

Hydrogen Sulfide Released by Dibutyl Dithiophosphate Increases Potato Growth and Yield

Luis Esquivel-Cervantes; Nina Zidack; Alanna Oiestad;
Ned Bowden; John M. Martin; Michael J. Giroux

Accessibility Disclaimer:

For a more accessible version of this document, please submit an accessibility request form through the Montana State University Library website.



Hydrogen Sulfide Released by Dibutyl Dithiophosphate Increases Potato Growth and Yield

Luis Esquivel-Cervantes¹ · Nina Zidack¹ · Alanna Oiestad¹ · Ned Bowden² · John M. Martin¹ · Michael J. Giroux¹

Received: 11 August 2025 / Accepted: 22 January 2026 / Published online: 24 February 2026
© The Author(s) 2026

Abstract

Hydrogen sulfide is a plant gasotransmitter that can increase plant growth. Application of compounds that slowly release hydrogen sulfide (H_2S) is emerging as a method to increase plant growth and yield. This study investigated how dibutyl dithiophosphate (DBDTP), which releases H_2S when metabolized by a plant, impacts potato growth and development. Two commonly grown potato varieties, Russet Burbank (RB) and Clearwater (CW), were grown under tissue culture, field, and growth chamber conditions with varying amounts of DBDTP. Potato root length was increased in tissue culture when DBDTP was included in the media at 0.025 and 0.1 mM. Potato plant height was also increased, though not significantly, at 0.05 mM DBDTP, while 0.2 mM DBDTP significantly reduced plant height. Under field conditions, potato yield was increased when DBDTP was applied as a 0.5 mM soil drench in 2023. Treated plants yielded 57.9 g per plant, compared to 33.5 g per plant for control plants, with a positive trend detected in 2024. Foliar sprays containing 0.5 mM DBDTP did not cause any visible damage to potato leaves when applied eight days after transplanting in growth chambers. DBDTP was associated with increased auxin pathway gene expression at seven and twelve days after DBDTP treatment. This study demonstrates the versatile ways in which DBDTP may be applied to improve potato growth under tissue culture, growth chamber, and field conditions.

Resumen

El sulfuro de hidrógeno es un gas transmisor vegetal que puede aumentar el crecimiento de las plantas. La aplicación de compuestos que liberan lentamente sulfuro de hidrógeno (H_2S) está surgiendo como un método para aumentar el crecimiento y el rendimiento de las plantas. Este estudio investigó cómo el dibutil ditiofosfato (DBDTP), que libera H_2S cuando es metabolizado por una planta, afecta al crecimiento y desarrollo de la papa. Dos variedades de papa sembradas comúnmente, Russet Burbank (RB) y Clearwater (CW), se cultivaron bajo cultivo de tejidos, campo y cámara de crecimiento, con diferentes cantidades de DBDTP. La longitud de la raíz de la papa aumentó en el cultivo de tejidos cuando se incluyó DBDTP en el medio a 0,025 y 0,1 mM. La altura de la planta de papa también aumentó, aunque no de forma significativa, a 0,05 mM de DBDTP, mientras que 0,2 mM de DBDTP redujo significativamente la altura de la planta. Bajo condiciones de campo, el rendimiento de papa aumentó cuando se aplicó DBDTP líquido al suelo de 0,5 mM en 2023. Las plantas tratadas produjeron 57,9 g por planta, en comparación con los 33,5 g por planta de las plantas testigo, con una tendencia positiva detectada en 2024. Las aspersiones foliares que contenían 0,5 mM de DBDTP no causaron daños visibles a las hojas de papa cuando se aplicaron ocho días después del trasplante en cámaras de crecimiento. DBDTP se asoció con un aumento de la expresión génica de la ruta de la auxina a los siete y doce días después del tratamiento con DBDTP. Este estudio demuestra las formas versátiles en que la DBDTP puede aplicarse para mejorar el crecimiento de la papa en cultivo de tejidos, cámaras de crecimiento y condiciones de campo.

✉ Michael J. Giroux
mgiroux@montana.edu

¹ Department of Plant Biosciences and Plant Pathology,
Montana State University, Bozeman, MT 59717, USA

² Department of Chemistry, University of Iowa, Iowa City,
IA 52242, USA

Introduction

Potatoes are an important food crop worldwide and an important source of carbohydrates, vitamins B1, B3, and C, iron, and essential minerals like potassium, phosphorus, and magnesium (Sharma et al., 2020). They are also a vital food

security crop (Ortiz & Mares, 2017). Improving characteristics such as root growth, plant height, biomass, yield, and response to environmental stress is fundamental to increase potato productivity (Nelson et al., 2013).

Different approaches have been proposed to address production challenges in agriculture. The use of growth enhancers to enhance plant growth is a relatively new area of research. This approach involves the application of hormones or hormone releasing compounds that interact with enzymes, phytohormones, and other plant gasotransmitters to activate biological processes to increase plant growth. Growth enhancers can positively influence crop development processes, including root growth, plant height, yield, and stress tolerance. (Dai et al. 2024; Ali et al., 2013)

Gasotransmitters can pass through cell membranes and are synthesized by plants to act as signaling molecules to regulate plant development. They can be applied to plants to affect their growth by interacting with different enzymatic pathways (Kolupaev et al., 2022). Although these chemicals exist as gases at room temperature, they can be administered as precursor chemicals that are liquids or solids which react within plants to release a gasotransmitter. This method of delivery is favored due to ease of handling and because applying gaseous compounds evenly is difficult (Wang, 2018; García Mata & Lamattina, 2013).

The primary plant gasotransmitters include nitrogen monoxide (NO), carbon monoxide (CO), and hydrogen sulfide (H_2S) (Kolupaev et al., 2022). H_2S levels are associated with a variety of physiological responses, including seed germination, plant growth, root development, environmental stress resistance, and delayed senescence (Dai et al. 2024; Li & Zhu, 2014; Bloem et al., 2004). Many H_2S releasing compounds are known including sodium hydrosulfide (NaSH), sodium sulfide (Na_2S), morpholin-4-ium 4-methoxyphenyl(morpholino) phosphinodithioate (GYY4137), L-thiovaline (TV), thioglycine, and dithiophosphates (DTPs) (Yang et al., 2022; Carter et al., 2019; Lisjak et al., 1988). These chemicals increase plant growth and tolerance to biotic and abiotic stresses when used appropriately. However, among them, only Dibutyl dithiophosphates (DBDTP) have been formulated for use in agriculture.

In the past five years, dithiophosphates have been tested on corn by irrigating corn with aqueous solutions containing dithiophosphates or by applying dithiophosphates as a sidedress fertilizer (Carter et al., 2019). Dithiophosphates are synthesized by reacting C_2 to C_{12} fatty alcohols with P_4S_{10} at elevated temperatures (Brown et al., 2021). Dithiophosphates degrade in aqueous solutions to safe and natural chemicals, including fatty alcohols and monopotassium phosphate (Brown et al., 2021; Ranasinghe Arachchige et al., 2022).

The use of affordable seed or soil additives that improve plant growth and yield is essential for increasing agronomic

yield. There have been no reports of the effects of any H_2S releasing compound on potato growth. Therefore, this research aimed to explore the effects of DBDTP on potato growth, yield, and gene expression. Several critical questions regarding the use of DBDTP were tested, including concentration and application method. We hypothesized that, when applied at the right concentrations and timing, DBDTP would enhance potato growth and that enhancement would result from upregulation of plant growth pathways.

Specifically, we tested DBDTP in potato tissue culture to evaluate its impact on root length, plant height, and fresh and dry weight of Russet Burbank (RB) and Clearwater Russet (CW) potatoes. We also investigated the effect of DBDTP on gene expression in tissue-culture RB plants. Field tests used evaluated DBDTP as a soil drench application in the field, examining its effects on potato yield, height, vigor, and biomass in the RB and CW varieties. Foliar applications of DBDTP were used to test for potato leaf phytotoxicity.

Materials and Methods

Plant Materials

Experiments were performed using two seed potato varieties commonly grown in MT: Russet Burbank (RB) and Clearwater (CW). Tissue-culture material for RB and CW was obtained from pathogen-free stock provided by the Montana State University Seed Potato Certification Program and maintained on Murashige and Skoog (MS) medium before experiments. Field trials utilized potato plantlets grown in tissue culture due to the importance of the first generation of tubers obtained from this method for MT seed potato growers.

Tissue Culture Trial

Potato plantlets were grown in-vitro from same-age nodes in Murashige & Skoog (MS) Basal Medium with Vitamins M519 (PhytoTech Labs, Lenexa, KS, USA), within $7.6 \times 7.6 \times 10$ cm Magenta GA-7 (Fisher, Waltham, MA, USA) containers to evaluate the effects of 0, 0.025, 0.05, 0.1, and 0.2 mM DBDTP. Because there are no previous reports on the use of DBDTP in potato tissue culture, these concentrations were chosen based on preliminary trials to span a gradient from undetectable to detrimental effects. There were five replications for each DBDTP concentration, with nine nodes in each Magenta container arranged as a Completely Randomized Design (CRD). This was replicated for the two potato varieties (Russet Burbank and Clearwater), and the experiment was repeated twice. Briefly, the tissue culture media was prepared using 20 g sucrose, 4.4 g MS

medium, and 2.3 g phytigel (Sigma-Aldrich, Inc., St. Louis, MO, USA) per liter of water. After autoclaving, the media were cooled to 65 °C for 45 min in a water bath before adding the DBDTP.

DBDTP solutions were filter sterilized using a 22 µm filter and added to the cooled media. Immediately after addition, ~60 mL medium was poured into Magenta vessels. Nine potato nodes were cut and added to each Magenta. Cuttings in containers were placed into a Conviron growth chamber CMP 6010 (Conviron, Pembina, ND, USA) and grown under 16 h of light at 22 °C and 8 h of darkness at 19 °C, with a light intensity of 250 PAR. After four weeks, plant height, root length, overall fresh weight, and dry weight were measured. Then, each plantlet was removed, cleaned of any adhering MS media, measured, weighed, and placed in a labeled envelope in a forced air drying room at 37 °C for one week and dry weight determined.

RNA Seq Experiment

Russet Burbank potato plantlets were grown in tissue culture using Magenta vessels. There were two treatments: 0 mM and 0.1 mM DBDTP. Plants were grown under normal conditions as described above. The 0.1 mM treatment was selected based on growth effects observed in preliminary tissue culture trials. Each treatment included 12 Magenta GA-7 vessels (three vessels for each of the four sampling times), with 15 nodes per vessel. Shoots without roots were collected at 6 h, 12 h, 3 days, and 7 days after treatment and frozen in liquid nitrogen. For each treatment × time point, three RNA samples (replicates) were prepared; each sample consisted of 12 shoots obtained by pooling four shoots from each of the three Magenta vessels, using different shoots for each replicate so that no plant contributed to more than one RNA sample. Separate sets of three Magenta vessels were used at each time point (i.e., the vessels harvested at 6 h were different from those harvested at 12 h, 3 d, and 7 d, to maintain sterility and to obtain sufficient tissue. Samples were stored at –70 °C until RNA extraction.

