Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1450790

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 7 - Issue 3: 298-304 / May 2024

ASSESSING ALUMINUM STRESS RESILIENCE IN COMMON BEAN ROOTS: PHENOTYPIC, HISTOCHEMICAL, AND *PVGST/PVPOD* GENE EXPRESSION ANALYSIS

Mehmet Zahit YEKEN[1]*

[1]*Bolu Abant İzzet Baysal University, Faculty of Agriculture, Department of Field Crops, 14030, Bolu, Türkiye*

Abstract: Common bean (*Phaseolus vulgaris* L.) is grown in various parts of the world. Aluminum (Al) toxicity poses a significant and widespread challenge in marginal areas with unfavorable soil qualities where common bean is grown. In acidic soils, stable forms of Al dissolve into the soil solution and inhibit root growth and function by injuring the root apex with phytotoxic ions. This leads to the development of a smaller root system, adversely affecting crop yield. In this study, the phenotypic evaluation for relative root elongation of 10 common bean genotypes/cultivars under Al stress (50 µM), the impact of Al toxicity using different histochemical dyes (Evan's blue and Schiff's reagent) and the expression levels of *PvGST* (Glutathione S-transferases) and *PvPOD* (peroxidase) genes in the root tissues of the most resistant/sensitive common beans under Al stress (50 µM) and control conditions (0 µM) were investigated. The maximum relative root elongation value (71.9%) was found in Önceler-98 cultivar, while the lowest value (14.1%) was obtained from Blksr-19 genotype. Histochemical applications used in the study supported phenotypic results. The cracks at the root tip and high blue color intensity were detected in Schiff's reagent and Evan's blue dyes in the Blksr-19, respectively. The expression levels of *PvGST* and *PvPOD* genes in the root tissue of the Blksr-19 (Al-sensitive) were highly upregulated at 24 h of Al stress treatment. The results revealed that these genes might be involved in the common bean root tissue's defense mechanism against Al stress for the first time. The findings herein will help plant breeders develop common bean cultivars tolerant to Al toxicity.

Keywords: Aluminum stress, Breeding, Gene expression

***Corresponding author:** Bolu Abant İzzet Baysal University, Faculty of Agriculture, Department of Field Crops, 14030, Bolu, Türkiye
E mail: yekenmehmetzahit@gmail.com (M. Z. YEKEN)
Mehmet Zahit YEKEN https://orcid.org/0000-0003-0490-371X

Received: March 11, 2024
Accepted: April 19, 2024
Published: May 15, 2024

Cite as: Yeken MZ. 2024. Assessing aluminum stress resilience in common bean roots: phenotypic, histochemical, and *PvGST/PvPOD* gene expression analysis. BSJ Agri, 7(3): 298-304.

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is among the most significant edible legume grains that can be consumed directly by humans. The seeds of common bean include protein, vitamins and minerals (Baloch and Nadeem, 2022; Yeken, 2023). The Food and Agriculture Organization (FAO) reported that on almost 35.9 million hectares, more than 27.7 million tons of common beans were produced in 2021 (FAO, 2023). This plant is produced on marginal land in different parts of the world when the potential yield is limited by adverse soil qualities (Eticha et al., 2010; Yang et al., 2011). Al toxicity is one of the most limiting factors in plant growth and development, particularly in acidic soils with a pH below 5 (Tóth et al., 2021). Toxic levels of soluble Al limit roots' development and function by damaging the root apex (Yang et al., 2011). These processes also impact other plant parts, resulting in a significant reduction in yield (Ambachew et al., 2023). Al toxicity can lead to poor root development, root browning, stunted plant growth, and reduced nutrient and water uptake efficiency by the roots (Bartoli et al., 2017). Al can bind to the cell wall, causing it to become rigid (Delhaize et al., 1993). The integrity of the cell membrane is evaluated using Evan's blue staining. In the staining process, living cells exclude the dye due to the semipermeable nature of cell membranes, but damaged cells are unable to eliminate the dye and so are stained blue (Ikegawa et al., 2000). The intensity of Evans blue's absorbance increases proportionally to the amount of cell membrane damage. On the other hand, Al can alter the lipid peroxidation in the plasma membrane and the calcium ion homeostasis, inhibit nutrients and water uptake, and reduces the photosynthetic rate (Tóth et al., 2021). The use of Schiff's reagent method allows for the identification of lipid peroxidation on the root surface. This technique was successfully applied in pea roots to identify aldehyde functions that originate from the peroxidation of membrane lipids and are attached to the membrane protein under Al stress (Yamamoto et al., 2001). Plants can improve their ability to remove ROS by increasing the activity of antioxidants, such as glutathione S-transferase and peroxidase, when exposed to oxidative stress (Mhamdi et al., 2010; Nanda et al., 2010). Glutathione S-transferases (GSTs) play significant roles in detoxifying xenobiotics and toxic lipid peroxides,

regulating signal transduction, defense against heavy metals and ozone damage, glucosinolate biosynthesis and metabolism in plants (Abdul Kayum et al., 2018). Additionally, they regulate plant growth and development (Jiang et al., 2010). On the other hand, peroxidase (POD) plays a crucial role during stress conditions in plants, removing hydrogen peroxide generated (Tóth et al., 2021). There are many studies on Al stress in common bean (Rangel et al., 2007; Eticha et al., 2010; Rangel et al., 2010; Yang et al., 2011; Butare et al., 2012; Bartoli et al., 2017; dos Santos Neto et al., 2020; Ambachew and Blair, 2021; Tóth et al., 2021; Ambachew et al., 2023). However, to the best of our knowledge, there is no study in the literature on the expression levels of *PvGST* and *PvPOD* genes under Al stress in common bean.

