Molecular Biology Reports (2025) 52:168
https://doi.org/10.1007/s11033-025-10274-z

ORIGINAL ARTICLE

Molecular docking and differential *rbcl* gene expression reveal variation in glyphosate herbicide tolerance of sugarcane varieties

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Received: 16 October 2024 / Accepted: 17 January 2025
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Abstract

Background Glyphosate is an extensively employed herbicide in agriculture, specifically for sugarcane cultivation. The situation is different with the extensive physiological and genetic effects exerted by this herbicide on a range of plant species, including sugarcane, whose model basis is still poorly characterized, although its primary mode of action, which acts on the EPSPS enzyme in the shikimic acid pathway, is completely elucidated. The current study was aimed at investigating the stability of glyphosate formulation, molecular interactions of glyphosate formulation with *rbcL* enzyme associated with chlorophyll metabolism, and its effects on varieties of sugarcane.

Methods and results The stability of a ground-up glyphosate formulation was assessed under accelerated storage conditions. Molecular docking was performed to analyze interactions between glyphosate derivatives and *rbcL*. Two sugarcane varieties (G84-47 and GT54-9) were treated with increasing glyphosate concentrations (0.2, 0.4, and 0.8 mg L^{-1}) to evaluate effects on chlorophyll content, plant height, herbicide tolerance, and *rbcL* expression. The formulation showed good stability with minor degradation (47.55–47.14%). N-Nitrosoglyphosate and 2-(phosphonomethylamino)acetic acid exhibited favorable binding affinities with *rbcL*. Glyphosate treatment reduced chlorophyll content and plant height dose-dependently, with G84-47 showing higher sensitivity. GT54-9 demonstrated higher herbicide tolerance in survival analysis. *rbcL* expression remained stable in G84-47 but was significantly upregulated in GT54-9 under high herbicide stress.

Conclusions This study reveals genetic variability in sugarcane responses to glyphosate, with variety-specific mechanisms underlying stress adaptation. The inducible *rbcL* expression in tolerant varieties provides insights into herbicide resistance mechanisms. These findings can inform marker-assisted breeding and improve agronomic practices for enhanced sugarcane cultivation resilience and sustainability.

Keywords Glyphosate · Molecular docking · *rbcL* · Sugarcane · Herbicide tolerance · Gene expression

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Published online: 28 January 2025

Introduction

Glyphosate, a broad-spectrum systemic herbicide, is used extensively in sugarcane cultivation worldwide. Although its mechanism of action involves inhibition of the enzyme 5- enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the shikimic acid pathway, the multifaceted effects of glyphosate on sugarcane growth, physiology, and metabolism remain incompletely understood. This study employed an integrated approach combining molecular docking, analytical quantification, and plant tissue culture to elucidate the mechanistic underpinnings and biological effects of glyphosate in sugarcane.

Molecular docking simulations were performed to investigate the binding interactions of glyphosate with EPSPS in sugarcane. Detailed analysis of docking poses revealed specific interactions between glyphosate and key residues within the EPSPS active site, providing insights into the molecular basis of enzyme inhibition. These findings align with a previous study [1]. A computational model demonstrated the favorable binding of glyphosate to the CP4 EPSPS enzyme in transgenic glyphosate-resistant sugarcane. Additionally, the potential binding of glyphosate to other critical enzymes, such as ribulose-1, and 5-bisphosphate carboxylase/oxygenase (RuBisCO), was explored to elucidate the secondary interactions affecting photosynthesis and carbon metabolism.

Analytical quantification techniques, including high-performance liquid chromatography (HPLC), were employed to determine glyphosate levels and shikimate accumulation in sugarcane tissues [2]. These measurements enabled the evaluation of EPSPS inhibition and established the relationships between glyphosate exposure and biochemical perturbations within the shikimic acid pathway.

Controlled tissue culture experiments were conducted to assess the phytotoxic effects of glyphosate on sugarcane at the cellular and whole-plant levels, similar to the approach used [3, 4]. The impact of glyphosate exposure on the growth parameters, chlorophyll content, photosynthetic performance, oxidative stress markers, and metabolite profiles was evaluated. These studies provided insights into glyphosate treatment's physiological and metabolic consequences, shedding light on the potential mechanisms underlying the observed phenotypic effects. By integrating molecular docking, analytical quantification, and plant tissue culture, this study aimed to elucidate the multifactorial effects of glyphosate on sugarcane, from its initial binding interactions with target enzymes to its downstream effects on growth, development, and metabolism.

This study's findings contribute to a better understanding of glyphosate's effects on sugarcane, which can guide the development of sustainable herbicide application practices and improve crop management strategies for this economically important crop.

Materials and methods

Analysis of the active ingredient and impurities in a glyphosate pesticide formulation

Pesticide sample

The study analyzed a glyphosate pesticide formulation (ground up 48% SL) with a registration number of 1016, obtained from the Pesticide Analysis Department, Central Agricultural Pesticide Laboratory.

High-performance liquid chromatography (HPLC) analysis

The active ingredient and impurity contents of the glyphosate pesticide sample were determined using high-performance liquid chromatography (HPLC) on an Agilent Technologies 1260 Infinity II system with a UV detector. Separations were carried out on an Eclipse plus C18 column (5 µm, 4.6 × 250 mm) at 30 °C. The mobile phase consisted of a mixture of acetonitrile and methanol (70:30, v/v) delivered at a flow rate of 1.3 mL min^{-1}. Detection wavelengths of 210 nm, 235 nm, and 254 nm were used to detect the active ingredients and impurities corresponding to the pesticide formulation. Retention times and peak areas were used for quantification.