RNA was extracted using the RNeasy Plant Mini Kit (QIAGEN, Germantown, MD, USA) following the manufacturer's instructions. The quality of the RNA was assessed using an RNA 6000 Nano Kit for the 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) before being sent for sequencing at LC Sciences (Houston, TX, USA). Sequence data was received, trimmed and low quality reads were removed. Sequence data was then imported using the NGen and ArrayStar programs within the DNASTAR software package (DNASTAR, Inc., Madison, WI, USA). Alignment and transcript expression used the *Solanum tuberosum* reference genome SolTub_3.0, downloaded from the Ensembl Plants FTP site (Yates et

al., 2022). Differentially expressed genes (DEG) and GO annotation were obtained using iDEP 2.0 (Ge et al., 2018) to identify significant changes in gene expression between treated and control untreated plants. Total raw counts per gene were submitted to the iDEP website, where only genes with a CPM (counts per million) > 0.5 and present in at least 2 samples were kept (for a 15–20 million read library). Differentially Expressed Genes (DEGs) were obtained based on the DESeq2 (Love et al., 2014) method using an FDR (False Discovery Rate) < 0.05 with at least a 2-fold expression change. Gene Ontology (GO) enrichment analysis was performed for DEGs for the GO Biological Process, GO Molecular Function, and GO Cellular Component categories, using the over-representation analysis based on a one-sided hypergeometric test with Benjamini–Hochberg FDR correction available in iDEP. A global pathway analysis was also carried out in iDEP to determine if pathways shifted up or down, even if many individual genes were not significant DEGs. Pathway analysis was done using the GAGE (Generally Applicable Gene-set Enrichment) method in iDEP with FDR < 0.05 for only the GO term Biological Process.

Field Trial

Russet Burbank (RB) and Clearwater (CW) potato plantlets were grown for four weeks in tissue culture as described above with the only changes being that 10.2 × 10.2 × 7.5 plastic containers were used and each contained 80 mL of MS medium and 20 potato nodes. DBDTP was included at 0.025, 0.05, 0.1, and 0.2 mM DBDTP.

After four weeks, plantlets were transplanted into trays in the greenhouse using 3.18 × 3.18 cm plugs made from a blend of peat moss, coco coir, and bio char (Gro Indoor, Sheridan, WY, USA). Before transplanting, DBDTP concentrations were applied a second time, applying at 0.025, 0.05, 0.1, and 0.2 mM by dipping plugs in a solution containing DBDTP and transplanting one plantlet per plug. A total of 80 plantlets were used for each treatment. All treatments received the same fertilization regime, with 20–20–20 (NPK) at a concentration of 200 ppm applied daily or as needed. The potatoes were grown in these plugs for four weeks. When plants reached a height of 12.7 to 15.2 cm, they were transplanted into the field.

The field trial was conducted at the Horticulture Farm in Bozeman, Montana, where an 8.8 × 29.3 m plot was used to transplant the potatoes in a randomized block split-plot design where variety was the main plot and DBDTP dose was the subplot. There were eight plants per plot, with 26.7 cm spacing between plants, 2.3 m long rows, 0.76 m spacing between rows, and 10 blocks.

Before planting, plants were dipped once in DBDTP solutions at concentrations adjusted for the larger plant

sizes, in contrast to tissue-culture conditions. In tissue culture, plants had received 0, 0.025, 0.05, 0.1, or 0.2 mM DBDTP. At transplanting, the same treatment groups were assigned to 0, 0.5, 1, 2, or 10 mM DBDTP, respectively; thus, each plant received one tissue-culture dose and one field dose at the corresponding level. These higher field concentrations were selected based on preliminary pea and wheat experiments (data not shown), where similar concentrations were tested. This scheme allowed us to test for a dose-response trial in potatoes under field conditions. One month after planting, a soil drench containing DBDTP was applied using the same concentrations as described above. Potato height and vigor were evaluated one and two months after transplanting. Plant vigor was scored visually on a 1–5 scale based on overall condition, strength, and greenness of the canopy, where one indicated a weak, small, pale plant and five indicated a large, robust, dark-green plant in excellent condition.

In 2023, the plot was harvested 2 months after planting due to nearby Potato Virus Y (PVY) infection, and after 3 months in 2024. The difference in time after planting contributed to the differences in the yield response between years. Plant biomass, tuber weight per plant, and number of tubers per plant were evaluated in both years.

Phytotoxicity Trial

Potential phytotoxic effects of DBDTP were tested on RB potato plantlets in growth chambers, utilizing the concentrations evaluated in the field drench applications as foliar treatments. This was done to ensure that DBDTP doses showing positive effects in field tests would not have negative impacts when applied in foliar application. In Montana State, potatoes are irrigated using fertigation, making it an efficient method for applying DBDTP. This trial will serve as a preliminary assessment for further experiments on DBDTP's foliar application.

The phytotoxicity of DBDTP was evaluated at four different times after transplanting, using plants grown in growth chambers. For the first experiment, the first three application times were one, eight, and fifteen days after transplanting (15 DAT), plantlets were grown for 4 weeks in tissue culture without DBDTP and then transplanted into 3.18 × 3.18 cm plug trays. For each time point, four trays were used (four replicates). Each tray contained five rows of seven plantlets; the five DBDTP doses (0, 0.5, 1, 2, and 10 mM) were randomly assigned to rows, and each row (seven plants) was considered one experimental unit. Separate trays were used at each application time, so plants treated at 1 DAT were different from those treated at 8 and 15 DAT.

For the fourth application time (1 month after transplanting, 1 MAT), four-week-old tissue culture plantlets

were transplanted into individual 10 cm pots and placed in a completely randomized design with 24 pots (one plant per pot) per DBDTP dose. The amount of water sprayed in these experiments was obtained based on a previous calibration. This calibration was done every time DBDTPs were applied, and plants were sprayed till runoff to ensure full coverage. All potato plants were hand-watered every day with 100 ppm 20-20-20 NPK fertilizer.

Shoot fresh weight was measured one MAT in the case of the first three application times, and one month after application in the case of the 1 MAT. Phytotoxicity was evaluated using the top five leaves with a visual scale of 1 to 5, where 1 indicated no burns on the leaves; 2 represented small individual burns on some leaves; 3 indicated at least one leaf with spots that merged into a bigger spot but covering no more than ¼ of the leaf; 4 implied bigger spots in at least one leaf covering more than ½ of the leaves but without dead leaves; and 5 represented at least one true leaf dead and more leaves with scores 3 and 4 or dead plant.

Statistical Analysis

The data were analyzed using analysis of variance (ANOVA) in R (R Foundation for Statistical Computing, Version 4.0.5, Vienna, Austria). The values are presented as the means of two independent experiments, analyzed either individually or collectively, depending on whether interactions between DBDTP concentration and trial repetition were observed. We obtained estimated marginal means using the emmeans package and performed planned pairwise contrasts comparing each DBDTP's doses with 0 mM, using Sidak adjustment for multiple testing. Differences with a P-value less than 0.05 were considered significant. Figures were created using Microsoft Excel.

For the tissue culture experiments, the data were analyzed by combining the two independent experiments in a mixed-effects model. Concentration, variety, and trial repetition were treated as fixed effects, along with their interactions, using the lmer function in R. Additionally, replication and the trial repetition were included as random effects. The assumptions (normality and constant variance) were met with all the response variables. The results were averaged over variety.

In the case of RNA-seq, the sequenced data were imported using the DNASTar (DNASTar, Madison, WI, USA) program for alignment and transcript levels. Total raw counts from DNASTar were analyzed using iDEP (Ge et al., 2018). Significant changes in gene expression between treated plants and the control group were evaluated employing DESeq2 within iDEP 2.0 for each time point. Plots were created in R Studio 4.4.1.

In the case of the field data, a mixed-effects model with dose and variety treated as fixed effects, and replication and

variety as random effects for a randomized block split plot design using the lme4 package in R (Bates et al., 2015) for each year were used to analyze data due to the huge difference in yield because of the early 2023 harvest. Results were averaged over variety. In 2023, only yield data were square-root-transformed before analysis to meet normality and constant variance assumptions, and estimated marginal means were back-transformed for interpretability. In 2024, normality, constant variance, were met, and no transformation was necessary.

In the phytotoxicity experiment, the data were analyzed using a linear mixed-effects model for both trials, and assumptions of normality and constant variance were met. The model included experimental repetition, DBDTP dose, application time, and all interactions as fixed effects, with random intercepts for block and for application time, experimental repetition, and block combination. For the one MAT evaluation, the model included experimental repetition, dose, and their interaction as fixed effects, and replication as a random effect.

Results

Effect of DBDTP on Potato Under Tissue Culture Conditions

The effect of DBDTP H₂S donor was evaluated first in tissue culture. The results from the two independent experiments and a combined mixed model, averaging over varieties, showed that root length significantly increased under all DBDTP treatments after four weeks in tissue culture when compared to the control group (Fig. 1A). These results suggest that DBDTP effectively promotes root development.

Shoot height did not follow the same trend; this was especially apparent at 0.2 mM DBDTP, where a significant negative effect was observed (Fig. 1A). At this concentration, fresh weight was also significantly lower than the 0 mM DBDTP control, although there was no significant difference in dry weight (Fig. 1B). The impact of DBDTP on root length was visually distinguishable between the treatments and the control with peak positive difference observed at 0.05 mM and significantly reduced root length at 0.2 mM DBDTP (Fig. 1C–G).

Effect of DBDTP on Gene Expression in Tissue Culture Potatoes

After six hours of DBDTP treatment, a total of 18,819 expressed genes were detected. There were 608 DEGs of which 470 were up-regulated, and 138 were down-regulated (Fig. 2A). Twelve hours after treatment, 19,057 expressed

genes were detected and there were 728 DEGs of which 534 were up-regulated, and 194 were down-regulated (Fig. 2B). Three days after treatment, 19,024 genes were expressed, 2211 were DEGs of which 1247 were up-regulated, and 964 were down-regulated (Fig. 2C). Seven days after treatment, 19,369 genes were expressed and 154 were DEGs of which 103 were up-regulated, and 51 were down-regulated (Fig. 2D).

The DEGs from each time point were annotated into the functional categories Biological Process, Molecular Function, and Cellular Component. The most interesting results were observed at twelve hours after treatment, a total of 40 pathways were found among the DEGs in the GO Biological Process, where 20 pathways were upregulated (Fig. 3). Nine out of 534 up-regulated DEGs were in the hormone transport pathway (GO:0009914), nine in the auxin transport pathway (GO:0060918), thirteen in the regulation of hormone levels pathway (GO:0010817), and eleven in the microtubule-based movement pathway (GO:0007017) (Fig. 3). A total of 34 pathways for the GO Molecular Function and 20 for the GO Cellular Component were found. Data corresponding to six hours, three days, and seven days after treatment are shown in the supplementary data (Supplementary Fig. 1, Supplementary Fig. 2, Supplementary Fig. 3). xxx.

