both doses evaluated (45 and 90 g ae ha^{-1}), while the RR2 cultivar showed an increase of up to 18% (Figure 3I).

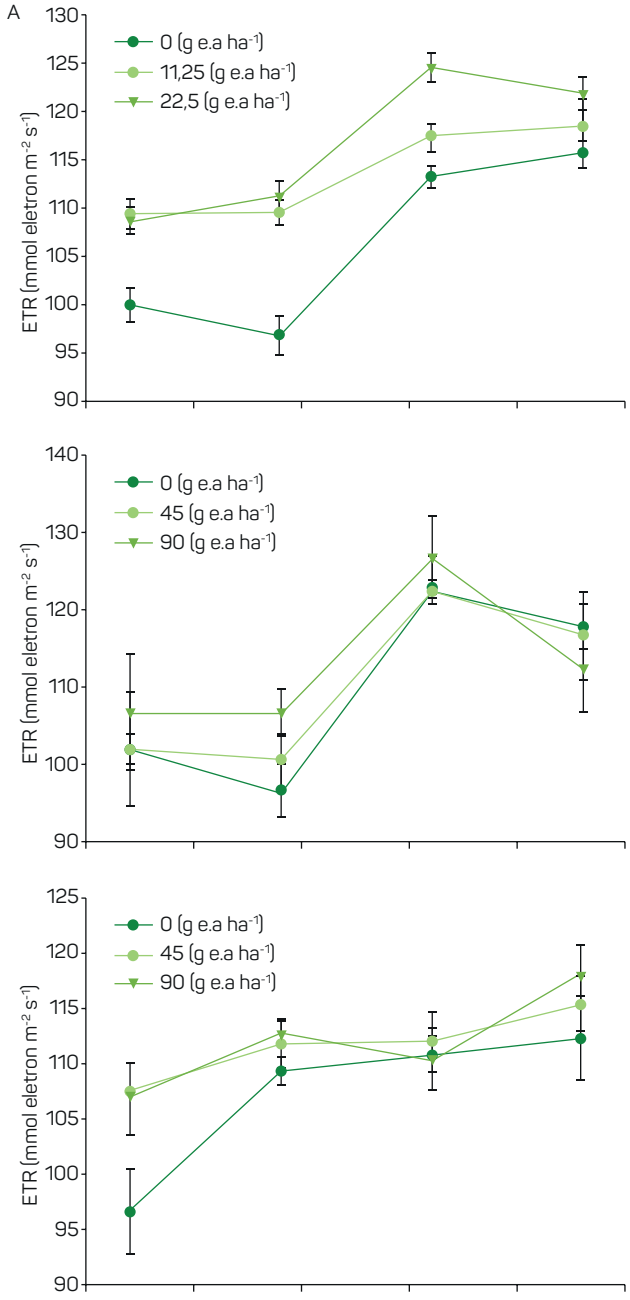

Figure 1 - Electron transport rate (ETR) in soybean cultivars NR (CD383), RR (BMX-Tornado), and RR2 (M5917) 10 days after application with low doses of glyphosate at 7, 14, 21, and 28 DAA. Mean ± confidence interval (5% probability)

3.3 Transcriptome Analysis

The alignment of mapped reads was ≥ 90% in the *Glycine max* reference genome, enabling the identification of expression in 31,515 genes. Out of this total, 587 were DEG ($p \leq 0.05$ and fold change ≥ 2), of which 229 and 358 genes were

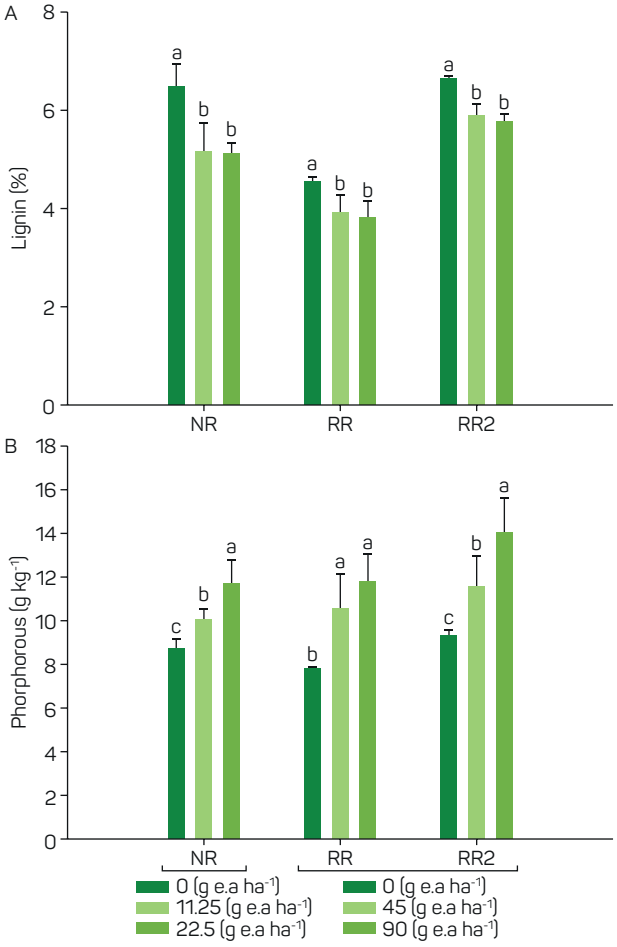

Figure 2 - Percentage of lignin (A) and phosphorus content (B) in soybean cultivars NR (CD383), RR (BMX-Tornado), and RR2 (M5917) 10 days after application with low doses of glyphosate. Mean ± confidence interval (5% probability). Same letters between within a cultivar did not differ from each other using the Tukey test ($p \leq 0.05$)