Molecular docking and reaction analysis

Protein and ligand preparation

The amino acid sequence of the large subunit of ribulose-1, 5-bisphosphate carboxylase/oxygenase (*rbcL*) from Saccharum hybrid (Sugarcane) (UniProt ID: Q41373) was retrieved from the UniProt database. Active site residues were predicted using the DeepSite server, and the protein was prepared for docking using AutoDockTools v1.5.7. The structures of N- Nitrosoglyphosate (PubChem CID: 41910) and 2-(phosphonomethylamino) acetic acid (PubChem CID: 3496) were obtained from PubChem and energy-minimized using the MMFF94 force field in Avogadro v1.2.0.

Molecular docking

Molecular docking between the prepared *rbcL* structure and ligand structures was performed using AutoDock Vina with an exhaustiveness of 8. The best conformations were

selected based on predicted binding affinity and visualized using BIOVIA Discovery Studio Visualizer v2020.

Forward reaction prediction

The RXN for Enzyme Catalysis IBM was used to predict the reaction between the docked ligands and chlorophyll a/b substrates. Reaction mechanisms and transformed ligands were analyzed.

In vitro assessment of glyphosate toxicity on sugarcane varieties

Plant materials and growth conditions

Two sugarcane varieties, GT54-9 and G84-47, were obtained from the Sugar Crops Research Institute, Agricultural Research Center, Egypt. Apical meristems from 5 to 6 months of healthy mother plants were used as explants for tissue culture experiments.

Tissue culture experiments

Explants were surface-sterilized with 30% (v/v) Clorox solution, mercuric chloride (0.2%), and sterile distilled water. Sterilized meristems were cultured on Murashige and Skoog (MS) medium supplemented with standard salts, vitamins, sucrose (3%, w/v), and agar (0.7%, w/v). Cultures were maintained at 25 ± 1 °C with a 16/8 h light/dark photoperiod and 55–60% relative humidity.

Glyphosate treatments

Glyphosate (48% SL) was added to the culture medium post-sterilization at final concentrations of 0.2, 0.4, and 0.8 mg L^{-1}. Explants were cultured on the glyphosate-supplemented MS media following the method [5].

Morphological trait analysis

- **Experimental Design**: Three different glyphosate treatments were applied (0.2, 0.4, and 0.8 mg L^{-1}), with each treatment consisting of three replicates.
- **Sample Composition**: Each replicate included three jars, and each jar contained three explants.
- **Measurement Parameters**: After 21 days of glyphosate treatment, we recorded chlorophyll content, shoot length, and LC_{50} to study the effects of glyphosate on the sugarcane varieties.

Gene expression analysis

RNA was extracted from treated samples, and DNase-treated RNA was used for cDNA synthesis. Quantitative real-time PCR (qRT-PCR) was performed using gene-specific primers for ribulose-1, 5- bisphosphate carboxylase (*rbcL*), and the actin housekeeping gene. Relative gene expression was calculated using the $2^{\wedge\text{-}\Delta\Delta Ct}$ method, normalizing target gene expression to the actin reference gene.

Statistical analysis

Data were summarized using descriptive statistics. Non-parametric tests (Mann-Whitney U and Kruskal-Wallis) were used for group comparisons. Survival analysis was performed using the Kaplan-Meier method. Statistical significance was defined as $p \leq 0.05$. Analyses were conducted using SPSS version 21.0 and Microsoft Excel 2016.

Results and discussion

Stability assessment of ground-up glyphosate SL formulation under accelerated storage conditions

Physical properties of ground-up formulation

The Ground-up glyphosate formulation (48% SL) exhibited good solution stability when subjected to accelerated storage conditions between 40 °F and 100 °F (approximately 4 °C to 38 °C) for 14 days in a glass bottle, corroborating previous findings [6]. The formulation remained a clear, homogenous solution with no visible precipitation, phase separation, or color change before and after storage, indicating that it maintained physical and chemical stability over the accelerated period. These observations are largely in line with existing literature on glyphosate formulation stability [7].

Determination of active ingredient content by high-performance liquid chromatography (HPLC)

The glyphosate content, quantified by HPLC, was 47.55% w/w initially and 47.14% w/w after 14 days at 54 °C, corresponding to a minor 0.41% decrease [8]. This change falls well within the established ± 5% limit for HPLC, suggesting negligible active ingredient degradation during accelerated storage [9]. Additionally, the consistent HPLC chromatographic profiles before and after storage, with no new peaks or significant changes in peak areas, provided further evidence for the stability of the formulation and the absence of major chemical degradation or impurity formation over

Table 1 ΔG (kcal mol-1) for each ligand with *rbcL*

Ligand	ΔG (kcal mol^{-1})	Docking score
N-Nitrosoglyphosate	-4.2	-101.08
2-(phosphonomethylamino)acetic acid	-5.4	-88.52

the 14 days at elevated temperatures. Supplemental Fig. 1, shows the HPLC chromatograms for the glyphosate standard and the active ingredient used (Ground-up 48%SL).

Level of impurities

N-nitroso glyphosate was considered a relevant impurity in the glyphosate sample, with maximum acceptable levels of 1 mg kg^{-1} and 1 g kg^{-1} glyphosate, respectively.

The sample was free from N-nitroso glyphosate (UND) under the analysis conditions before and after storage. Supplemental Fig. 2, shows HPLC chromatograms of the N-Nitrosoglyphosate standard and the Ground-up 48%SL. The satisfactory physical stability and active ingredient retention demonstrated by the Ground-up glyphosate SL formulation when subjected to accelerated storage at 54 °C for 14 days suggests suitability for extended shelf life under normal ambient conditions. These findings align with and reinforce prior research on the stability of glyphosate formulations.

Molecular docking reveals interactions of glyphosate derivatives with the *rbcL* active site and their potential roles in chlorophyll metabolism

Molecular docking simulations were performed to investigate the binding interactions and potential reactivity of the two glyphosate derivatives, N-nitroso glyphosate and 2-(phosphonomethylamino) acetic acid, with the *rbcL* enzyme involved in chlorophyll biosynthesis. The predicted binding affinities (ΔG) and docking scores of these ligands with *rbcL* are presented in Table 1. N-Nitrosoglyphosate exhibited a binding affinity of -4.2 kcal mol^{-1} (docking score: −101.08), while 2-(phosphonomethylamino) acetic acid displayed a slightly higher affinity of -5.4 kcal mol^{-1} (docking score: -88.52), suggesting favorable interactions with the target protein. These results agree with a previous study [10], which found N-nitroso glyphosate to have a somewhat higher predicted binding affinity to *rbcL*, suggesting that the additional nitroso group allows for additional favorable electrostatic interactions.