Overall, the enriched biological processes differed between time points, indicating that transcriptional levels change over time after DBDTP treatment. At 6 h, enriched upregulated Biological Process terms were mainly associated with cellular responses to phosphate starvation, nutrient and external stimuli, steroid metabolic process, and multiple “negative regulation” terms related to proteolysis, hydrolase activity, and molecular function (Supplementary Fig. 1). At 12 h, enrichment shifted toward hormone and growth-related processes, including auxin transport, microtubule based movement, and reorganization of the cell wall and other cellular components (Fig. 3). At 3 d, only a small number of processes remained significantly up-regulated, predominantly protein modification (Supplementary Fig. 2). At 7 d after treatment, enrichment was dominated by glutathione metabolic processes together with many terms describing negative regulation of protein and macromolecule metabolism, proteolysis, and catalytic activity (Supplementary Fig. 3). Although the DBDTP expression patterns changed over time, it was noticed that early (6 h) and late (7 d) responses both involve proteolysis and enzymatic activity.

Results from the Generally Applicable Gene-set Enrichment (GAGE) analysis on GO Biological Process gene sets at 12 h after treatment showed significant enrichment of plant growth related pathways, including auxin export across the plasma membrane (GO:0010315; 10 genes),

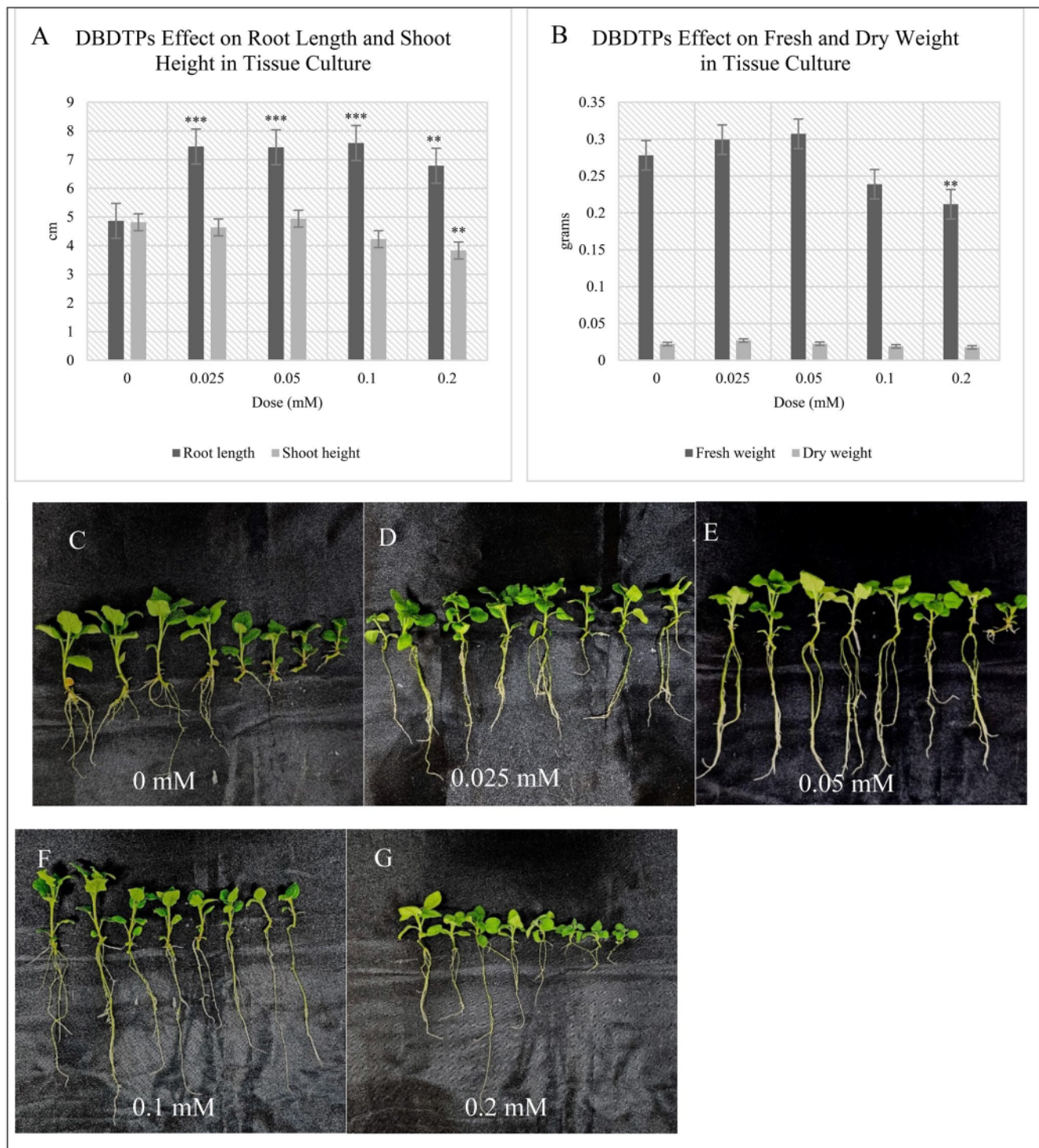


Fig. 1 Effect of DBDTP on potato plantlets of two cultivars (Russet Burbank and Clearwater) under tissue culture conditions after four weeks. (A) Potato root length and shoot height in response to DBDTP concentrations (B) Potato fresh and dry weight in response to DBDTP concentrations, (C) Plantlets grown under 0 mM DBDTP control, (D) Plantlets grown under 0.025 mM DBDTP, (E) Plantlets grown under 0.05 mM DBDTP, (F) Plantlets grown under 0.1 mM DBDTP, (G)

Plantlets grown under 0.2 mM DBDTP. Each data point in the figure represents the estimated mean ($\pm$ SE) from two trials. Error bars represent the standard error (SE) of the estimated marginal means. A single asterisk (*) indicates a significant difference from the control (0 mM DBDTP) at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis

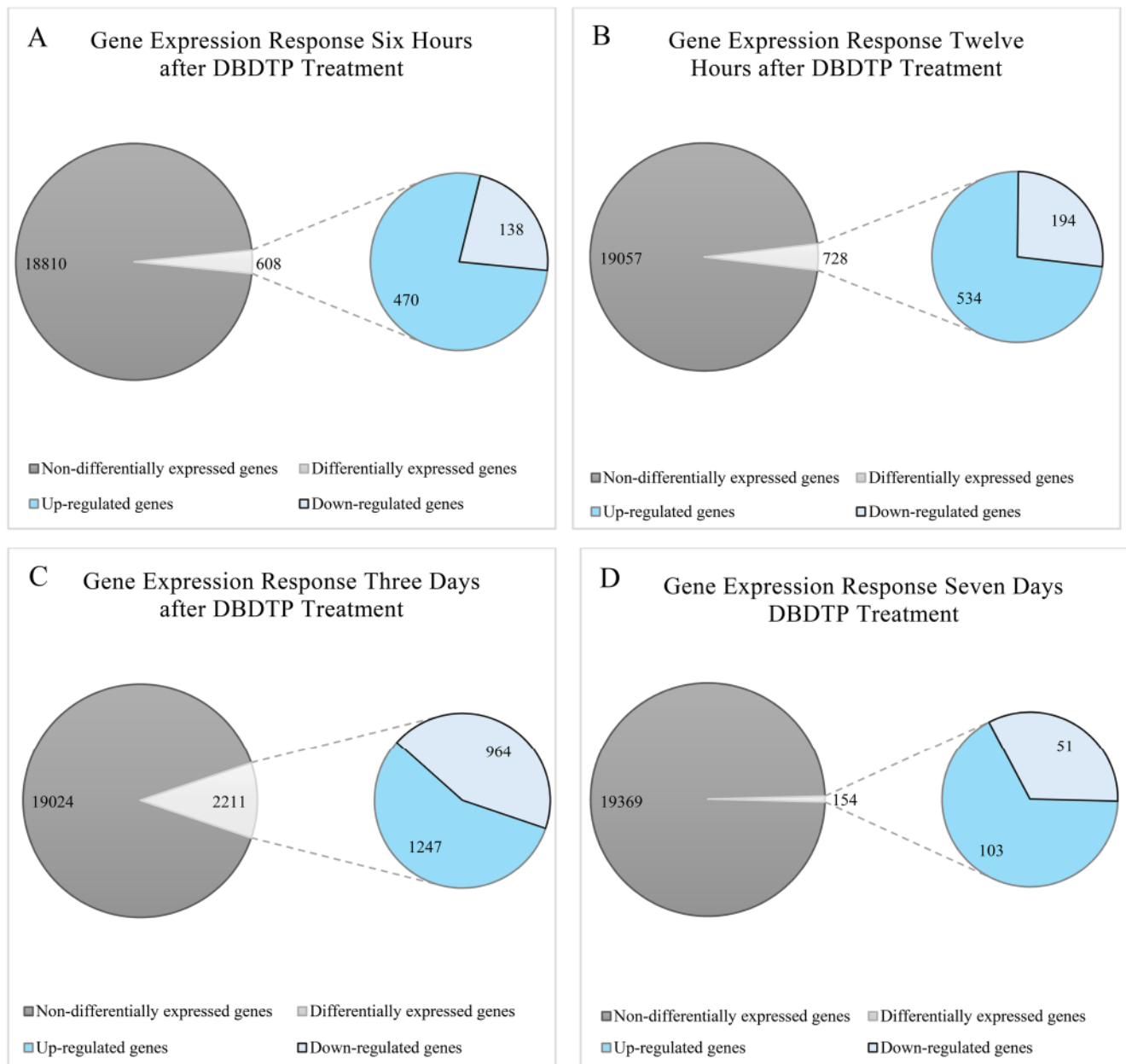


Fig. 2 Effect of DBDTP on gene expression at different time points in Russet Burbank potato shoots in tissue culture. **(A)** Number of DEGs between 0.1 mM and 0 mM DBDTP six hours after treatment. **(B)** Number of DEGs between 0.1 mM and 0 mM DBDTP twelve hours

after treatment, **(C)** Number of DEGs between 0.1 mM and 0 mM DBDTP three days after treatment, **(D)** Number of DEGs between 0.1 mM and 0 mM DBDTP seven days after treatment. The shown DEGs are expression level relative to 0 mM DBDTP

microtubule-based process (GO:0007017; 147 genes), microtubule-based movement (GO:0007018; 42 genes), and cell wall organization (GO:0071555; 160 genes) (Fig. 4). These GAGE results were similar with the GO over-representation analysis of DEGs from the DESeq2 differential expression analysis (Fig. 3), with both approaches showing pathways associated with plant growth.