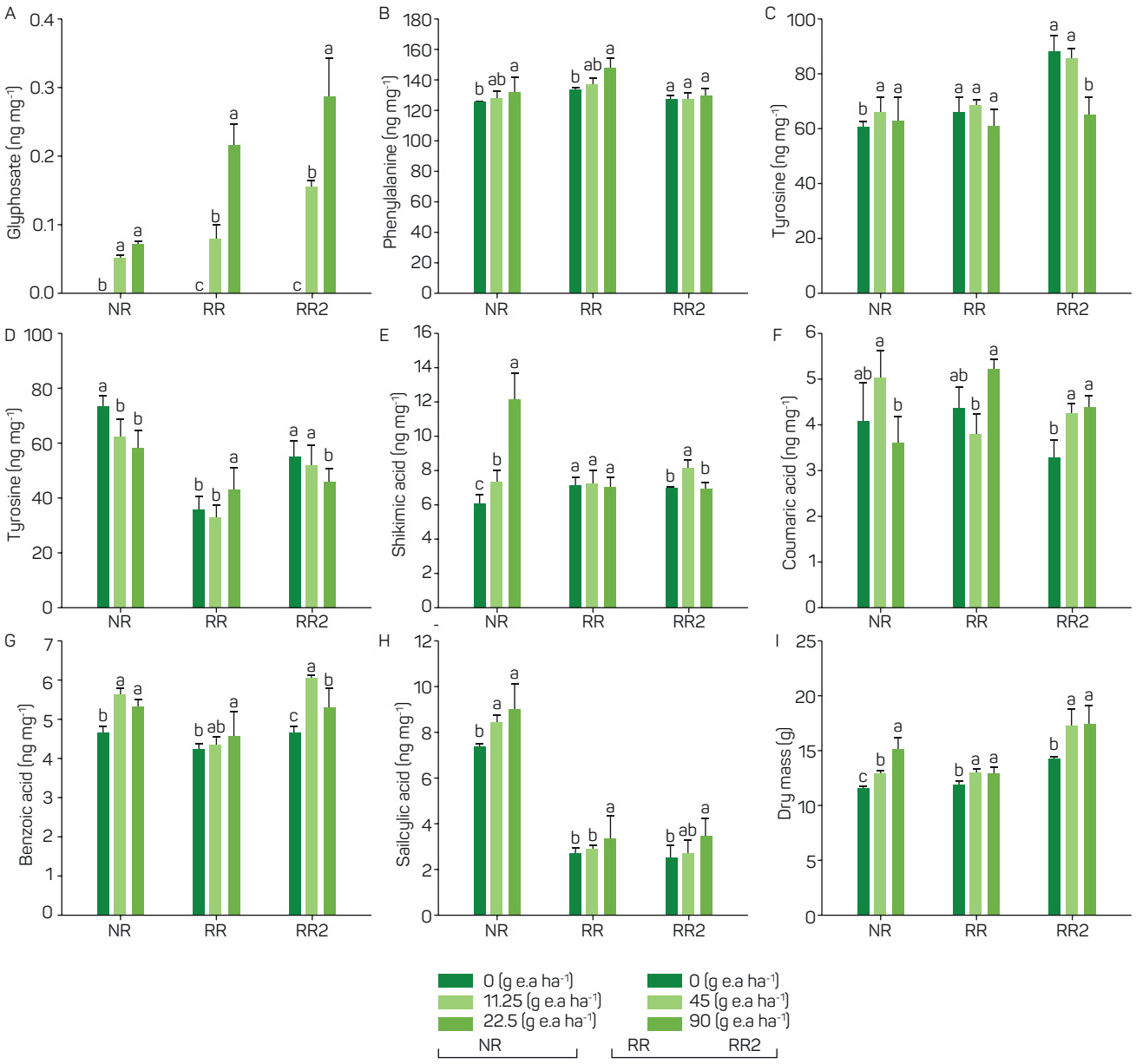

Figure 3 - Content of aromatic amino acids, main components of the shikimic acid pathway, and biomass accumulation in soybean cultivars NR (CD383), RR (BMX-Tornado), and RR2 (M5917) 10 days after application with low doses of glyphosate. A) Glyphosate, B) phenylalanine, C) tyrosine, D) tryptophan, E) Shikimic acid, F) coumaric acid, G) benzoic acid, H) salicylic acid, and I) Dry mass. Mean ± confidence interval (5% probability). Same letters between within a cultivar did not differ from each other using the Tukey test ($p \leq 0.05$)

up-regulated and down-regulated, respectively (Figure 4A). PCA indicated differences in gene expression among soybean cultivars (NR, RR, and RR2) and glyphosate dose groups (0, 11.25, 22.5 ae ha^{-1} for NR and 0, 45, and 90 ae ha^{-1} for RR and RR2), demonstrating varied responses of cultivars to glyphosate application (Figure 4B, C). Therefore, cultivars were analyzed separately comparing only with their respective control.

The gene ontology (GO) enrichment analysis of DEGs showed pathways affected by glyphosate compared to their controls (Figure 5). For the NR cultivar, enriched pathways included metabolic processes of flavones, UDP-rhamnose, UDP-glucuronate, glycosaminoglycan, triglycerides, and jasmonic acid, along with genes involved in meristematic development and the regulation of auxin signaling. Notably, pathways related to the shikimic acid route, flavone metabolic processes, and auxin signaling regulation were prominently enriched. Among these, the flavone metabolic pathway, produced from phenylalanine, was affected by glyphosate, with the LOC100810341 gene enriching this pathway by 99.7%. Similarly, the regulation of auxin signaling pathway, crucial for plant growth and development, was influenced, with one DEG, AUX22, enriching the pathway by 32.2%. Other enriched pathways

not directly linked to the shikimic acid pathway were also identified, suggesting broader impacts of glyphosate application (Figure 5, Table 1).