A detailed analysis of the docking poses in Fig. 1**(A)** revealed specific interactions between the ligands and *rbcL*. For N-nitroso glyphosate, key interactions include hydrogen bonding with GLN 155, carbon-hydrogen bonding with TYR 111, attractive charge interactions with GLU 89, and pi-cation/anion/donor hydrogen bonding with TRP 112. In contrast, 2-(phosphonomethylamino) acetic acid forms a hydrogen bond with THR 158, a carbon-hydrogen bond with PHE 95, and an unfavorable amino acid interaction with PHE 58. The additional hydrogen bonds formed by 2-(phosphonomethylamino) acetic acid with catalytic residues

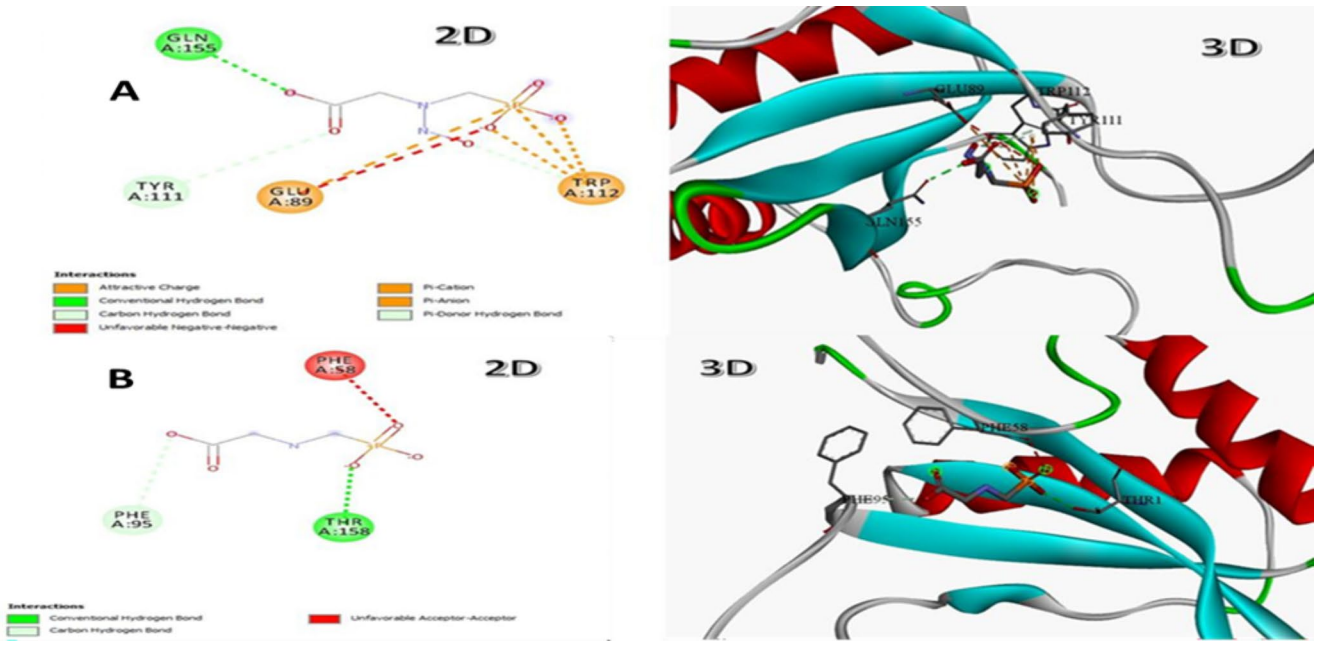

Fig. 1 2D and 3D Structures of N-Nitrosoglyphosate (**A**) and 2-Phosphonomethylamino Acetic Acid (**B**) Ligands Interacting with a Protein

such as THR 158 likely contribute to improved binding complementarity, despite an unfavorable interaction with PHE 58 Fig. 1**(B)**, which aligns with the findings [11], which predicted that this ligand would bind more strongly due to its smaller size, fitting better within the narrow *rbcL* active site.

Computational predictions of the potential reactions between the ligands and chlorophyll a/b Fig. 2 provided insights into their roles in chlorophyll metabolism. The amino group of 2- (phosphonomethylamino) acetic acid was proposed to facilitate the elimination of the C18 vinyl group from chlorophyll a, forming a new C17 = O double bond and a product resembling chlorophyll b. This transformation was accompanied by $Mg2^{+}$-mediated decarboxylation of the carboxylate group of the ligand and reduction of the B-ring ketone. In contrast, the docked nitroso group of N-nitroso glyphosate enabled a direct nucleophilic attack on C17 and the generation of a new C-N bond. These findings suggest distinct pathways for chlorophyll transformation by the two ligands, highlighting the importance of ligand structure in dictating enzymatic reactivity, which agrees with previous studies demonstrating the influence of ligand structure on enzymatic reactions [12].

Docking simulations and computational predictions suggest that these glyphosate derivatives could modulate chlorophyll metabolism through distinct reactivity patterns, potentially influencing the enzyme's catalytic activity and product formation. Although computational modeling can provide valuable mechanistic hypotheses, experimental validation is crucial to confirm the predicted mechanisms and assess their biological relevance [13]. Furthermore, the implications of these predicted mechanisms on the functional properties of *rbcL* and photosynthesis may vary depending on the specific biochemical context and the environmental conditions. Additional studies exploring the downstream effects of chlorophyll modification by different ligands on photosynthetic efficiency and plant physiology could provide further insight into the biological significance of these findings [14].