Results from GAGE at 6 h after treatment showed that DBDTP significantly upregulated many defense and detoxification related pathways, including fungal response, glutathione

metabolic process, hydrogen peroxide catabolic process, and other terms for cellular detoxification or negative regulation of proteolysis (Supplementary Fig. 4). At 3 d, only down-regulated pathways related to photosynthesis were expressed (Supplementary Fig. 5). Finally, at 7 d, enrichment changed to upregulated pathways associated with response to auxin, photosynthesis, and photosynthetic light reaction (Supplementary Fig. 6). Overall, GAGE provided a pathway-level view of the DBDTP response in potato, even when only a fraction of the genes in a given pathway met the DEG threshold.

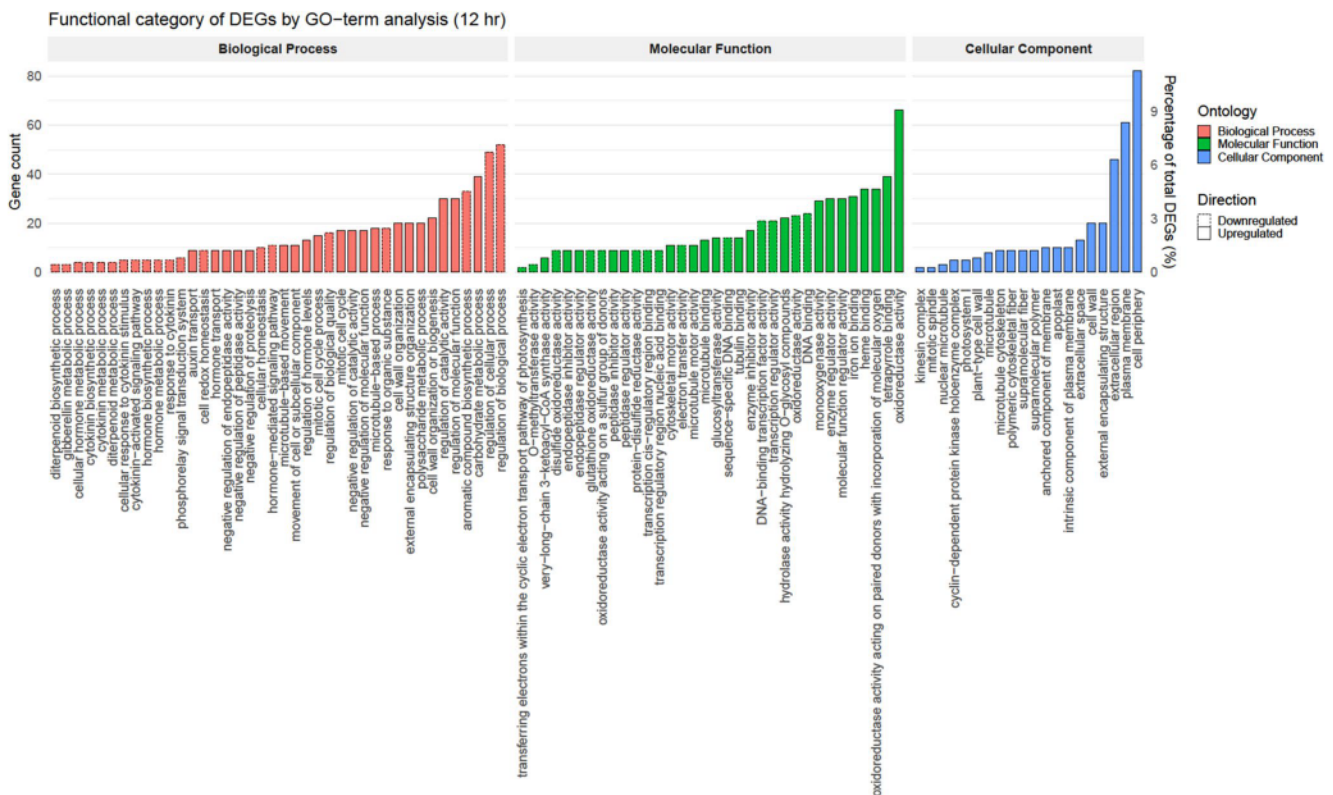


Fig. 3 Significant DEGs pathways for GO terms Biological Process, Molecular Function, and Cellular Component at twelve hours after treatment. Significant pathways are represented on the X-axis; the

number of DEGs annotated in each pathway was presented on the left Y-axis; and the right Y-axis showed the percentage of DEGs annotated in each pathway

Impact of DBDTP on Potato Under Field Conditions

The impact of DBDTPs on RB and CW potato plants was evaluated in 2023 and 2024 by measuring plant height and vigor at one and two months after transplanting. The number of tubers per plant, tuber weight per plant, and above ground biomass at harvest were also recorded. Potatoes were harvested two months after transplanting in 2023. In 2024 they were harvested three months after transplanting. This timing difference significantly affected the response variables, potatoes at two months after transplanting were at the tuber filling stage and three months after transplanting were already at the maturity stage. The results of the experiments were analyzed separately for each year and averaged over variety.

Effect of DBDTP on height, vigor, biomass, Tubers Per Plant, and Grams Per Plant in 2023

The results showed that the application of DBDTP at 10 mM significantly reduced plant height and vigor of field grown plants at one and two months after transplanting compared to the 0mM control (Fig. 5A, B). In contrast, the 0.5 mM and 2 mM concentrations led to positive trends between 10 and

17% for plant height and vigor at 1 month and 2 months after transplanting. As shown in Fig. 5C, the addition of 10 mM DBDTP decreased biomass per plant compared to the 0 mM treatment. On the other hand, the 2 mM and 0.5 mM treatments increased biomass compared to the control (0 mM), although these increases were also not statistically significant. Analysis of variance indicated a non-significant difference in the number of tubers for dose; however, the 0.5 mM treatment produced nearly one additional tuber per plant (Fig. 5D). In 2023, tuber weight per plant was significantly increased at 0.5 mM DBDTP by 24 g versus the control (Fig. 6A).

Effect of DBDTP on height, vigor, biomass, Tubers Per Plant, and Grams Per Plant in 2024

In 2024, DBDTP did not have a significant effect on height compared to the control at either one or two months after transplanting (Fig. 7A). The only significant observation was for plant vigor at one month after transplanting at 10 mM DBDTP, showing a negative impact (Fig. 7B). Plant biomass exhibited a positive trend at 0.5 mM DBDTP (Fig. 7C).

In terms of the impact of DBDTP concentration on tuber number per plant in 2024, the effect of 2 mM and 0.5 mM



Fig. 4 Global pathway enrichment analysis, twelve hours after DBDTP treatment. Dot size represents the number of expressed genes involved in each pathway. Different dot colors represented the adjusted P value of each pathway; the pathways with Adj P value ≤ 0.05 were signifi-

cantly enriched pathways. Arrow simples denote down regulation and closed circles upregulation. Rich Factor is the ratio between the number of expressed genes and all pathway annotated genes

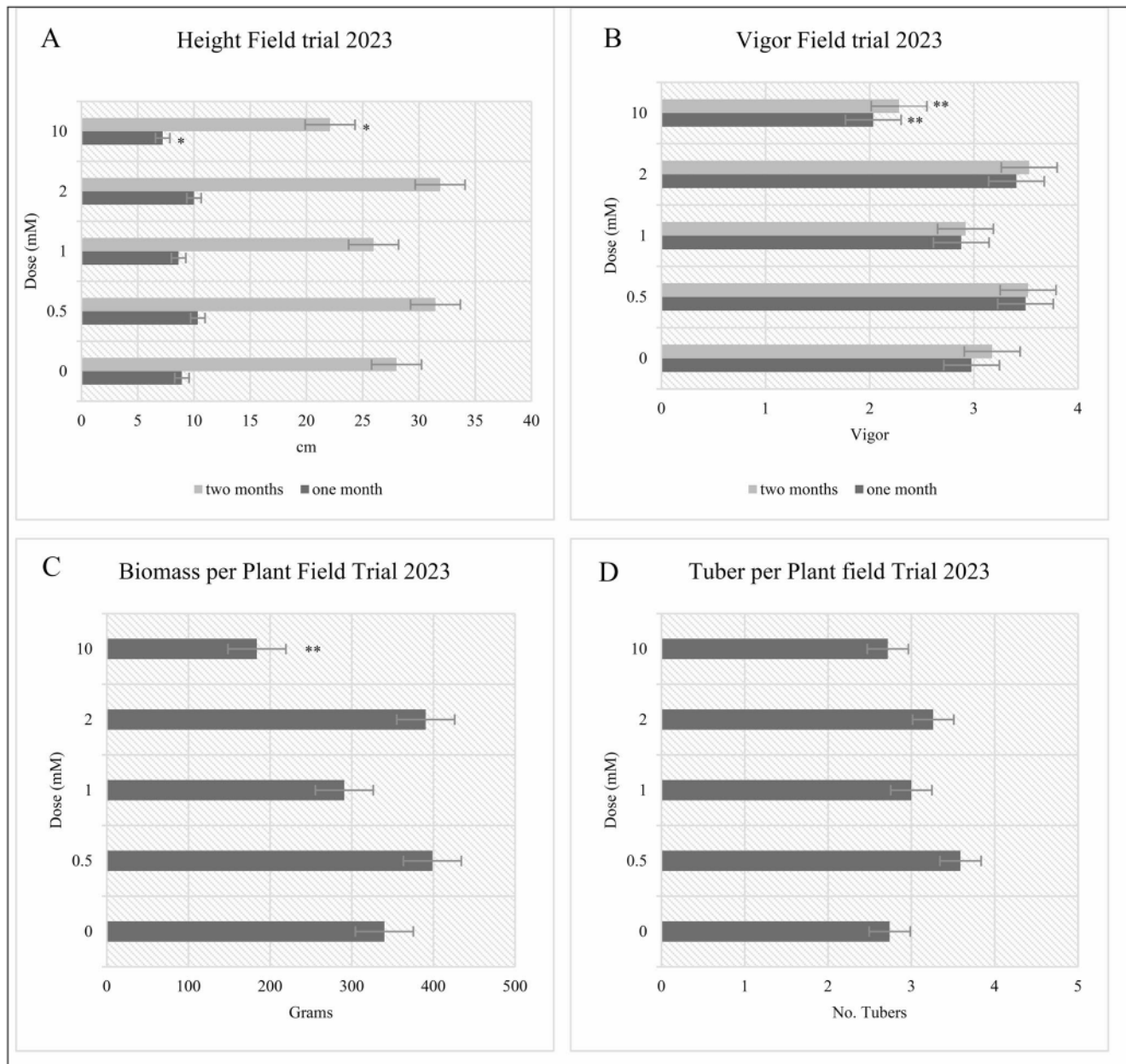


Fig. 5 Plant height, vigor, biomass, and tubers per plant of two cultivars (Russet Burbank and Clearwater) under field conditions for five concentrations of DBDTP in 2023. (A) Plant height measured one and two months after transplanting per DBDTP concentration, (B) Plant vigor assessed one and two months after transplanting per DBDTP concentration. (C) Plant biomass in grams per plant per DBDTP concentration. (D) Number of tubers per plant per DBDTP treatment. Each data point in the figure represents the estimated mean ($\pm$ SE) from the

first trial conducted in 2023, based on measurements from eight plants per treatment across ten blocks. Error bars represent the standard error (SE) of the estimated marginal means. A single asterisk (*) indicates a significant difference from the control (0 mM DBDTP) at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis

(Fig. 7D) was similar to the effect observed in the previous year (Fig. 5D), where these two concentrations increased the number of tubers per plant.

here was a significant effect on tuber weight in 2024 (ANOVA P value=0.046), though this significant effect was not detected between control and treatments (Table 1).