For the RR cultivar, the pathways exhibiting increased gene expression include calcium and zinc transport, lignin catabolism, response to oxidative and osmotic stress, elongation of long-chain fatty acids, DNA ligase activity, sucrose metabolism, and regulation of salicylic acid signaling. Notably, the lignin catabolism pathway, which has an association with the shikimic acid pathway and

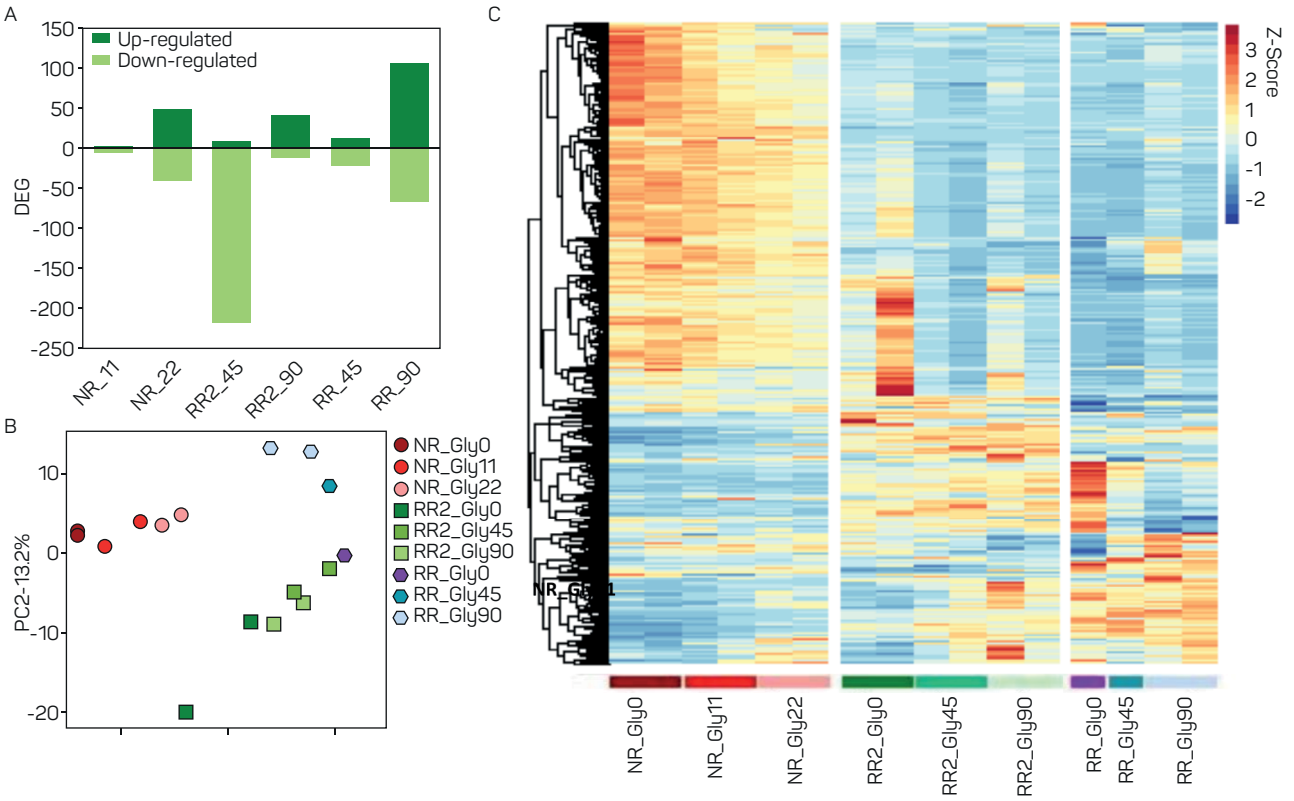

Figure 4 - A) Differentially expressed genes (DEG), B) PCA analysis of differential gene expression results, and C) DEG heatmap in soybean cultivars NR (CD383), RR (BMX-Tornado), and RR2 (M5917) 10 days after application with low doses of glyphosate