In conclusion, molecular docking analysis and computational predictions offer valuable insights into the potential interactions between glyphosate derivatives and the *rbcL* active site and their roles in chlorophyll biosynthetic pathways. Although both N-nitroso glyphosate and 2- (phosphonomethylamino)acetic acid exhibited favorable binding affinities, the latter formed more favorable interactions within the active site, potentially contributing to improved binding complementarity. The predicted reaction mechanisms suggest distinct pathways for chlorophyll transformation, highlighting the importance of the ligand structure

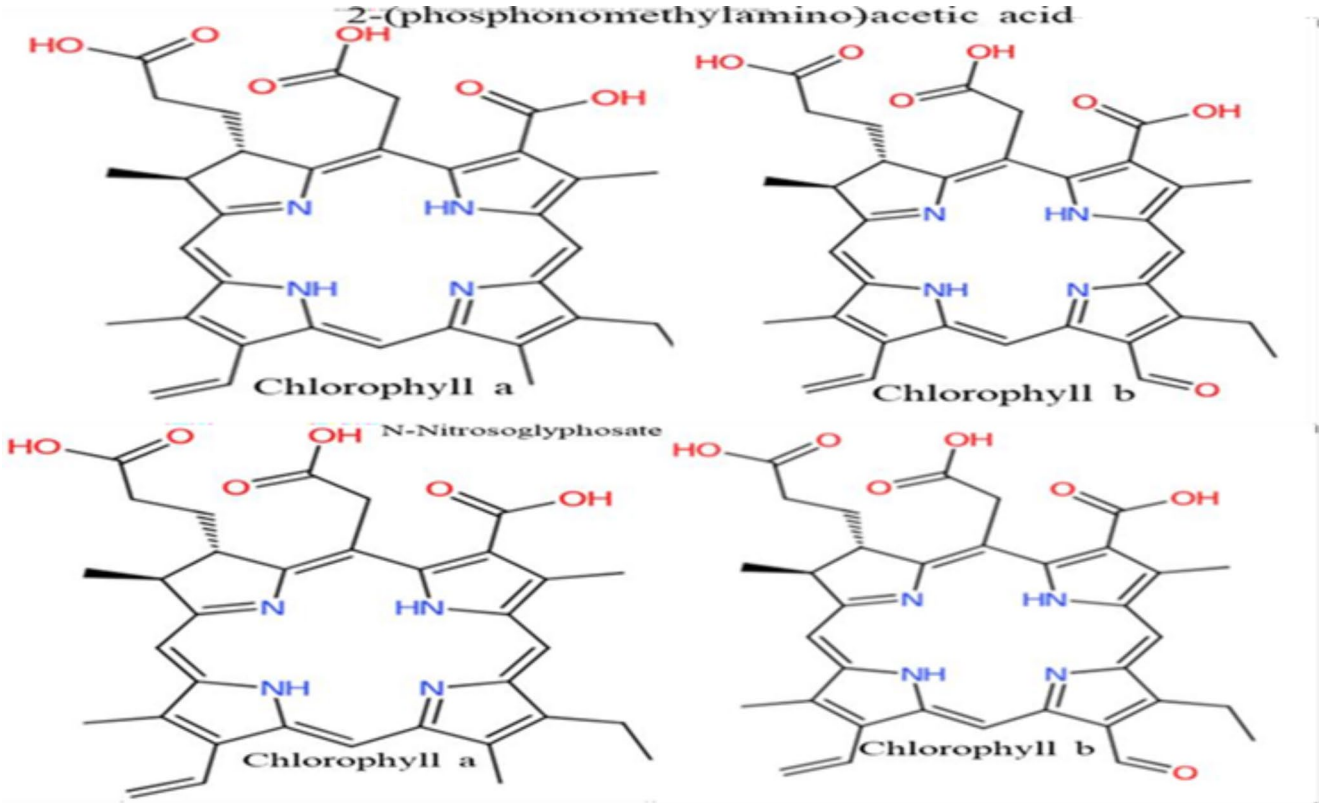

Fig. 2 Docking Analysis of 2-(Phosphonomethylamino) Acetic Acid and N-Nitrosoglyphosate Interactions with Chlorophyll a and Chlorophyll b: Comparative Confidence Scores

in dictating enzymatic reactivity. However, further experimental validation and exploration of the downstream effects are warranted to fully understand these findings' biological implications.

Comparative effects of glyphosate on chlorophyll, growth, and herbicide tolerance in sugarcane varieties

Chlorophyll content

This study investigated the effect of glyphosate herbicide on chlorophyll content in two sugarcane varieties, G84-47 and GT54-9. Treatment with increasing glyphosate concentrations (0.2, 0.4, and 0.8 mg L^{-1}) led to dose-dependent reductions in chlorophyll content in both varieties Supplemental Tables 1**and** Fig. 3**(A)).**

Supplemental Table 1 describes the Kruskal-Wallis Test (Non-parametric data) between all Concentrations with Multiple Comparisons (Mann-Whitney Test) within the entire Concentrations for each parameter in both G84-47 and GT54-9 varieties. There is no significant difference between the Concentrations which have the same letter for each parameter within each variety.

In G84-47, chlorophyll content decreased from 25.08±3.70 µg mL^{-1} in the control to 14.09±9.89, 9.12±6.86, and 2.40±5.03 µg mL^{-1} at 0.2, 0.4, and 0.8 mg L^{-1} glyphosate, respectively Supplemental Table 1. GT54-9 exhibited a similar trend, with chlorophyll decreasing from 23.24±3.33 µg mL^{-1} in the control to 19.54±3.65, 13.66±6.06, and 7.01±5.79 µg mL^{-1} at ascending glyphosate doses Supplemental Table 1.