Nonetheless, DBDTP at 0.5 mM and 1 mM showed a trend in increasing tuber weight compared to the control (Fig. 6B).

Overall, results from 2023 to 2024 showed a consistent increasing trend in vigor, biomass, number of tubers per plant, and tuber weight per plant at 0.5 mM.

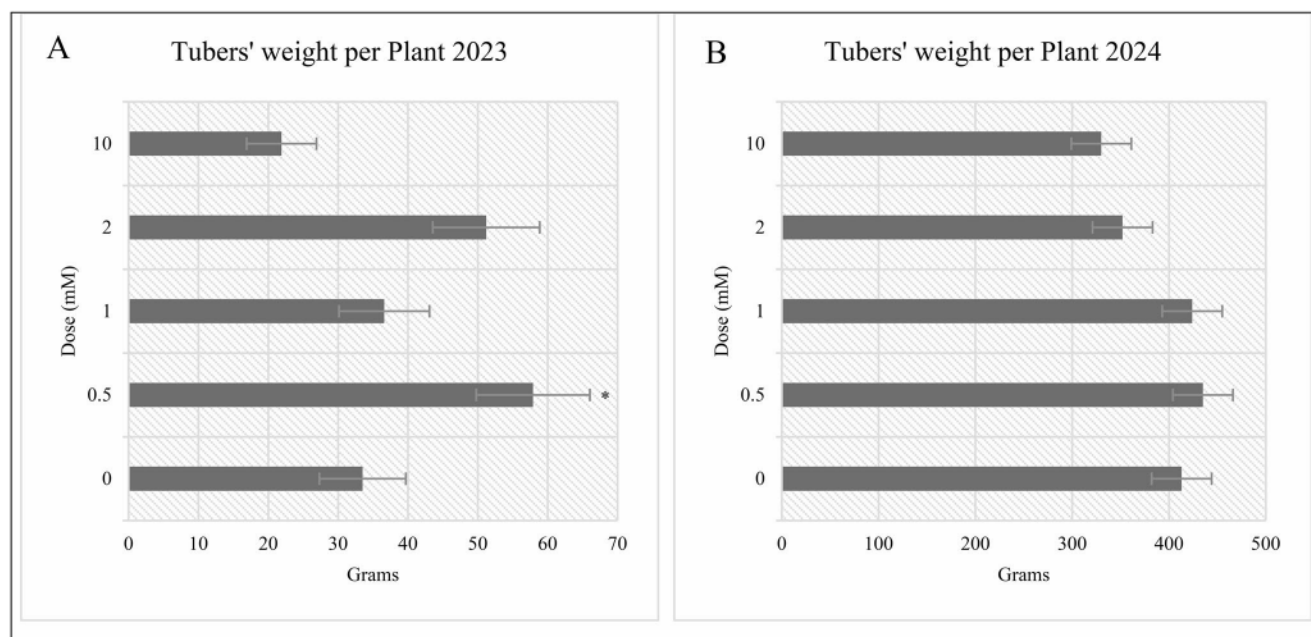


Fig. 6 Potato tubers' weight per plant in 2023 and 2024 of two cultivars (Russet Burbank and Clearwater) under field conditions at harvest after following DBDTP treatments. (A) Effect of four total DBDTP applications on tuber weight (grams per plant) in potatoes harvested in 2023, two months after transplanting. (B) Effect of four total DBDTP applications on tuber weight (grams per plant) in potatoes harvested in 2024, three months after transplanting. Each data point represents the estimated mean ($\pm$ SE) from separate trials conducted in 2023 and

2024, based on measurements from eight plants per treatment across ten blocks. Error bars represent the standard error (SE) of the estimated marginal means. A single asterisk (*) indicates a significant difference from the control (0 mM DBDTP) at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis

Effect of Foliar DBDTP Application

A significant interaction between the DBDTP dose and application time was observed, indicating that the same dose has different phytotoxicity at different developmental times. Thus, applying the same DBDTP dose earlier or later may reduce or increase damage. Results presented in Fig. 8A indicates that DBDTP foliar treatments induced phytotoxicity at one, eight, and 15 days after transplanting at concentrations of 1, 2, and 10 mM compared to the 0 mM control. For the 0.5 mM concentration, a notable phytotoxic effect was observed only one day after transplanting but not at eight and fifteen days after transplanting compared to the control. After one month, the analysis revealed a significant interaction between the two rounds of the experiment. Figure 8B illustrates that DBDTP produced effects like those observed at one, eight, and 15 days after transplanting, with concentrations of 1, 2, and 10 mM showing significant phytotoxicity. Interestingly, the 0.5 mM concentration did not exhibit phytotoxicity.

For shoot fresh weight, the mixed model indicated a marginal dose $\times$ application-time interaction (P value=0.07). Tukey post hoc tests showed that DBDTP significantly reduced shoot fresh weight at the higher doses when treatments were compared to control. At 1 and 15 days after

transplanting, plants sprayed with 10 mM DBDTP had significantly lower shoot fresh weight than the control, whereas no differences were detected at eight DAT or at doses ≤ 2 mM (Fig. 8C). One month after transplanting, shoot fresh weight again significantly decreased at 2 and 10 mM DBDTP compared with 0 mM (Fig. 8D).

Our findings indicate that the foliar application of DBDTP needs to be carried out with caution, as high concentrations cause significant foliage damage (Figs. 9A-C and 10A-E).

Discussion

In plants, it has been reported that treatment with H_2S increased seed vigor, root activity, the contents of photosynthetic pigments, plant growth and development, and stress tolerance of plants (Dai et al. 2024; Li, Min, & Zhou, 2016; Ali et al., 2013). H_2S plays a vital role in promoting the growth of primary roots as well as the development of lateral and adventitious roots. Furthermore, exogenous applications of H_2S have been shown to lead to the accumulation of auxins, which are critical for root growth (Mukherjee & Corpas, 2020; Wu et al., 2021). In tissue culture experiments summarized in this study, we confirmed that the application of DBDTP induced root growth by significantly increasing

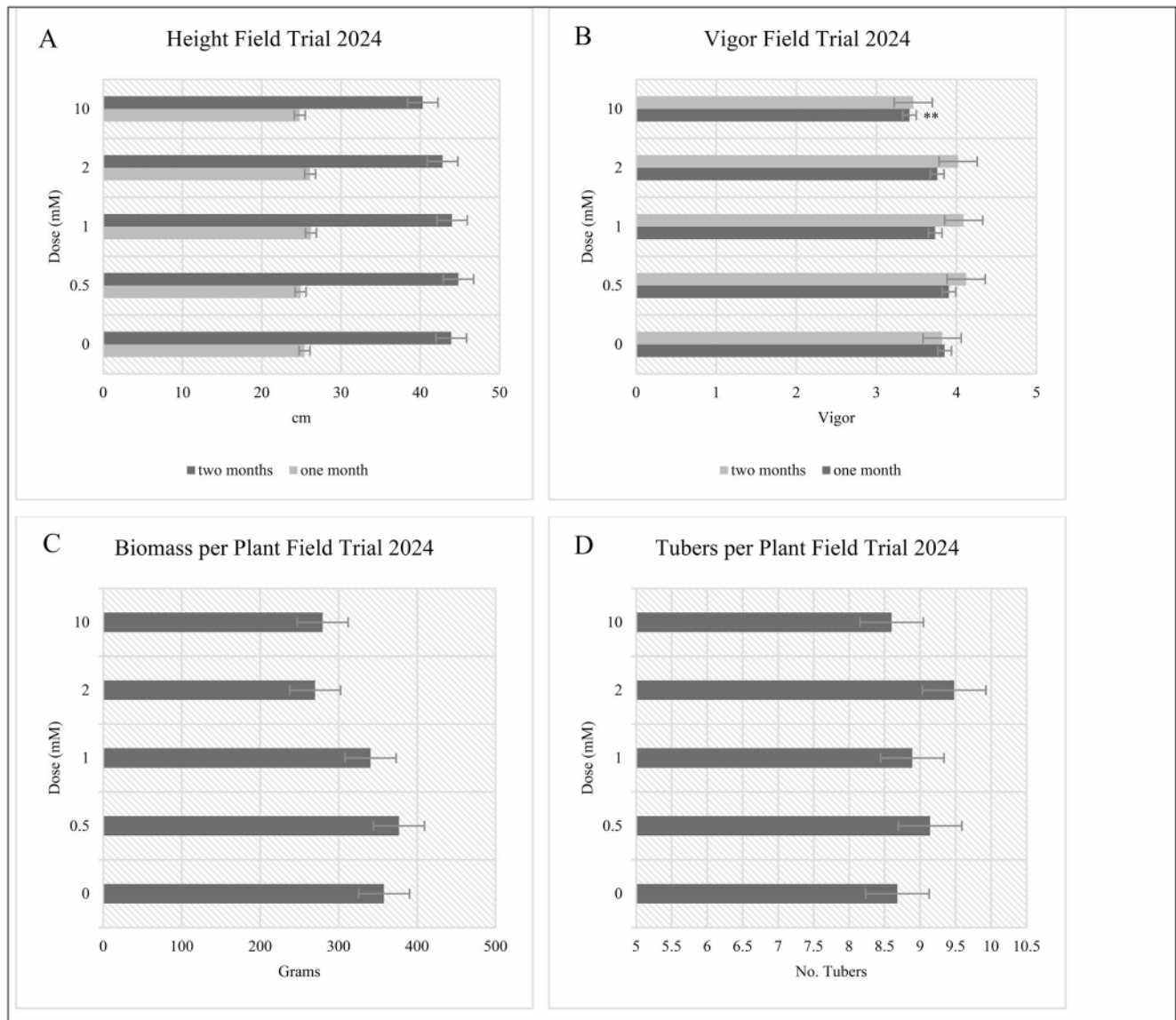


Fig. 7 Plant height, vigor, biomass, and tubers per plant of two cultivars (Russet Burbank and Clearwater) under field conditions for five concentrations of DBDTP in 2024. (A) Plant height measured one and two months after transplanting per DBDTP concentration, (B) Plant vigor assessed one and two months after transplanting per DBDTP concentration. (C) Plant biomass in grams per plant per DBDTP concentration. (D) Number of tubers per plant per DBDTP treatment. Each data point in the figure represents the estimated mean ($\pm$ SE) from the

second trial conducted in 2024, based on measurements from eight plants per treatment across ten blocks. Error bars represent standard error (SE) of the estimated marginal mean. A single asterisk (*) indicates a significant difference from the control (0 mM DBDTP) at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis