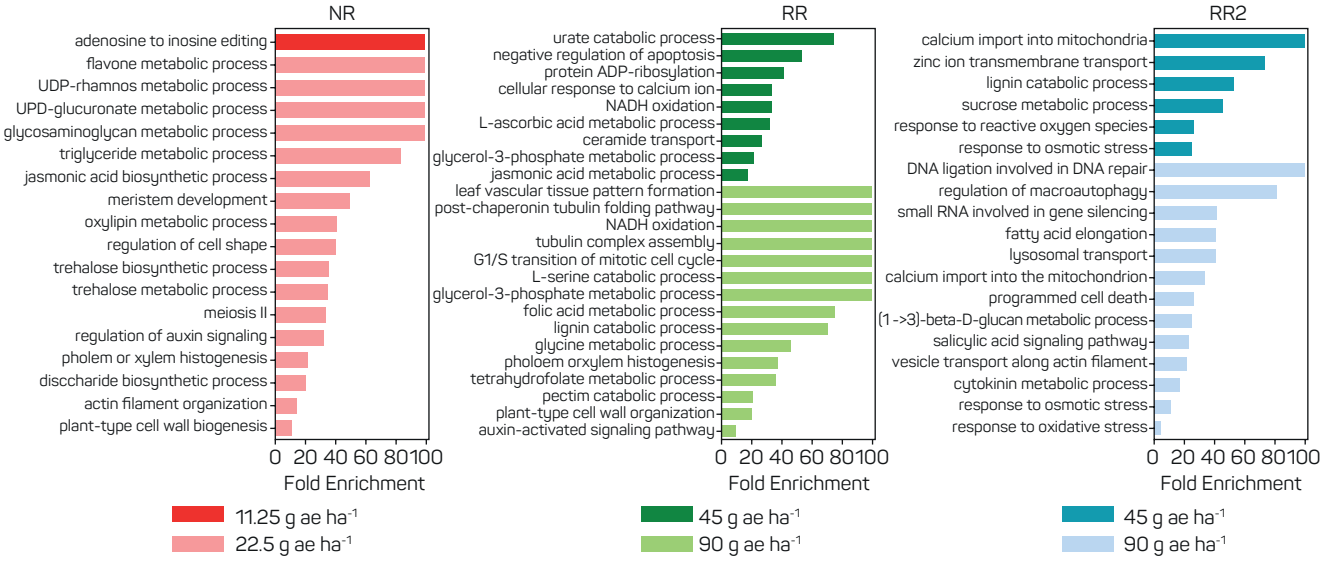

Figure 5 - GO Enrichment of complete biological process of differentially expressed genes (DEG) in soybean cultivars NR (CD383), RR (BMX-Tornado), and RR2 (M5917) 10 days after application with low doses of glyphosate

Table 1 - GO enrichment of complete biological process of DEGs from soybean cultivars 10 days after application of subdoses 11.25 and 22.5 g ae ha^{-1} of glyphosate via foliar application.

GO complete biological process	# genes	DEG	Expected	(E)	P
Cultivar NR (CD383) – 11.25 g ea ha^{-1}					
Adenosine to inosine editing	9	1	0.01	>100	0.001
Cultivar NR (CD383) – 22.5 g ea ha^{-1}					
Flavonoid metabolic process	10	1	0.01	99.7	0.011
UDP-rhamnose metabolic process	10	1	0.01	99.7	0.011
UDP-glucuronate metabolic process	10	1	0.01	99.7	0.011
Glycosaminoglycan biosynthetic process	10	1	0.01	99.7	0.011
Triglyceride biosynthetic process	24	2	0.02	83.1	0.001
Jasmonic acid biosynthetic process	16	1	0.02	62.3	0.016
Meristematic development	20	1	0.02	49.9	0.020
Oxylipin metabolic process	49	2	0.05	40.7	0.001
Regulation of cell shape	50	2	0.05	39.9	0.001
Trehalose biosynthetic process	28	1	0.03	35.6	0.028
Trehalose metabolic process	29	1	0.03	34.4	0.029
Meiosis II	30	1	0.03	33.3	0.031
Auxin-mediated signaling	31	1	0.03	32.2	0.031
Phloem or xylem histogenesis	48	1	0.05	20.8	0.047
Disaccharide biosynthetic process	50	1	0.05	19.9	0.046
Actin filament organization	138	2	0.14	14.5	0.008
Cell wall biogenesis	186	2	0.19	10.7	0.015
Cellular carbohydrate biosynthetic process	309	2	0.31	6.5	0.039
Cell division	312	2	0.31	6.4	0.039
Carboxylic acid biosynthetic process	644	3	0.65	4.7	0.027
Cultivar RR (BMX-Tornado) – 45 g ea ha^{-1}					
Calcium import into mitochondria	17	1	0.01	>100	0.007
Zinc transport via transmembrane	36	1	0.01	73.9	0.013
Lignin catabolic process	51	1	0.02	52.1	0.019
Sucrose metabolic process	58	1	0.02	45.9	0.021
Response to reactive oxygen species	104	1	0.04	25.6	0.038
Response to osmotic stress	106	1	0.04	25.1	0.390
Cultivar RR (BMX-Tornado) – 90 g ea ha^{-1}					
DNA ligase	4	1	0.01	>100	0.009
Regulation of macroautophagy	7	1	0.01	81.4	0.013
Fatty acid stretching	14	1	0.02	40.7	0.026
RNA involved in gene silencing	41	3	0.07	41.7	0.001
Lysosomal transport	14	1	0.02	40.7	0.026
Metabolic process of very long chain fatty acids	24	1	0.04	23.7	0.042
Calcium import into mitochondria	17	1	0.03	33.5	0.031
Symbiont-induced cell death	22	1	0.04	25.9	0.039
Metabolic process of (1->3)-beta-D-glucan	23	1	0.04	24.8	0.041
Regulation of the salicylic acid signaling pathway	25	1	0.04	22.8	0.044
Cytokinin metabolic process	70	2	0.12	16.3	0.007
Transport of vesicles along the actin filament	26	1	0.05	21.9	0.046
Response to reactive oxygen species	385	3	0.68	4.4	0.031
Response to osmotic stress	106	2	0.18	10.8	0.015
Cultivar RR2 (M5917) – 45 g ea ha^{-1}					
Urate catabolic process	5	1	0.01	74.5	0.016
Protein ADP-ribosylation	9	1	0.02	41.5	0.026