Plant height

This study also evaluated the effects of glyphosate on plant height in two sugarcane varieties, G84-47 and GT54-9. Treatment with increasing herbicide concentrations resulted in dose-dependent reductions in plant height in both varieties Supplemental Tables 1**and** Fig. 3**(A).**

In G84-47, plant height declined from 10.69±3.00 cm in the control to 7.69±6.01, 7.28±5.88, and 2.00±4.01 cm with increasing glyphosate doses of 0.2, 0.4, and 0.8 mg L^{-1}, respectively Supplemental Table 1. GT54-9 exhibited a similar trend, with plant height decreasing from 23.52±2.93 cm in the control to 17.93±2.13, 11.41±4.68, and 7.70±4.28 cm at ascending glyphosate concentrations Supplemental Table 1.

Varietal differences in herbicide tolerance

The decline in chlorophyll content and plant height was more pronounced in G84-47 than in GT54- 9, suggesting a higher sensitivity of G84-47 to glyphosate. These findings are consistent with previous research demonstrating variable responses of different plant varieties to herbicide exposure [15].

Fig. 3 Comparative Analysis of Chlorophyll Content and Plant Height in Sugarcane Varieties G84-47 and GT54-9 (**Pic A**: Various Glyphosate Concentrations; **Pic B**: Comprehensive Concentration and Variety Overview)

Although this study provides valuable insights into herbicide tolerance in sugarcane, some limitations should be acknowledged. Experiments focused on the short-term effects of glyphosate exposure, and longer-term studies are needed to assess the sustainability of herbicide management practices in sugarcane production systems [16].

Differential responses of sugarcane varieties to glyphosate herbicide exposure

The interaction between sugarcane varieties and glyphosate herbicide

The responses of two sugarcane varieties, G84-47 and GT54-9, to increasing concentrations of glyphosate herbicide, were investigated by measuring the changes in chlorophyll content and plant height. A significant interaction between herbicide concentration and sugarcane variety was observed for both chlorophyll content and plant height Supplemental Table 2.

Supplemental Table 2 shows the interaction between the chlorophyll content. In the control without herbicide, chlorophyll levels were 25.08±3.70 µg mL^{-1} in G84-47 and 23.24±3.33 µg mL^{-1} in GT54-9. At 0.2 mg L^{-1} glyphosate, chlorophyll in G84-47 decreased to 14.09±9.89 µg mL^{-1}, while GT54-9 exhibited a less severe decline to 19.54±3.65 µg mL^{-1}. This pattern continued at 0.4 mg L^{-1} (G84- 47: 9.12±6.86 µg mL^{-1}, GT54-9: 13.66±6.06 µg mL^{-1}) and 0.8 mg L^{-1} (G84-47: 2.40±5.03 µg mL^{-1}, GT54-9: 7.01±5.79 µg mL^{-1}).

For plant height Supplemental Table 2, control values were 10.69±3.00 cm for G84-47 and 23.52±2.93 cm for GT54-9. At each glyphosate concentration, the reductions in both chlorophyll content and plant height were significantly greater in the G84-47 than in the GT54-9 variety.

Figure 3**(B)** illustrates that variety G84-47 showed a greater decline in chlorophyll content and plant height with increasing herbicide concentration than GT54-9. This interaction demonstrates the higher sensitivity of G84-47 to the herbicide. It suggests that it has a lower innate tolerance than GT54-9, potentially because of genetic differences influencing herbicide metabolism or target site sensitivity.

Overall, these results demonstrate an interaction between sugarcane variety and herbicide tolerance, with G84-47 being more sensitive to growth reduction by glyphosate than GT54-9. Further research on the genetic and physiological factors underlying the differential herbicide sensitivity in sugarcane is warranted.

Comparative survival analysis of sugarcane varieties G84-47 and GT54-9 under different treatment concentration levels

Survival analysis using the Kaplan-Meier test revealed significant differences in the survival estimates between sugarcane varieties G84-47 and GT54-9 under different treatment concentrations Fig. 4.

At a 0.2 concentration level, the survival estimate (mean) for G84-47 was 2.63 with a 95% confidence interval of 2.34 to 2.92, while for GT54-9, it was 2.93 with a 95% confidence interval of 2.78 to 3.07. The difference between the two varieties was statistically significant (χ^2 = 4.735, p =.0.030) Table 2.

Similarly, at the 0.4 concentration level, the survival estimate for G84-47 was 2.63 (95% CI: 2.35 to 2.91), and for GT54-9, it was 2.85 (95% CI: 2.65 to 3.05). The difference