Table 1 Planned contrasts comparing each DBDTP dose with the 0 mM control for tuber weight per plant in 2024 (g per plant) in two potato cultivars (Russet Burbank and Clearwater). Pairwise contrasts were obtained from the emmeans of the mixed model and adjusted using the Sidak method

Contrast	Estimate	SE	Df	t.ratio	p.value
Dose 0 vs. Dose 0.5	22.2	41.2	72	0.54	0.97
Dose 0 vs. Dose 1	10.9	41.2	72	0.26	0.99
Dose 0 vs. Dose 2	-60.3	41.2	72	-1.46	0.47
Dose 0 vs. Dose 10	-82.8	41.2	72	-2.01	0.18

root length for all tested concentrations. Specifically, at low concentrations, 0.025 mM increased root length by 53.3%, 0.05 mM by 52.9%, and 0.1 mM by 56.0%. Even at the highest concentration of 0.2 mM, root length was increased by 39.5% (Fig. 1A). Previous studies demonstrated that H_2S promotes the formation of adventitious roots in cucumbers by inducing the expression of genes related to haem oxygenase-1 (*HO-1*), which is part of a root development signaling pathway (Lin et al., 2012). H_2S also increases the number

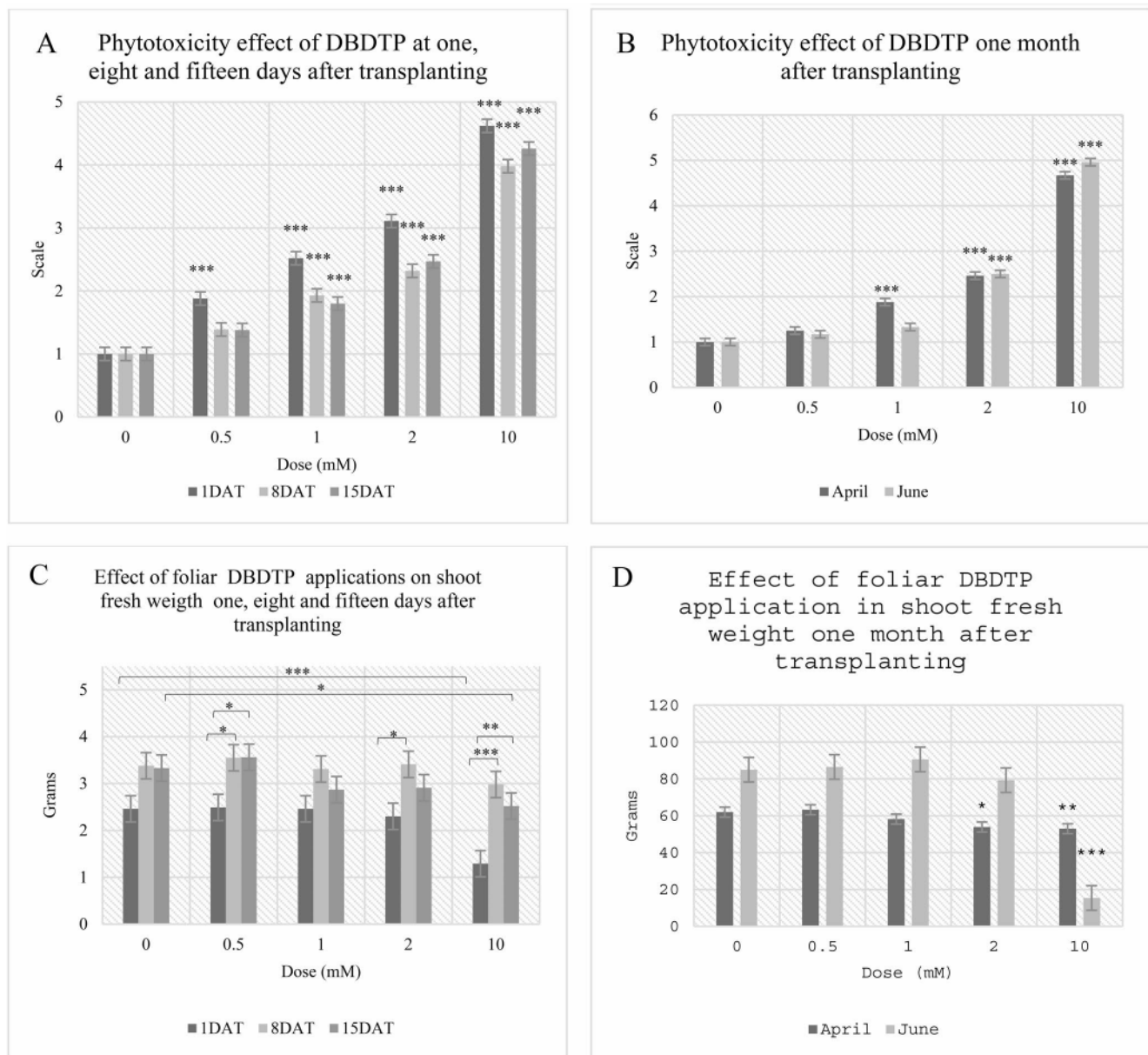


Fig. 8 Effect of DBDTP as foliar applications on Russet Burbank potato plantlets at different application times. **(A)** Visual assessment of phytotoxicity at one, eight, and fifteen days after transplanting for five DBDTP treatments from two trials analyzed together. **(B)** Visual assessment of phytotoxicity one month after transplanting for both trials (April and June 2024) analyzed separately. **(C)** Fresh weight per plant for the two rounds of the experiment at one, eight, and 15 days after transplanting analyzed together, significance versus control and within same treatments. **(D)** Fresh weight per plant at one month after

transplanting application for the two rounds of the experiment analyzed separately. Each data point in the figure represents the estimated mean ($\pm$ SE) from two trials. Error bars represent the standard error (SE) of the estimated marginal means. A single asterisk (*) indicates a significant difference from the control (0 mM DBDTP) at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis

of lateral roots, total root length, and the total root surface area (Jia et al., 2015; Wu et al., 2021). Larger root systems are crucial for plants to acquire water and maximize nutrient uptake from the soil, while also providing support and stress tolerance (Epstein, 1977). Despite root length increases, our findings indicate that DBDTP can negatively impact shoot height and fresh weight when applied at high levels, such as

0.2 mM. At this concentration, shoot height was reduced by 20.37% and fresh weight by 22.22% (Fig. 1A and B). Alternatively, a positive trend was observed at 0.05 mM DBDTP for height (2.70%) and fresh weight (11.11%). In the case of dry weight, there was a non-significant negative effect at 0.2 mM and a non-significant positive effect of 18.18% at 0.025 mM.



Fig. 9 Phytotoxicity damage on Russet Burbank potato plantlets due to foliar application of five DBDP treatments at one, eight, fifteen, and one month after transplanting on potato plants grown in plugs. (A) Phytotoxicity at one day after transplanting with doses 0.5, 0, 10, 1,

and 2 mM (left to right), (B) Phytotoxicity eight days after transplanting with doses 0, 10, 1, 2, and 0.5 mM (left to right), (C) Phytotoxicity fifteen day after transplanting with doses 0, 1, 2, 0.5, and 10 mM (left to right)

Wu et al. (2021) demonstrated, based on RNA-Seq, that in peach roots, exogenous H_2S led to a 44.50% increase in the concentration of endogenous auxin. In another study under in vitro conditions in potatoes, Kolachevskaya et al. (2014) created transgenic lines harboring the *Agrobacterium* auxin biosynthesis gene *tms1*. Expression of *tms1* was detected mostly in tubers rather than in shoots, and a positive correlation was observed between *tms1* expression, Indoleacetic acid (IAA) content in tubers, and stimulation

of tuber formation, implying an important role of auxins in potato tuberization. Auxins are known for being key root development regulators. Our tissue culture results demonstrated DBDTP application in tissue culture increased root length. This enhancement may be due to the ability of H_2S to increase auxin.

The results from the RNA-Seq at twelve hours after treatment revealed that H_2S regulates the expression of DEGs related to auxin pathways (Fig. 3), supporting previous

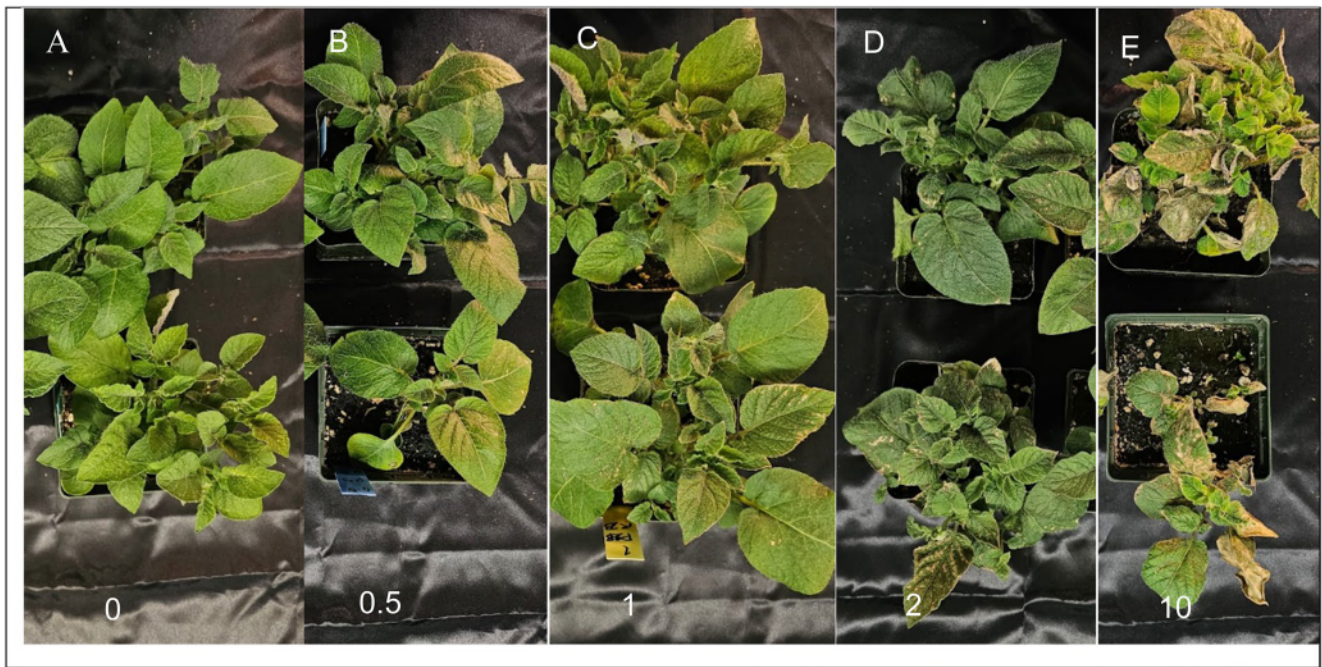


Fig. 10 Phytotoxicity damage on Russet Burbank potato plantlets due to foliar applications of DBDTP treatments one month after potato transplanting, (A) 0 mM DBDTP treatment, (B) 0.5mM DBDTP treat-

ment, (C) 1 mM DBDTP treatment, (D) 2 mM DBDTP treatment, (E) 10 mM DBDTP treatment

studies (Lin et al., 2012; Mukherjee & Corpas, 2020; Wu et al., 2021) where exogenous applications of H_2S have led to the accumulation of auxins and the expression of auxin related genes. Global pathway analysis also showed auxin-related pathways that are upregulated at twelve hours and seven days after treatment (Fig. 4, Supplementary Fig. 6), indicating again that DBDTPs are involved in the process of root growth. Enhancing auxin content in young tissue is primordial, as research has demonstrated that young true leaves are an important source for the IAA required for the formation of lateral root primordia in *Arabidopsis* (Bhalerao et al., 2002; Ljung et al., 2002). Increasing auxin concentration may increase potato yields, given the role of auxins in potato tuberization (Kolachevskaya et al., 2014). Therefore, testing DBDTP application on a larger scale in greenhouses and field experiments, could lead to methods for increased potato production.