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Negative regulation of the apoptotic process	7	1	0.02	53.2	0.021
Jasmonic acid metabolic process	42	2	0.11	17.7	0.020
L-ascorbic acid metabolic process	23	2	0.06	32.4	0.031
Cellular response to calcium ion	11	1	0.03	33.9	0.031
NADH oxidation	11	1	0.03	33.9	0.039
Ceramide transport	14	1	0.04	26.6	0.047
Glycerol-3-phosphate metabolic process	17	1	0.05	21.9	0.005
Response to bacteria	128	3	0.34	8.7	0.007
Immune system process	284	4	0.76	5.2	0.007
Cultivar RR2 (M5917) – 90 g ea ha^{-1}					
Formation of leaf vascular tissue	9	1	0.01	>100	0.005
Postchaperonin tubulin folding pathway	9	1	0.01	>100	0.005
NADH oxidation	11	1	0.01	>100	0.006
Tubulin complex set	13	1	0.01	>100	0.007
G1/S transition of the mitotic cell cycle	14	1	0.01	>100	0.008
L-serine catabolic process	16	1	0.01	>100	0.009
Glycerol-3-phosphate metabolic process	17	1	0.01	>100	0.009
Glycine metabolic process	39	1	0.02	46.2	0.022
Folic acid metabolic process	24	1	0.01	75.1	0.013
Lignin catabolic process	51	2	0.03	70.7	0.001
Tetrahydrofolate metabolic process	50	1	0.03	36.0	0.027
Regulation of the G2/M transition of the mitotic cell cycle	33	1	0.02	54.6	0.018
Phloem or xylem histogenesis	48	1	0.03	37.5	0.026
Pectin catabolic process	174	2	0.10	20.7	0.043
Cell wall organization	89	1	0.05	20.2	0.048
Auxin signaling pathway	590	3	0.33	9.2	0.042

glyphosate, displayed increased expression post-application, with LOC100806093 being DEG and enriching the pathway by 52.1%. Additionally, the regulation of salicylic acid signaling pathway, implicated in defense responses, was enriched. Glyphosate can influence salicylic acid levels via the shikimic acid route, as seen in increased levels due to differential expression of the LOC100786006 gene. Other pathways, such as calcium transport and fatty acid metabolism, also was enriched, providing insights into calcium content and fatty acid regulation post-glyphosate application (Figure 5, Table 1).

For cultivar RR2, a greater number of genes were observed to be expressed in various metabolic pathways, including lignin catabolism, cellular response to calcium, formation of vascular tissue in leaves, NADH oxidation, glycerol and glycine metabolism, and auxin activity. Notably, two important pathways within the shikimic acid route were enriched: lignin catabolism and regulation of auxin signaling. The lignin catabolism pathway exhibited significant enrichment, with the presence of the DEGs LOC100806093 and LOC100785427, suggesting reduced lignin levels in soybean plants of cultivar RR2 following glyphosate application. Similarly, the regulation of auxin signaling pathway showed enrichment, with LOC100784373, LOC100813003, and LOC100805743 being DEGs, indicating alterations in auxin signaling in response to glyphosate application (Figure 5, Table 1).

4. Discussion

The ETR increase in the soybean cultivars in response to low glyphosate doses, especially in the NR and RR2 cultivars, suggests adjustments to the photosynthetic processes to boost growth and biomass production. Low glyphosate doses can stimulate photosynthesis, possibly increasing metabolic activity and triggering detoxification and protective processes against oxidative stress (Cedergreen, Olesen, 2010). ETR, in conjunction with ΦPSII, are indicators of the electron balance in the photosystem (Santos et al., 2022); therefore, an ETR can show that the photosynthesis apparatus is more efficient in response to glyphosate, which may be converted into higher yield (Cedergreen, Olesen, 2010). Studies have shown that doses below 45 g ae ha^{-1} increase ETR in soybeans and other crops such as barley, coffee, sugarcane, and eucalyptus (Cedergreen et al., 2016; Silva et al., 2016; Nascentes et al., 2018; Santos et al., 2022).

The decrease in lignin levels observed in soybean cultivars may stem from a reduction in phenylalanine levels, the primary substrate for lignin synthesis (Zobiole et al., 2010;

Duke et al., 2018). However, if such a reduction in amino acids occurred, it likely took place before 10 DAA, as evidenced by the increase in phenylalanine following glyphosate application during this period. Additionally, cultivars RR and RR2 exhibited DEGs related to lignin catabolism, accounting for the reduction in lignin content in these cultivars. Conversely, in the NR cultivar, glyphosate did not impact the lignin pathway, despite the observed reduction in lignin percentage in the plants. Reductions in lignin content may sufficiently allocate more carbon for saccharide production, thereby promoting plant growth (Velini et al., 2008; Jalal et al., 2021).

Although no DEGs or pathways related to P absorption were observed, the content of this nutrient increased, likely due to competition between the phosphate group of glyphosate and P (Mervosh, Balke, 1991). When the cell senses a P deficiency, highly specific phosphate transporter genes are expressed, leading to an increase in P levels in the plant (Rouached et al., 2010; Pereira et al., 2019). However, in P deficiency tests, no positive glyphosate hormesis responses were observed compared to a high-P control (Cedergreen et al., 2016), supporting the hypothesis that P dynamics in the plant may be one of several processes stimulated by hormesis when content of this nutrient is adequate.

Taken together, the increase in P and the reduction in lignin suggest broader shifts in metabolic resource allocation triggered by sublethal glyphosate stress. The partial inhibition of EPSPS may lead to a transient deficit of aromatic compounds (such as phenylalanine), which in turn can activate compensatory secondary metabolic pathways, including the phenylpropanoid pathway (Maeda, Dudareva, 2012). This activation could alter carbon and nitrogen allocation patterns, possibly favoring nitrogen redistribution toward amino acid biosynthesis and growth-related processes (Liu et al., 2025). In this context, the increase in P content may support these secondary responses, as phosphorus is essential for energy transfer and biosynthetic reactions. These findings align with the hormesis literature, where low doses of stressors such as glyphosate stimulate metabolic adjustments that enhance growth or stress resilience (Belz, Duke, 2014; Cedergreen, 2008). Incorporating these physiological and molecular changes into the hormetic framework provides a more comprehensive understanding of plant responses to low-dose glyphosate exposure.