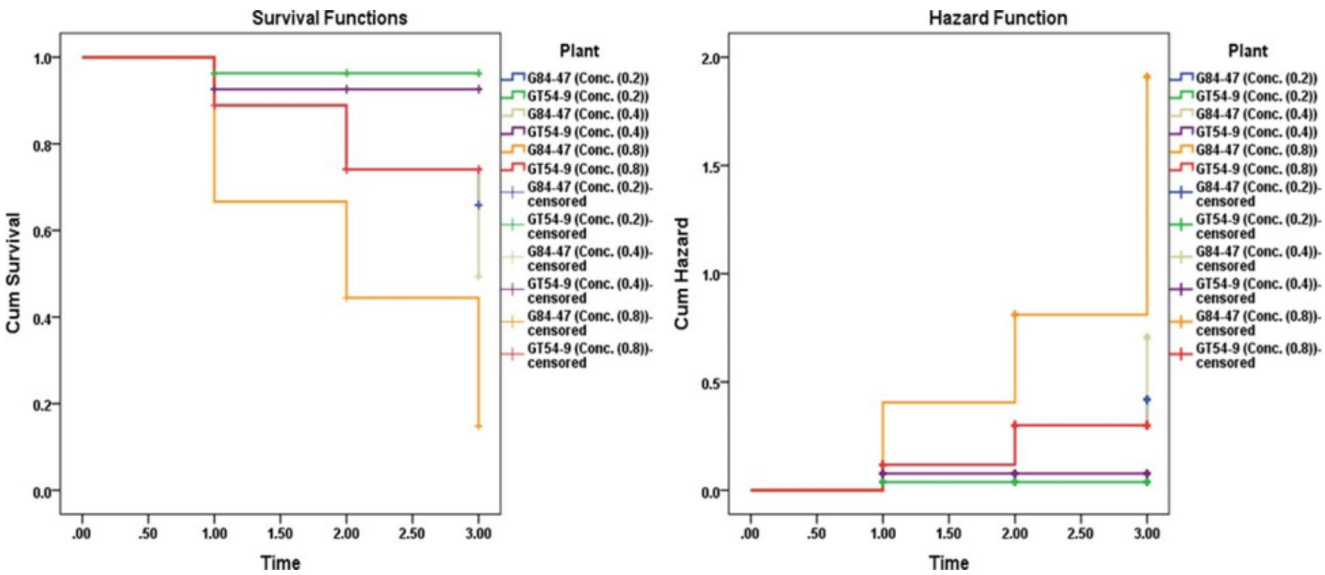

Fig. 4 Survival and Hazard time for sugarcane varieties G84-47 and GT54-9 under different concentration levels

Table 2 Survival analysis for sugarcane varieties G84-47 and GT54-9 in each concentration using the Kaplan-Meier test

Concentrations	Plants	Estimate Mean	S.E.	95% CI		Chi- Square	*P* value
				Lower	Upper		
Conc. (0.2)	G84-47	2.63	0.15	2.34	2.92	4.735	0.030
	GT54-9	2.93	0.07	2.78	3.07		
Conc. (0.4)	G84-47	2.63	0.14	2.35	2.91	4.852	0.028
	GT54-9	2.85	0.10	2.65	3.05		
Conc. (0.8)	G84-47	2.11	0.17	1.77	2.45	10.876	0.001
	GT54-9	2.63	0.14	2.36	2.90		

between varieties was statistically significant (χ^2 = 4.852, p=0.028) Table 2.

At the highest concentration level of 0.8, the survival estimate for G84-47 was 2.11 (95% CI: 1.77 to 2.45), while for GT54-9, it was 2.63 (95% CI: 2.36 to 2.90). The difference between varieties was highly significant (χ^2 = 10.876, p=0.001) Table 2.

These results indicated that the sugarcane variety GT54-9 exhibited better survival estimates than G84-47 under all treatment concentrations, with the most pronounced difference observed at the highest concentration level of 0.8. However, it is crucial to acknowledge that these results might be influenced by various factors such as environmental conditions, soil properties, and management practices, which could potentially affect the survivability of sugarcane varieties differently [17].

Differential *rbcL* gene expression reveals contrasting immune responses in sugarcane varieties under herbicide stress

Plants employ compensatory mechanisms to adapt to environmental stresses, such as herbivore damage or exposure to herbicides, by activating various molecular pathways and regulatory mechanisms that help mitigate the impact of the stress. These compensatory mechanisms can provide a competitive advantage concerning other plants in the presence of the stressor [18–20]. For example, in response to herbivore damage, plants can exhibit compensatory growth, which involves increasing their intrinsic rate of biomass increase as a response to the damage. This compensatory growth ability is maintained at the expense of a reduced growth rate without damage, whereas the ordinary plant type has a higher growth rate [21, 22].

Differential expression of the *rbcL* gene between the two sugarcane varieties, G84-47 and GT54-9, revealed distinct patterns in their immune responses, stress adaptation mechanisms, and compensatory strategies in response to herbicide exposure. For the G84-47 variety, *rbcL* expression remained relatively stable across different herbicide concentrations, with mean levels of 0.32, 0.19, and 0.27 at 0.2, 0.4, and 0.8 concentrations, respectively (P=0.861, Supplemental Table 3. This lack of significant variation suggests that the immune and stress response mechanisms in G84-47 cells may be less responsive to changes in herbicide levels within the tested range, potentially rendering it more vulnerable to environmental challenges Fig. 5.

In contrast, the GT54-9 variety exhibited a significant dose-dependent increase in *rbcL* expression in response to herbicide treatment (P=0.002, Supplemental Table 3. At the highest 0.8 concentration, the mean *rbcL* expression was 6.33, nearly 28 times higher than the 0.23 mean expression at 0.4 concentration. This drastic upregulation of *rbcL* expression in GT54-9 plants under high herbicide stress implies an inducible and dynamic immune response, potentially serving as a protective mechanism against the adverse effects of the applied stimulus.

The *rbcL* gene encodes the large subunit of RuBisCO (Ribulose-1,5-bisphosphate carboxylase/oxygenase), which is the primary enzyme responsible for carbon fixation during photosynthesis. RuBisCO catalyzes the first major step of carbon fixation, enabling the incorporation of atmospheric carbon dioxide into organic compounds, which are then utilized in the subsequent reactions of the Calvin cycle to produce energy-rich molecules like glucose [23, 24].

In the context of glyphosate exposure, as observed in the study, plants may upregulate certain genes, such as the *rbcL* gene, to enhance carbon fixation and photosynthesis, compensating for the herbicide's inhibitory effects on metabolic pathways like the shikimate pathway. Glyphosate, being a broad-spectrum herbicide, is known to inhibit the shikimate pathway, which is a crucial metabolic pathway present in plants and some microorganisms but absent in animals. The shikimate pathway is responsible for the biosynthesis of aromatic amino acids and other essential metabolites. By disrupting this pathway, glyphosate can negatively impact various physiological processes in plants, including photosynthesis.