A positive trend in plant height and vigor was observed for potato plants treated with DBDTP in 2023 field (Fig. 5A, B). Treatment with 0.5 mM DBDTP increased height by 15.9% and vigor by 17.2% compared to the control in the first month, while 2 mM treatment improved these responses by 12% and 14.5%, respectively. Additionally, the 0.5 mM and 2 mM DBDTP treatments led to a 12.3% and 13.8% increase in height and a 10.8% and 11.2% increase in vigor when compared to the control at the second month time-point. In 2024, we observed again a small non-significant effect on height due to DBDTP; one month after transplanting, the

1 mM treatment increased height by 3.1% compared to the control. Meanwhile, two months after transplanting, height was increased by 2.0% and vigor by 7.8% (Fig. 7A) when 0.5 mM was applied. While these results were not significant, they support information reported by other authors on the positive effects of H_2S on plant growth.

A significant negative effect on height and vigor was observed in 2023 when the highest concentration of DBDTP (10 mM) was used (Fig. 5A, B). Plant height in the first and second months was reduced by 19% and 21% respectively, compared to the control treatment (0 mM). However, in 2024, the only significant negative effect was observed for vigor in the first month at 10 mM DBDTP, with a reduction of 10.5% (Fig. 7B). In many studies, it has been reported that the use of high concentrations of H_2S can have a negative impact on plant growth. Thompson & Kats, (1978) found that the use of 3,000 ppm H_2S in greenhouses caused leaf lesions, defoliation, and reduced growth. High levels of the H_2S donor NaHS inhibit auxin transport and lead to changes in root system development. This inhibition occurs by repressing primary root growth through the activation of a signal transduction pathway involving the accumulation of reactive oxygen species (ROS), activation of *Mitogen-Activated Protein Kinase 6* (MPK6), and production of nitric oxide (NO) (Jia, Hu, Fan, & Li, 2015; Zhang, Luo, Wang, & Xu, 2017). Furthermore, seeds pre-soaked in 8.0 mM NaHS delayed germination (Dai et al., 2024).

The highest treatment (10 mM DBDTP) exhibited a significant negative effect on biomass, affecting fresh weight by 45.9% per plant compared to the control in 2023. However, the use of 0.5 mM and 2 mM concentrations showed a positive, non-significant effect and increased fresh weight by 17.3% and 14.9% respectively (Fig. 5C). While not significant, the fresh weight of plants treated with DBDTP in 2024 also trended positive. Fresh weight was reduced by 21.8% when treated with 10 mM DBDTP, while treatment at 0.5 mM increased fresh weight by 5.3% compared to 0 mM (Fig. 7C).

In previous studies, it was shown that using dibutyl dithiophosphate significantly increased biomass of corn plants by 39% when 1 mg per plant of dibutyl dithiophosphate was used. However, higher concentrations (100 mg and 200 mg) had a negative impact on biomass and height of the plants (Carter et al., 2019). Additionally, Carter et al. (2018) found that using H₂S with the donor GYY-4137 significantly boosted the growth of radishes, peas, and lettuce. But once again, higher concentrations had a negative effect on plant growth. Together, these two studies by Carter et al. (2018, 2019) revealed a similar pattern where low concentrations increased growth, some middle concentrations had a similar effect as the control, and higher concentrations led to reduced growth. Our findings align with those of these authors, indicating that the highest concentrations resulted in negative effects on plant growth, while low concentrations had a positive impact.

Concerning the number of tubers per plant, the difference for the treatments approached statistical significance (P -value=0.054) in 2023 but not in 2024 (P -value=0.54). In 2023, 0.5 mM and 2 mM increased the number of tubers by 31.0% and 19.1% compared to the control treatment (Fig. 5D), while in 2024 the same concentrations increased tubers by 5.3% and 9.2%, suggesting once more the role of DBDTP in promoting plant developmental processes.

In 2023, we obtained a significant effect on tuber weight per plant, where 0.5 mM significantly increased weight by 72.8% per plant, and 2 mM treatment increased tuber weight by 52.8% compared to the control treatment (Fig. 6A). In 2024, the results did not show a significant effect; however, 0.5 mM was 5.3% higher than the control (Fig. 6B). Furthermore, the 10 mM concentration reduced tuber weight per plant by 29.8% in 2023 and 20.1% in 2024 compared to 0 mM.

As mentioned before, PVY infection was detected in an adjacent field in 2023 leading to an earlier harvest, whereas no PVY symptoms were observed in the 2024 trial. It is therefore possible that the stronger DBDTP effects on tuber weight observed in 2023, but not in 2024, reflect a benefit of H₂S that is greater in early plant development. It is also possible that some of our plants in 2023, though asymptomatic

were virus infected and that the positive impact of DBDTP was magnified in the stressed plants. Exogenous H₂S can inhibit fungal spore germination and disease development (Wu et al., 2018) and induce the expression of biotic-defense-related genes (Shi et al., 2015). H₂S can also suppress viral replication and lesion development in tobacco infected with TMV (Pang et al., 2021). We believe that DBDTP may have partially mitigated PVY-associated yield losses in 2023, whereas this benefit was not apparent in the PVY-free conditions of 2024. However, our experiment was not designed to quantify disease incidence or to test this mechanism directly, so specific studies will be required to confirm this hypothesis.

An interesting pattern was observed in our data from 2023 for all response variables. The 0.5 mM treatment increased the responses and 1 mM treatment showed results similar to the control. Subsequently, the 2 mM treatment again enhanced the responses, while the 10 mM treatment negatively affected them. Similar trends have been reported by other authors. Carter et al. (2018, 2019) showed comparable patterns in their studies on corn plant height and mass, radish root weight, pea shoot height and fresh weight, and lettuce biomass. In these cases, the lowest concentration had a positive effect, an intermediate concentration resembled the control, other intermediate concentrations enhanced plant growth, and the highest concentrations negatively impacted the plants. In 2024 our data only showed this pattern for the response variables tubers per plant, height, and vigor one month after transplanting, but not for the other variables.

These results emphasize the necessity of defining appropriate concentrations of DBDTP for field application and that this will likely vary for crop species. As indicated by all response variables, using concentrations that are too high has toxic effects on plants, leading to reduced height, vigor, and yield. This observation confirms the findings of many authors who have noted that while exogenous H₂S can have positive effects on plants at low concentrations, it can be phytotoxic at higher levels, negatively impacting germination, root growth, and other aspects of plant growth and development (Gupta et al., 2021; Li et al. 2012; Zhang, Luo, Wang, & Xu, 2017).

We observed that concentrations of 1, 2, and 10 mM can lead to significant damage when applied at one, eight, and fifteen days, and one month after transplanting compared to the control (Fig. 8). On the other hand, 0.5 mM concentration exhibited a similar response to the control group for the eight and fifteen DAT group and for the one month after transplanting group; This highlights the importance of establishing timing and concentration recommendations for foliar applications. Furthermore, our results indicate that the 1 mM concentration generally caused significant foliage damage. The 0.5 mM concentration generally did not

exhibit significant phytotoxicity. It only caused significant damage (22%) at 1 DAT compared to the control plants, but the damage remained under 10% for subsequent application times. These results indicate that 0.5 mM might be suitable for application in foliar trials one week after transplanting.

Conclusion

The application of hydrogen sulfide donor DBDTP under different experimental conditions showed fascinating results, indicating its potential for further investigation. In tissue culture trials, DBDTP significantly improved root length across all tested concentrations (0.025, 0.05, 0.1, and 0.2 mM). While height and fresh weight did not show significant improvements, the 0.05 mM concentration demonstrated a slight enhancement in these traits. RNA-seq results showed the upregulation of genes in the GO term Biological Process for auxin pathways at twelve and seven days after DBDTP treatment. In field trials, the application of DBDTP enhanced plant vigor, height, biomass, tubers per plant, and yield under 0.5 mM concentration, with an increase of 5% in key traits such as tuber weight per plant compared to the control. Higher concentrations, such as 10 mM, had a detrimental impact on plant performance, significantly affecting the evaluated traits.

The phytotoxicity trial results indicates that 0.5 mM is the most appropriate concentration for foliar applications, as it did not cause visible damage when applied eight DAT. Additional research is needed to refine phytotoxicity assessments by incorporating more physiological parameters such as dry weight, height, and yield, alongside visual ratings.

These findings reinforce DBDTP's potential to enhance plant growth and yield. Further research is needed to optimize its application in agricultural systems such as fertigation or tuber treatment in potato production to provide full benefits as a fertilizer additive and ensure appropriate doses and timings.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s12230-026-10041-1>.

Author Contributions Experiment conceptualization was performed by A.O., N.B., M.J.G. and methodology determined by by L.E.-C., N.Z., A.O., J.M.M., M.J.G. Formal Analysis and investigation by L.E.-C., and M.J.G. Resources obtained by N.B., N.Z. and M.J.G. Data curation by L.E.-C., A.O., J.M.M. and paper first draft and data visualizations created by L.E.-C. and A.O. Project supervision by M.J.G. and funding acquisition by N.B. and M.J.G. All authors reviewed the manuscript and offered edits and suggestions.

Funding We acknowledge funding from the Montana Department of Agriculture, the USDA Specialty Crop Program and the Montana Agricultural Experiment Station.

Data Availability Data is provided within the manuscript and supplementary information files.