The increases in phenylalanine observed in NR and RR soybeans, along with tyrosine in NR soybeans and tryptophan in RR soybeans, may stem from a physiological response to glyphosate-induced stress (Brito et al., 2018). This could be attributed to the partial interruption of the EPSPS by the herbicide, leading to shikimic acid accumulation (Reddy et al., 2010). As a consequence, the elevated shikimate levels can subsequently be converted into higher phenylalanine levels. Another possible explanation for the elevated levels of these amino acids could be the breakdown of plant proteins, releasing their constituent amino acids (Zulet-González et al., 2020). Conversely, the decrease in tyrosine levels in the RR2 cultivar and tryptophan in NR and RR2 soybean cultivars may also reflect a physiological response, as these amino acids could be rapidly utilized in plant processes. These amino acids serve as precursors to various compounds that regulate both growth and defense mechanisms in plants, including condensed tannins, anthocyanins, vitamin E, indoleacetic acid (IAA), salicylic acid, flavones, isoflavones, phenylpropanoids, and coumarins, all of which play crucial roles in plant development (Jalal et al., 2021; Almeida et al., 2024). Hence, the presence of these compounds can enrich pathways identified through RNA-Seq analysis.

The increases of shikimic acid in NR soybean plants proved the inhibition of EPSPS by glyphosate (Reddy et al., 2010). In GS plants, shikimic acid accumulation can be up to 300 times higher than in GR plants (Zelaya et al., 2011). Although the doses tested in this study do not allow direct comparison, there was no accumulation of shikimic acid in the RR and RR2 cultivars, due to the insensitivity of the EPSPS carried by these transgenic plants (Zelaya et al., 2011; Bidóia et al., 2024). In contrast, higher concentrations of phenolic acids, such as coumaric and benzoic acid, may aid soybean plants in recovering from oxidative stress (Kumar et al., 2023), as indicated by enriched pathways observed in the RR cultivar by RNA-Seq. On the other hand, salicylic acid is involved in the activation of pathogen resistance genes (Farhangi-Abriz, Ghassemi-Golezani, 2018). Therefore, the presence of these acids in soybean plants may confer greater adaptability to the environment, suggesting that the application of low doses of glyphosate is beneficial in this regard.

The increase of biomass in soybean cultivars by the low glyphosate may be related to the enrichment of the pathway regulating auxin signaling and lignin catabolism (Almeida et al., 2024). However, the enrichment and interaction of other pathways such as the increase in P content, the increase in electron flow, the increase in some essential amino acids and the increase in coumaric, benzoic and salicylic acids may also have contributed to the growth and accumulation of biomass of soybean plants. In other words, it is difficult to attribute this stimulating effect to just a single factor, which demonstrates the complexity of understanding glyphosate hormesis, which can vary between species and cultivars of the same species (Belz et al., 2011; Abbas et al., 2017), as found in this study, in which each type of soybean cultivar presented different physiological and metabolic changes. Hormetic stimulation of both specific and non-specific adaptive mechanisms is part of the inducible adaptation of plants to stress, helping them cope with unpredictable environmental changes (Erofeeva, 2022).

This study has provided valuable insights into the response of soybeans to low doses of glyphosate that induce hormetic effects. However, further research is needed to

explore genes that differentiate NR, RR, and RR2 cultivars at their physiological and metabolite levels through co-expression network analysis. Additionally, identifying the hormonal and transcription factors that regulate glyphosate tolerance/sensitivity in RR, RR2, and NR soybeans remains an important area for investigation.

5. Conclusions

Low-dose glyphosate treatment in soybean plants altered several metabolic and physiological pathways, as well as enriched DEGs, especially those related to the shikimic acid pathway. Key changes include reduced lignin levels and increased biomass production. While both ETR and P content increased with glyphosate, no enriched genes or pathways were linked to these changes, suggesting that they are just some of the multiple processes stimulated and triggered in response to oxidative stress caused by this herbicide. The effects on metabolic pathways and enriched DEGs varied by soybean cultivar and glyphosate dose, showing that glyphosate hormesis is complex and depends on both plant factors (cultivar, accession, or biotype) and the interaction of biotic (physiological and metabolic factors inherent to the plant) and abiotic (dose, environment, etc.) factors. However, the growth and biomass increase were consistent across all cases.

Author's contributions

EDV, and CAC: conceptualization. FHK, DMR, VGCP, and RNC: methodology and Investigation. FHK, RAC, VGCP, RCN, and RAC: collection and analysis of the data and validation. FHK, and RAC: software, formal analysis and writing—original draft preparation. FHK, DMR, VGCP, RCN, EDV, and RAC: writing—review and editing, RAC; visualization. EDV, and CAC: supervision, project administration and funding.

