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GENE EXPRESSION DYNAMICS IN ALFALFA UNDER LEAD-INDUCED STRESS

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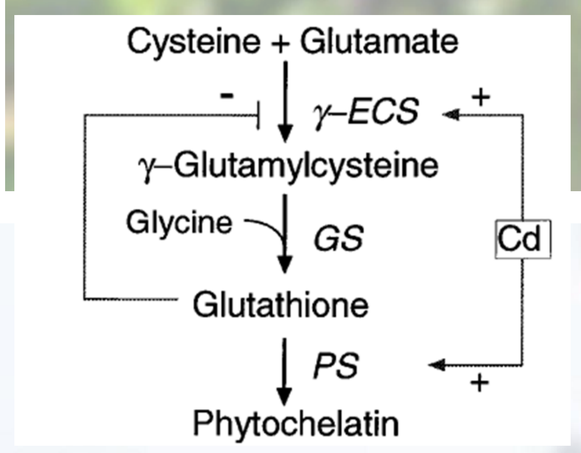
Heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As) and copper (Cu) are chemical elements that can become harmful to plants when found in high concentrations. Although some metals, like copper and zinc, are essential micronutrients in small quantities, their excessive accumulation can severely impact plant health and disrupt environmental balance.

Gene expression in response to heavy metal stress plays a crucial role in plant adaptation and survival. High concentrations of metals like cadmium (Cd) and lead (Pb) can be toxic to cells, disrupting metabolic and physiological functions. To mitigate this stress, plants activate complex genetic regulatory networks, including genes involved in heavy metal homeostasis, detoxification, chelation, oxidative stress response, and transcriptional regulation.

Phytochelatins, chelating proteins that bind toxic metals and reduce their harmful effects, are not directly produced by the expression of a heavy metal tolerance gene. Instead, they result from a metabolic pathway that utilizes glutathione as a substrate, involving the enzymes γ-glutamylcysteine synthetase, glutathione synthetase, serine acetyltransferase, and cysteine synthetase.



This study aimed to evaluate the expression of genes encoding these enzymes in alfalfa plants exposed to varying lead concentration.

REVERS-TRANSCRIPTASE PCR

BIOLOGICAL MATERIALS

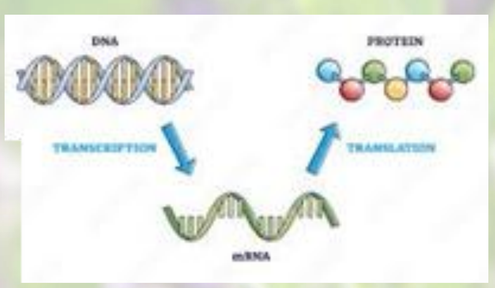
Two alfalfa varieties – Sigma and Satelit

The Pb treatment was applied when the plants were 4 weeks old -Pb 10, 20, 50, 100, and 500 ppm

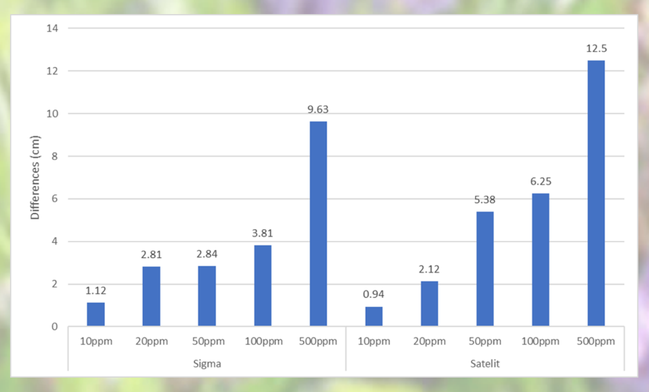
Total RNA extraction - RNAgents Total RNA Isolation System kit from Promega.

RT-PCR - Accessquick RT-PCR System from Promega.

Gene	Primer name	Primers sequences
γ-glutamilcistein-sintetaza	GGS	F: CTTAGTGGAGCCCCTCTGGAA
		R: CTGGAAACCAATCCCCAAAAA
glutation-sintetaza	glu1	F: CAATCTTCTGCTGTCAAATGCCCTTCAA
		R: GCTTTTCTAACAATATCCGAGTCATCCA
Mt EF1 α	EF 1	F: ATTCCAAAGGCGGCTGCATA
		R: CTTTGCTTGGTGCTGTTTAGATGG



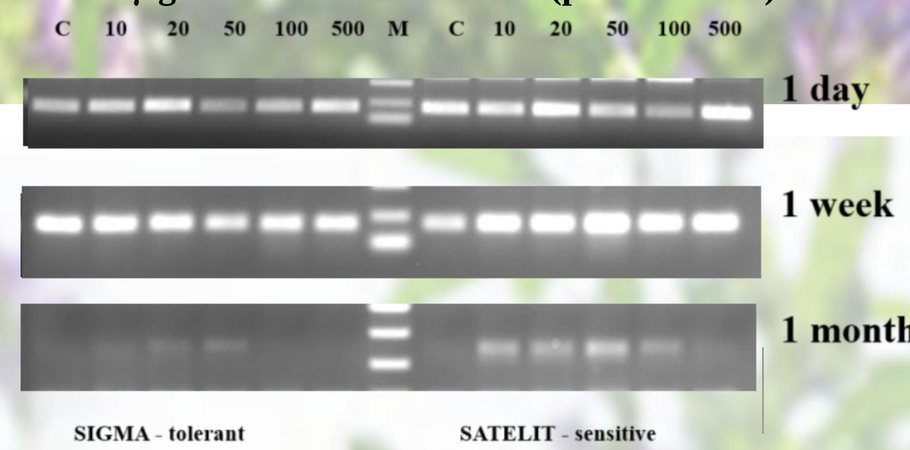
Results and discussions



Growth differences between the variants treated with different concentrations of lead and the control variant.

Gene expression analysis was performed using the RT-PCR (Reverse Transcription PCR) method. Gene expression was comparatively studied for each treatment variant in both the tolerant and the sensitive cultivar. To observe the variation in gene expression over different treatment durations, molecular tests were conducted after one day, one week, and one month of treatment.

Gene γ-glutamilcistein-sintetase (primers ECS)



After 1 day of treatment, a strong gene expression is observed in the sensitive cultivar treated with 500 ppm Pb.
After 1 week, no differences are observed between the tolerant and sensitive cultivars, with pronounced expression of the γ-glutamylcysteine synthetase gene in all plants.
After 1 month of treatment, gene expression is very weak and leveled across all sample

Gene glutation sintetase (primers GS)

After one day of treatment, the sensitive cultivar exposed to 500 ppm Pb exhibited a pronounced gene expression.
After one week of treatment, elevated expression of the glutathione synthetase gene was observed in plants treated with 500 ppm Pb, in both the tolerant and sensitive cultivars.
After one month of treatment, gene expression levels became uniform across all samples.

Conclusions

Gene expression varied depending on the cultivar (tolerant vs. sensitive), the treatment duration, and the Pb concentration in the substrate. Individual variation was also observed among plants of the same cultivar.

The most pronounced effect of Pb treatment was recorded after one day, when a marked upregulation of γ-glutamylcysteine synthetase and glutathione synthetase genes was observed. The sensitive cultivar consistently exhibited higher expression levels.

After prolonged exposure (1 month), gene expression stabilized, with no significant differences observed between cultivars, Pb concentrations, or among individual plants. Research on the expression of genes involved in phytochelatin biosynthesis will continue, with the present study serving as a foundation and starting point for future investigations.