The differential regulation of the *rbcL* gene observed between the sugarcane varieties G84-47 and GT54-9 suggests that they employ different strategies in mounting a compensatory response to glyphosate stress. The significant upregulation of *rbcL* in GT54-9, particularly the 8-fold increase at a specific glyphosate concentration (0.4 mg/L), indicates a potential compensatory mechanism to maintain

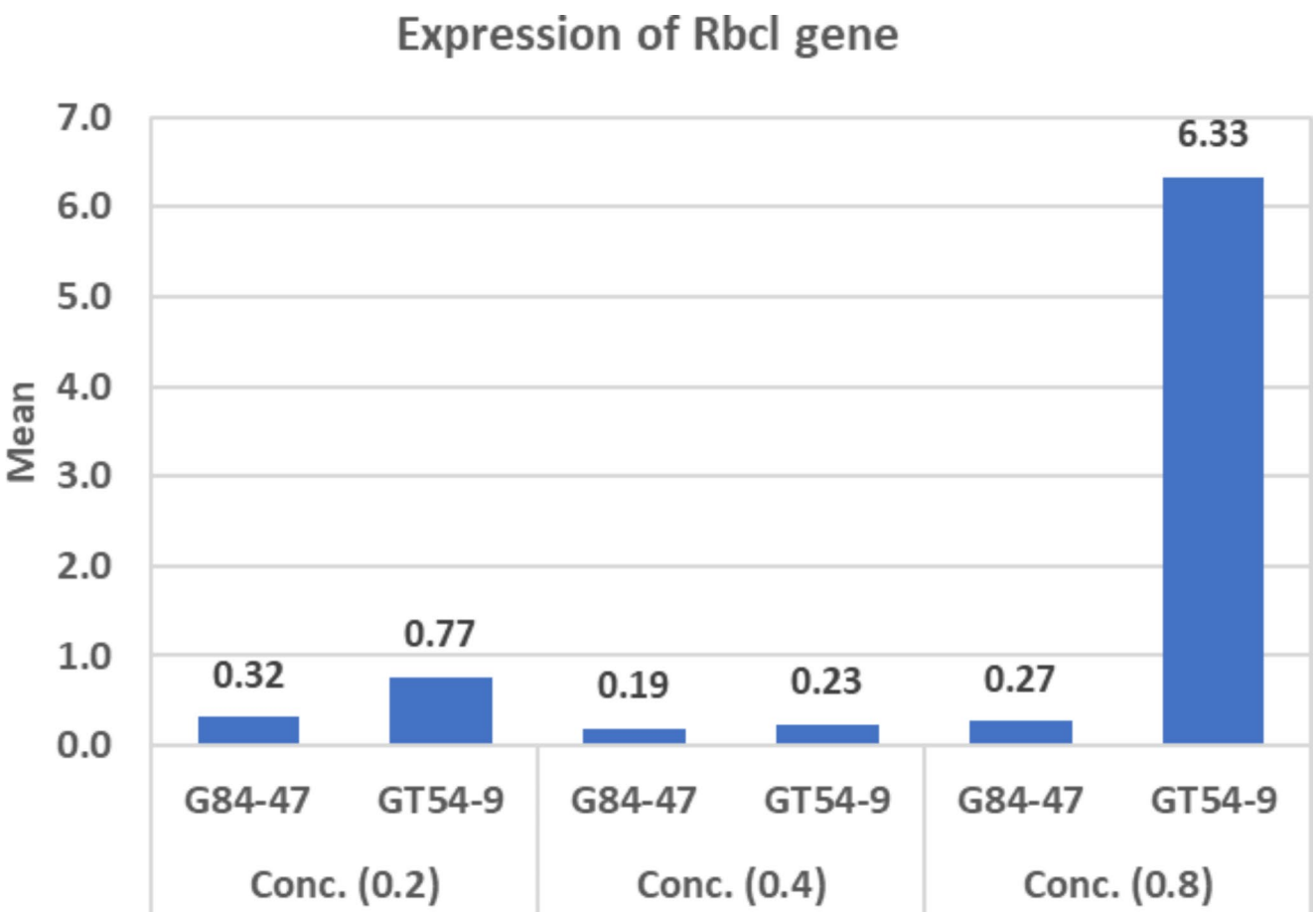

Fig. 5 Mean effect of Interaction between different Concentrations for expression of *rbcl* gene

photosynthetic efficiency and sustain growth in the presence of the herbicide.

During plant immune responses, photosynthesis is often downregulated to divert resources toward defense mechanisms. However, some biotic and abiotic stresses can induce *rbcL* expression as part of the stress response pathway, modulating redox balance, energy production, and carbohydrate metabolism during immune activation [24–26]. The observed upregulation of *rbcL* in GT54-9 under high herbicide concentrations is consistent with this pattern, suggesting the activation of defense pathways and resource allocation toward stress mitigation.

This upregulation of key genes, such as *rbcL*, can help plants maintain essential functions and sustain growth even in the presence of stressors like herbicides. By increasing the production of enzymes like RuBisCO, plants can potentially counteract the negative impacts of glyphosate on carbon fixation and energy production, which are crucial for overall plant growth, development, and survival under adverse conditions.

These findings highlight the variety-specific nature of the immune regulation and compensatory mechanisms in sugarcane, with GT54-9 displaying a more dynamic and responsive strategy than G84-47. The upregulation of *rbcL* expression in GT54-9 at higher herbicide concentrations suggests a proactive defense strategy and compensatory response, potentially aimed at mitigating the adverse effects of stressors, such as glyphosate. Conversely, the relatively stable *rbcL* expression in G84-47 indicated a less flexible immune response, which may render it more vulnerable to environmental challenges Fig. 5.

Overall, compensatory mechanisms, including the differential regulation of genes like *rbcL*, are vital strategies that allow plants to adapt to changing environmental conditions and stressors, ensuring their survival and productivity in challenging circumstances. The invasion and competitiveness of plants employing such compensatory mechanisms depend on factors like their compensatory growth ability, intrinsic growth rate, and the intensity of the stressor (e.g., herbivore attack rate or herbicide concentration).