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Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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References

Abbas T, Nadeem MA, Tanveer A, Chauhan BS. Can hormesis of plant-released phytotoxins be used to boost and sustain crop production? Crop Prot. 2017;93:69–76. Available from: https://doi.org/10.1016/j.cropro.2016.11.020

Agathokleous E, Calabrese EJ, Fotopoulos V. Low-dose stress promotes sustainable food production. npj Sustain Agricult. 2024;2:1-6. Available from: https://doi.org/10.1038/s44264-024-00026-0

Almeida AM, Marchiosi R, Abrahão J, Constantin RP, Santos WD, Ferrarese-Filho O. Revisiting the shikimate pathway and highlighting their enzyme inhibitors. Phytochem Rev. 2024;23:421-57. Available from: https://doi.org/10.1007/s11101-023-09889-6

Belz RG, Cedergreen N, Duke SO. Herbicide hormesis: can it be useful in crop production? Weed Res. 2011;51(4):321-32. Available from: https://doi.org/10.1111/j.1365-3180.2011.00862.x

Belz, R.G., Duke, S.O., 2014. Herbicides and plant hormesis. Pest Manag Sci. 2014;70(5):698–707. Available from: https://doi.org/10.1002/ps.3726

Bernards MA, Susag LM, Bedgar DL, Anterola AM, Lewis NG. Induced phenylpropanoid metabolism during suberization and lignification: a comparative analysis. J. Plant Physiol. 2000;157(6):601-7. Available from: https://doi.org/10.1016/S0176-1617(00)80002-4

Bidóia VS, Costa YKS, Gratão PL, Carbonari CA, Duke SO, Carvalho LB. Effects of glyphosate on glyphosate-resistant maize growth and metabolic parameters in the greenhouse and field. Crop Forage Turfg Manag.2024;10(2). Available from: https://doi.org/10.1002/cft2.20308

Brito IP, Tropaldi L, Carbonari CA, Velini ED. Hormetic effects of glyphosate on plants. Pest Manag Sci. 2018;74(5):1064-70. Available from: https://doi.org/10.1002/ps.4523

Cedergreen N, Hansen NK, Arentoft BW. The influence of nitrogen and phosphorous status on glyphosate hormesis in *Lemna minor* and *Hordeum vulgare*. Eur J Agron. 2016;73:107-17. Available from: https://doi.org/10.1016/j.eja.2015.11.003

Cedergreen N, Olesen CF. Can glyphosate stimulate photosynthesis? Pestic Biochem Physiol. 2010;96(3):140-8. Available from: https://doi.org/10.1016/j.pestbp.2009.11.002

Cedergreen, N. Is the growth stimulation by low doses of glyphosate sustained over time? Environ Pollut. 2008;156,1099–1104. Available from: https://doi.org/10.1016/j.envpol.2008.04.016

Cheng X, Li H, Tang Q, Zhang H, Liu T, Wang Y. Trends in the global commercialization of genetically modified crops in 2023. J Integr Agric. 2024;23(12):3943-52. Available from: https://doi.org/10.1016/j.jia.2024.09.012

Companhia Nacional de Abastecimento – Conab. [Adjustments in the corn and soybean acreage result in a production of 295.45 million tons in the 2023/2024 crop season]. Notícias. Jul 19, 2024[access Feb 6, 2025]. Portuguese. Available from: https://www.conab.gov.br/ultimas-noticias/5531-ajustes-na-area-de-milho-e-soja-resultam-em-uma-producao-de-295-45-milhoes-de-toneladas-na-safra-2023-2024

Duke SO, Belz RG, Carbonari CA, Velini ED. Understanding herbicide hormesis: Evaluating its positive and negative aspects with emphasis on glyphosate. Adv Weed Sci. 2025;43:e020250104. from: https://doi.org/10.51694/AdvWeedSci/2025;43:00006

Duke SO, Rimando AM, Reddy KN, Cizdziel JV, Bellaloui N, Shaw DR et al. Lack of transgene and glyphosate effects on yield, and mineral and amino acid content of glyphosate-resistant soybean. Pest Manag. Sci. 2018;74:1166-73. Available from: https://doi.org/10.1002/ps.4625

Duke SO. The history and current status of glyphosate. Pest Manag Sci. 2018;74(5):1027-34. Available from: https://doi.org/10.1002/ps.4652

Erofeeva EA. Environmental hormesis of non-specific and specific adaptive mechanisms in plants. Sci Total Environ. 2022;804. Available from: https://doi.org/ 10.1016/j.scitotenv.2021.150059

Farhangi-Abriz S, Ghassemi-Golezani K. How can salicylic acid and jasmonic acid mitigate salt toxicity in soybean plants? Ecotoxicol Environ Saf. 2018;147:1010-6. Available from: https://doi.org/10.1016/j.ecoenv.2017.09.070

Freitas-Silva LL, Araújo HH, Meireles CS, Silva LC. Plant exposure to glyphosate-based herbicides and how this might alter plant physiological and structural processes. Botany. 2021;100:473-80. Available from: https://doi.org/10.1139/cjb-2021-0033

Jalal A, Oliveira Junior JC, Ribeiro JS, Fernandes GC, Mariano GG, Trindade VDR et al. Hormesis in plants: physiological and biochemical responses. Ecotoxicol Environ Saf. 2021;207:1-20. Available from: https://doi.org/10.1016/j.ecoenv.2020.111225

Krenchinski FH, Costa RN, Pereira VGC, Bevilaqua NC, Alcántara-de la Cruz R, Velini ED et al. Glyphosate hormesis induced by treatment via seed stimulates the growth and biomass accumulation in soybean seedlings. Sci Total Environ. 2024a;918:170387. Available from: https://doi.org/10.1016/j.scitotenv.2024.170387

Krenchinski FH, Pereira VGC, Giovanelli BF, Cesco VJS, Alcántara-de la Cruz R, Velini ED et al. Glyphosate hormesis improves agronomic characteristics and yield of glyphosate-resistant soybean under field conditions. Agronomy. 2024b;14(7):1-14. Available from: https://doi.org/10.3390/agronomy14071559