Declarations

Competing Interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Ali, S., M.A. Farooq, S. Hussain, T. Yasmeen, G.H. Abbasi, and G. Zhang. 2013. Alleviation of chromium toxicity by hydrogen sulfide in barley. *Environmental Toxicology and Chemistry* 32 (10): 2234–2239. <https://doi.org/10.1002/etc.2309>.
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models Using lme4. *Journal of Statistical Software*, 67(1). <https://doi.org/10.18637/jss.v067.i01>
- Bhalerao, R.P., J. Eklöf, K. Ljung, A. Marchant, M. Bennett, and G. Sandberg. 2002. Shoot-derived auxin is essential for early lateral root emergence in *Arabidopsis* seedlings. *The Plant Journal* 29 (3): 325–332. <https://doi.org/10.1046/j.0960-7412.2001.01217.x>.
- Bloem, E., A. Riemenschneider, J. Volker, J. Papenbrock, A. Schmidt, I. Salac, S. Haneklaus, and E. Schnug. 2004. Sulphur supply and infection with *Pyrenopeziza brassicae* influence L-cysteine desulphydrase activity in *Brassica napus* L. *Journal of Experimental Botany* 55 (406): 2305–2312. <https://doi.org/10.1093/jxb/erh236>.
- Brown, E.M., N.P.R. Ranasinghe Arachchige, A. Paudel, and N.B. Bowden. 2021. Synthesis, stability, and kinetics of hydrogen sulfide release of dithiophosphates. *Journal of Agricultural and Food Chemistry* 69 (43): 12900–12908. <https://doi.org/10.1021/acs.jafc.1c04655>.
- Carter, J.M., E.M. Brown, J.P. Grace, A.K. Salem, E.E. Irish, and N.B. Bowden. 2018. Improved growth of pea, lettuce, and radish plants using the slow release of hydrogen sulfide from GYY-4137. *PLoS One* 13 (12): e0208732. <https://doi.org/10.1371/journal.pone.0208732>.
- Carter, J.M., E.M. Brown, E.E. Irish, and N.B. Bowden. 2019. Characterization of dialkyl dithiophosphates as slow hydrogen sulfide releasing chemicals and their effect on the growth of maize. *Journal of Agricultural and Food Chemistry* 67 (43): 11883–11892. <https://doi.org/10.1021/acs.jafc.9b04398>.
- Dai, J., D. Wen, H. Li, J. Yang, X. Rao, Y. Yang, J. Yang, C. Yang, and J. Yu. 2024. Effect of hydrogen sulfide (H₂S) on the growth and development of tobacco seedlings in absence of stress. *BMC Plant Biology*. <https://doi.org/10.1186/s12870-024-04819-w>.
- Epstein, E. 1977. The role of roots in the chemical economy of life on Earth. *BioScience* 27 (12): 783–787. <https://doi.org/10.2307/1297754>.
- García Mata, C., and L. Lamattina. 2013. Gasotransmitters are emerging as new guard cell signaling molecules and regulators of leaf

- gas exchange. *Plant Science* 201: 66–73. <https://doi.org/10.1016/j.plantsci.2012.11.007>.
- Ge, S. X., Son, E. W., & Yao, R. (2018). iDEP: an integrated web application for differential expression and pathway analysis of RNA-Seq data. *BMC Bioinformatics*, 19(1). <https://doi.org/10.1186/s12859-018-2486-6>
- Gupta, S. K., Marwa, N., Pandey, A. K., Zhang, Y.-B., & Zhang, J.-L. (2021). Hydrogen sulfide homeostasis in plants: An overview. Elsevier EBooks, 341–363. <https://doi.org/10.1016/b978-0-323-85862-5.00017-8>
- Jia, H., Hu, Y., Fan, T., & Li, J. (2015). Hydrogen sulfide modulates actin-dependent auxin transport via regulating ABPs results in changing of root development in Arabidopsis. *Scientific Reports*, 5(1). <https://doi.org/10.1038/srep08251>
- Kolachevskaya, O.O., V.V. Alekseeva, L.I. Sergeeva, E.B. Rukavtsova, I.A. Getman, D. Vreugdenhil, Y.I. Buryanov, and G.A. Romanov. 2014. Expression of auxin synthesis gene *tms1* under control of tuber-specific promoter enhances potato tuberization in vitro. *Journal of Integrative Plant Biology* 57 (9): 734–744. <https://doi.org/10.1111/jipb.12314>.
- Kolupaev, Y.E., Y.V. Karpets, M.A. Shklyarevskiy, T.O. Yastreba, S.H. Plohovska, A.I. Yemets, and Y.B. Blume. 2022. Gasotransmitters in plants: Mechanisms of participation in adaptive responses. *The Open Agriculture Journal*. <https://doi.org/10.2174/18743315-v16-e2207050>.
- Li, Z.B., and L. Zhu. 2014. Hydrogen sulfide donor sodium hydrosulfide-induced accumulation of betaine is involved in the acquisition of heat tolerance in maize seedlings. *Brazilian Journal of Botany* 38 (1): 31–38. <https://doi.org/10.1007/s40415-014-0106-x>.
- Li, Z.-G., M. Gong, and P. Liu. 2012. Hydrogen sulfide is a mediator in H₂O₂-induced seed germination in *Jatropha curcas*. *Acta Physiologiae Plantarum* 34 (6): 2207–2213. <https://doi.org/10.1007/s11738-012-1021-z>.
- Li, Z.-G., Min, X., & Zhou, Z.-H. (2016). Hydrogen Sulfide: A Signal Molecule in Plant Cross-Adaptation. *Frontiers in Plant Science*, 7. <https://doi.org/10.3389/fpls.2016.01621>
- Lin, Y.-T., M.-Y. Li, W.-T. Cui, W. Lu, and W.-B. Shen. 2012. Haem oxygenase-1 is involved in hydrogen sulfide-induced cucumber adventitious root formation. *Journal of Plant Growth Regulation* 31 (4): 519–528. <https://doi.org/10.1007/s00344-012-9262-z>.
- Lisjak, M., T. Teklic, I.D. Wilson, M.E. Wood, M. Whiteman, and J.T. Hancock. 1988. Abstracts of eleventh annual meeting, Japanese Society of Biorheology. *Biorheology* 25 (6): 893–915. <https://doi.org/10.3233/bir-1988-25610>.
- Ljung, K., R.P. Bhalerao, and G. Sandberg. 2002. Sites and homeostatic control of auxin biosynthesis in Arabidopsis during vegetative growth. *The Plant Journal* 28 (4): 465–474. <https://doi.org/10.1046/j.1365-3113x.2001.01173.x>.
- Love, M.I., W. Huber, and S. Anders. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* 15 (12): 550. <https://doi.org/10.1186/s13059-014-0550-8>.
- Mukherjee, S., and F.J. Corpas. 2020. Crosstalk among hydrogen sulfide (H₂S), nitric oxide (NO) and carbon monoxide (CO) in root-system development and its rhizosphere interactions: A gaseous interactome. *Plant Physiology and Biochemistry* 155: 800–814. <https://doi.org/10.1016/j.plaphy.2020.08.020>.
- Nelson, G.C., H. Valin, R.D. Sands, P. Havlik, H. Ahammad, D. Deryng, J. Elliott, S. Fujimori, T. Hasegawa, E. Heyhoe, P. Kyle, M. Von Lampe, H. Lotze-Campen, D. Mason d'Croz, H. van Meijl, D. van der Mensbrugghe, C. Müller, A. Popp, R. Robertson, and S. Robinson. 2013. Climate change effects on agriculture: Economic responses to biophysical shocks. *Proceedings of the National Academy of Sciences* 111 (9): 3274–3279. <https://doi.org/10.1073/pnas.1222465110>.
- Ortiz, O., & Mares, V. (2017). The Historical, Social, and Economic Importance of the Potato Crop. *Compendium of Plant Genomes*, 1–10. https://doi.org/10.1007/978-3-319-66135-3_1
- Pang, Z., H. Ye, D. Ma, X. Tu, L. Yi, and Z. Xi. 2021. A H₂S-specific ultrasensitive fluorogenic probe reveals TMV-induced H₂S production to limit virus replication. *ChemBioChem* 22 (13): 2292–2299. <https://doi.org/10.1002/cbic.202100138>.
- Ranasinghe Arachchige, N. P. R., E. M. Brown, and N. B. Bowden. 2022. Sustained release of hydrogen sulfide from di(t-butanol) dithiophosphate phenethylamine salt encapsulated into poly(lactic acid) microparticles to enhance the growth of radish plants. *ACS Agricultural Science & Technology* 2 (5). <https://doi.org/10.1021/acscagascitech.2c00179>.
- Sharma, S., Jaiswal, A. K., & Jaiswal, S. (2020). Potato. Elsevier EBooks, 339–347. <https://doi.org/10.1016/b978-0-12-812780-3.00021-0>
- Shi, H., T. Ye, N. Han, H. Bian, X. Liu, and Z. Chan. 2015. Hydrogen sulfide regulates abiotic stress tolerance and biotic stress resistance in Arabidopsis. *Journal of Integrative Plant Biology* 57 (7): 628–640. <https://doi.org/10.1111/jipb.12302>.
- Thompson, C.R., and G. Kats. 1978. Effects of continuous hydrogen sulfide fumigation on crop and forest plants. *Environmental Science & Technology* 12 (5): 550–553. <https://doi.org/10.1021/es60141a001>.
- Wang, R. 2018. Overview of Gasotransmitters and the Related Signaling Network. In *Gasotransmitters*, 1–28. The Royal Society of Chemistry. <https://doi.org/10.1039/9781788013000-00001>.
- Wu, W., Zhang, C., Chen, L., Li, G., Wang, Q., & Shi, J. (2018). Inhibition of hydrogen sulfide and hypotaurine on Monilinia fructicola disease in peach fruit. *Acta Horticulturae*, 1194(38), 257–266. <https://doi.org/10.17660/actahortic.2018.1194.38>
- Wu, X., A. Du, S. Zhang, W. Wang, J. Liang, F. Peng, and Y. Xiao. 2021. Regulation of growth in peach roots by exogenous hydrogen sulfide based on RNA-Seq. *Plant Physiology and Biochemistry* 159: 179–192. <https://doi.org/10.1016/j.plaphy.2020.12.018>.
- Yang, Z., X. Wang, J. Feng, and S. Zhu. 2022. Biological functions of hydrogen sulfide in plants. *International Journal of Molecular Sciences* 23 (23): 15107. <https://doi.org/10.3390/ijms232315107>.
- Yates, A.D., J. Allen, R.M. Amode, A.G. Azov, M. Barba, A. Becerra, J. Bhai, L.I. Campbell, M. Carbajo Martinez, M. Chakiachvili, K. Chougule, M. Christensen, B. Contreras-Moreira, A. Cuzick, L. Da Rin Fioretto, P. Davis, N.H. De Silva, S. Diamantakis, S. Dyer, and P. Flicek. 2022. Ensembl genomes 2022: An expanding genome resource for non-vertebrates. *Nucleic Acids Research* 50 (1): D996–D1003. <https://doi.org/10.1093/nar/gkab1007>.
- Zhang, P., Q. Luo, R. Wang, and J. Xu. 2017. Hydrogen sulfide toxicity inhibits primary root growth through the ROS-NO pathway. *Scientific Reports*. <https://doi.org/10.1038/s41598-017-01046-2>.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.