These results align with those of previous research emphasizing the genetic diversity and adaptive responses of plant species to herbicide exposure [24, 25, 27]. The findings also underscore the importance of understanding the

molecular mechanisms underlying immune regulation and compensatory mechanisms in sugarcane, as they can inform breeding strategies aimed at developing resilient varieties capable of withstanding herbicidal stressors. Further investigations are warranted to elucidate the intricate pathways involved in *rbcL* induction, immune modulation, and compensatory mechanisms in sugarcane, paving the way for targeted interventions to enhance crop resilience and productivity under stressful conditions.

Methodological considerations and future directions

We utilized molecular docking, analytical quantification, and plant tissue culture experiments as an integrated approach to covering a broad spectrum of glyphosate action in sugarcane varieties. Although the results illustrate distinct phenotypic and molecular responses to glyphosate exposure, several salient methodological considerations and limitations merit discussion.

Study design considerations

A combination of approaches was utilized in a variety of experimental designs to determine the effects of glyphosate. However, there are several limitations that we must address:

1. The study is based on two sugarcane genotypes (GT54-9 and G84-47), which is a relatively narrow sampling of genetic diversity in sugarcane germplasm. While these varieties exhibited distinct differential responses, screening more varieties would be needed to confirm the general applicability of our results.
2. Our experiments mainly recorded acute responses to glyphosate exposure. Studies in the long term are needed to evaluate:

- Long-term impacts on plant growth and development.
- Possible formation of tolerance mechanisms.
- Conclusion and implications for sustainable herbicide management.

1. These analyses suggested that target predictions from molecular docking could guide future studies. Still, the biological relevance of the interactions would ultimately need to be confirmed through assorted biochemical and other in vivo experiments.

The mechanisms underlying differential responses

The different responses of GT54-9 and G84-47 to glyphosate exposure underscore the role of genetic variation in herbicide tolerance. High herbicide stress, likely upregulates specific tolerance pathways; *rbcL* is positively correlated with GT54-9 tolerance. There are several reasons to study this further:

1. Molecular markers and pathways associated with herbicide response.
2. Gene expression changes in temporal dynamics.
3. Genetic determinants affecting differential herbicide tolerance.
4. Possible inter-pathway cross-talk between herbicide response pathways and other stress response mechanism.

Agricultural practice implications

These results have significant implications for cane weed management. The variety-specific responses to glyphosate observed in this research indicate that herbicide application strategies would benefit from being tailored to specific varieties. However, there are a few considerations that warrant further exploration:

1. Environmental factors that can affect herbicide efficacy and plant response.
2. Interaction between herbicide tolerance and other agronomically important traits.
3. Molecular markers for herbicide tolerance to assist breeding programs.
4. Management for a variety of specific herbicides with economic context.

Future research directions

From our results and the knowledge gaps identified, we recommend the following priority areas for future research:

1. Notions of genetic diversity in response to herbicides should be expanded to the screening of more varieties.
2. Biochemical and structural biology approaches for validation of molecular interactions.
3. Long-term field studies evaluating the sustainability of variety-specific management strategies for herbicides.
4. Potential non-target effects on beneficial soil microorganisms and ecosystem function.
5. Advancing better formulations that will maximize efficacy with less environmental exposure.

These research pathways will facilitate the development of more sustainable and effective weed tire-management strategies for sugarcane cropping systems, but they are antithetical to the paths to knowledge discovery identified by our surveys.

Conclusion

This study evaluated the stability and interactions of glyphosate derivatives, as well as the differential responses of sugarcane varieties to glyphosate exposure. Key findings include the ground-up glyphosate formulation demonstrating physical and chemical stability, with a minor 0.41% decrease in active ingredient content over 14 days at 54 °C. Molecular docking analysis revealed favorable binding of the glyphosate derivatives to the *rbcL* enzyme, with 2-(phosphonomethylamino) acetic acid exhibiting a binding affinity of -5.4 kcal/mol.

Experiments with sugarcane varieties G84-47 and GT54-9 showed a significant interaction between variety and herbicide tolerance. At 0.8 mg/L glyphosate, chlorophyll content declined from 25.08 to 2.40 µg/mL in G84-47, compared to 23.24 to 7.01 µg/mL in GT54-9. Survival analysis confirmed GT54-9's higher tolerance, with mean survival estimates of 2.63 and 2.11 for G84-47 versus 2.93 and 2.63 for GT54-9 at 0.2 and 0.8 mg/L, respectively. Differential *rbcL* gene expression revealed a 28-fold upregulation in GT54-9 at 0.8 mg/L glyphosate, compared to relatively stable expression in G84-47.

Although our integrated approach yielded valuable insights, several critical limitations and avenues for future research are noted. These include a broader variety of screening, long-term field studies, and the validation of molecular interactions by biochemical approaches. The differences in glyphosate response among varieties could lead to the development of variety-specific herbicide management strategies but must be employed judiciously considering the environment and economics.

The findings underscore the genetic diversity and adaptive mechanisms of sugarcane to herbicides, offering valuable information that can inform breeding programs and sustainable weed control strategies. Research to come in the area we suggest will help generate a better application and environmental profile of herbicide usage in the sugarcane system.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11033-025-10274-z.

Acknowledgements Not applicable.

Author contributions Kawthr S. M. Allam, the MSc student, conducted experiments and funded gene expression and HPLC analyses. Ayman H. Atta served as the primary academic supervisor. Mahmoud Magdy, Adel M. Abd El-Aal, Nasr S. Khalil, and Mohamed A. Rashed acted as academic supervisors, guiding the research process. Reham I. Abdelhamid contributed to tissue culture experiments, supplied sugarcane varieties for the study, and assisted with molecular docking. Additionally, Abdelhamid played a key role in data interpretation, bioinformatics analysis, and manuscript preparation.

Funding This research did not receive any external grants or funds from agencies in the public, commercial, or not-for-profit sectors. All work was performed in the Tissue Culture laboratory, at Sugar Crops Research Institute **(SCRI).**

Data availability No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate There is no ethical issue in this research.

Competing interests The authors declare no competing interests.

Conflict of interest There is no conflict of interest among the authors.

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