Kumar K, Debnath P, Singh S, Kumar N. An overview of plant phenolics and their involvement in abiotic stress tolerance. Stresses. 2023;3(3):570-85. Available from: https://doi.org/10.3390/stresses3030040

Li M, Li H, Sun A, Wang L, Ren C, Liu J et al. Transcriptome analysis reveals key drought-stress-responsive genes in soybean. Front Genet. 2022;13:1-17. Available from: https://doi.org/10.3389/fgene.2022.1060529

Liu H, Gao X, Fan W, Fu X. Optimizing carbon and nitrogen metabolism in plants: From fundamental principles to practical applications. J Integr Plant Biol. 2025;67(6):1447-66. Available from: https://doi.org/10.1111/jipb.13919

Majed N, Li Y, Gu AZ. Advances in techniques for phosphorus analysis in biological sources. Curr. Opin. Biotechnol. 2012;23(6):852-9. Available from: https://doi.org/10.1016/j.copbio.2012.06.002

Maeda H, Dudareva N. The shikimate pathway and aromatic amino acid biosynthesis in plants. Annu Rev Plant Biol. 2012:63:73-105. Available from: https://doi.org/10.1146/annurev-arplant-042811-105439

Mervosh TL, Balke NE. Effects of calcium, magnesium, and phosphate on glyphosate absorption by cultured plant cells. Weed Sci. 1991;39(3):347-53. Available from: https://doi.org/10.1017/S0043174500073045

Nascentes RF, Carbonari CA, Simões PS, Brunelli MC, Velini ED, Duke SO. Low doses of glyphosate enhance growth, CO_2 assimilation, stomatal conductance and transpiration in sugarcane and eucalyptus. Pest Manag Sci. 2018;74(5):1197-205. Available from: https://doi.org/10.1002/ps.4606

Novaes E, Kirst M, Chiang V, Winter-Sederoff H, Sederoff R. Lignin and biomass: a negative correlation for wood formation and lignin content in trees. Plant Physiol. 2010;154(2):555-61. Available from: https://doi.org/ 10.1104/pp.110.161281

Pereira FCM, Tayengwa R, Alves PLDCA, Peer WA. Phosphate status affects phosphate transporter expression and glyphosate uptake and transport in grand eucalyptus (*Eucalyptus grandis*). Weed Sci. 2019;67(1):29-40. Available from: https://doi.org/10.1017/wsc.2018.58

Reddy KN, Bellaloui N, Zablotowicz RM. Glyphosate effect on shikimate, nitrate reductase activity, yield, and seed composition in corn. J Agric Food Chem. 2010;58(6):3646-50. Available from: https://doi.org/10.1021/jf904121y

Rouached H, Arpat AB, Poirier Y. Regulation of phosphate starvation responses in plants: signaling players and cross-talks. Mol Plant. 2010;3(2):288-99. Available from: https://doi.org/10.1093/mp/ssp120

Santos JCC, Silva DMR, Amorim DJ, Rosa VR, Santos ALF, Velini ED et al. Glyphosate hormesis attenuates water deficit stress in safflower (*Carthamus tinctorius* L.) by modulating physiological and biochemical mediators. Sci Total Environ. 2022;810. Available from: https://doi.org/10.1016/j.scitotenv.2021.152204

Silva FML, Duke SO, Dayan FE, Velini ED. Low doses of glyphosate change the responses of soyabean to subsequent glyphosate treatments. Weed Res. 2016;56(2):124-36. Available from: https://doi.org/10.1111/wre.12189

Umburanas RC, Kawakami J, Ainsworth EA, Favarin JL, Anderle LZ, Dourado-Neto D et al. Changes in soybean cultivars released over the past 50 years in southern Brazil. Sci Rep. 2022;12:1-14. Available from: https://doi.org/10.1038/s41598-021-04043-8

Van Soest PJ. Use of detergents in analysis of fibrous feeds 3: study of effects of heating and drying on yield of fiber and lignin in forages. J Assoc Off Agric Chem. 1965;48(4):785-90. Available from: https://doi.org/10.1093/jaoac/48.4.785

Velini ED, Alves E, Godoy MC, Meschede DK, Souza RT, Duke SO. Glyphosate applied at low doses can stimulate plant growth. Pest Manag Sci. 2008;64(4):489-96. Available from: https://doi.org/10.1002/ps.1562

Zelaya IA, Anderson JAH, Owen MDK, Landes RD. Evaluation of spectrophotometric and HPLC methods for shikimic acid determination in plants: models in glyphosate-resistant and -susceptible crops. J Agric Food Chem. 2011;59(6):2202-12. Available from: https://doi.org/10.1021/jf1043426

Zhang X, Liu CL. Multifaceted regulations of gateway enzyme phenylalanine ammonia-lyase in the biosynthesis of phenylpropanoids. Mol Plant 2015;8(1):17-27. Available from: https://doi.org/10.1016/j.molp.2014.11.001

Zobiole LHS, Bonini EA, Oliveira RS, Kremer RJ, Ferrarese-Filho O. Glyphosate affects lignin content and amino acid production in glyphosate-resistant soybean. Acta Physiol Plant. 2010;32:831-83. Available from: https://doi.org/10.1007/s11738-010-0467-0

Zulet-González A, Barco-Antoñanzas M, Gil-Monreal M, Royuela M, Zabalza A. Increased glyphosate-induced gene expression in the shikimate pathway is abolished in the presence of aromatic amino acids and mimicked by shikimate. Front. Plant Sci. 2020;11:1-11. Available from: https://doi.org/10.3389/fpls.2020.00459