The improvement of new common bean cultivars with resistance to Al stress is one of the main goals of breeding programs (Butare et al., 2012; Ambachew and Blair, 2021). The existence of genetic variation in common bean in response to Al toxicity has led plant breeders to discover genotypes better suited to Al stress and to improve cultivars with better agronomic features (dos Santos Neto et al., 2020). In soils contaminated with Al, tolerant cultivars provide a sustainable and cost-effective solution that can increase yield gains. For this purpose, this study aimed to (1) assess the phenotypic variation for relative root elongation of common bean genotypes/cultivars under Al stress, (2) determine the impact of Al toxicity using different histochemical dyes, and (3) investigate the expression levels of *PvGST* and *PvPOD* genes in the root tissues of the most resistant/sensitive common beans under Al stress and non-stress conditions. Findings herein could help to develop modern cultivars with Al-toxicity resistance in common bean breeding programs.

2. Materials and Methods

2.1. Plant materials

The eight common bean genotypes (Blksr-14, Blksr-19, Brs-4, Brs-23, Brs-24, Dzc-2, Dzc-3, and Ylv-14) collected different provinces of Türkiye (Tübitak Project ID: 115R042) and two commercial cultivars (Bulduk and Önceler-98) were used as plant materials in this study.

2.2. Plant Growth, Al treatment, phenotypic evaluation and histochemical staining

Al exposure to common bean seedlings was performed as described earlier with some modifications (Kariya et al., 2017). The experiment was conducted in three replicates. Briefly, seeds of common bean genotypes/cultivars were surface-sterilized using domestic bleach (5% sodium hypochloride), and sown in the sterile peat soil. The sowing trays were watered to moistened peat. The growing conditions were 25°C and 16-h light [at ~150 µmol photons m^{-2} s^{-1}] and 8-h dark photoperiod. Four days after germination, seedlings were uprooted carefully and thoroughly washed without damaging the roots under running tap water. Al treatment media contained 500 µM $CaCl_2$ supplied with $AlCl_3$ at 50 µM and pH adjusted with 1 N HCl at 4.5 for 24 h. At the same time, control (without Al) media just contained 500 µM $CaCl_2$ and pH adjusted with 1 N HCl at 4.5 for 24 h. Magenta boxes (H 100 mm × W 60 mm × D 60 mm) were covered with black PVC tape to protect the roots from light except for the lid side which was left open. Seedlings were clamped in between slits of sponge and left floating over the water surface. Aeration was provided continuously by an air pump over 24 h during the Al treatment.

Root lengths of seedlings were measured using a ruler before and after the Al exposure. Relative root elongation (RRE) was measured using the following formula (equation 1).

$$RRE = (root\ elongation\ with\ Al / root\ elongation\ without\ Al)\ x\ 100 \quad (1)$$

After phenotypic evaluation, the impact of Al toxicity was detected using Evan's blue for plasma-membrane integrity and aldehyde detection using Schiff's reagent essentially as described earlier by Yamamoto et al. (2001). Briefly, roots were washed with distilled water thoroughly and stained with Schiff's reagent for 20 min. After staining, the roots were rinsed with a sulfite solution. To detect integrity of plasma-membrane, roots were stained with Evan's blue solution (0.025% [w/v] for 10 min. After staining, roots were washed extensively to remove excess of the dye. After Schiff's reagent and Evan's blue staining, root sections of 10 mm were observed under a light microscope Leica DM1000 LED (Leica, Weztlar, Germany).

2.3. Plant Growth and Al Treatment for Gene Expression Analysis

The most Al tolerant (cultivar; Önceler-98) and sensitive (genotype; Blksr-19) common beans were selected for gene expression analysis results of the relative root elongation and histochemical staining. The genotype and cultivar were grown again as mentioned above and treated with Al similarly. After treatment, the root samples were collected at 24 hours for both the treated and control groups. The collected samples were maintained at −80°C for RNA isolation.

2.4. RNA extraction, DNase treatment and cDNA synthesis

According to the manufacturer's instructions, total RNA was extracted from 150 mg of all root samples using NucleoZOL reagent (MACHEREYNAGEL GmbH, Dueren, Germany). Then, Thermo Fischer Scientific RNase-Free DNase was used to conduct DNase digestion on the resulting RNA. Following the manufacturer's protocols, using the Thermo Fisher Scientific RevertAid First Strand cDNA Synthesis Kit, complementary DNA (cDNA) was generated from 2 µg of total RNA. The resulting cDNA samples were diluted, and then for quantification studies stored at -20 °C. Using a DS-11 FX+ series spectrophotometer (Denovix Inc., Wilmington, DE, USA), nucleic acid measurements was performed.

2.5. Primers for Quantitative Real-Time PCR (qRT-PCR)

The primers of *PvGST* and *PvPOD* genes previously described by Oliveria et al. (2015) in *P. vulgaris* were used in the study. The *β-TUB* was previously used as the reference gene for the qRT-PCR analysis in common bean under Al stress (Eticha et al., 2010). Thus, it was involved in this study as the reference gene. The sequences of the primers used in the study are given in Table 1.

Table 1. Sequences of primers utilized for qRT-PCR investigation

Primers	Forward/Reverse	Sequences	References
PvGST (Glutatione S-transferase)	F	AGCTCTTCAAGGACACTGAGCCAA	Oliveira et al. (2015)
	R	AAAGGCTGTGGATGCTGCACTAGA	
PvPOD (Peroxidase)	F	TCCTTTTCAGCACTTTCACT	
	R	AGAAAGCAGTGTTCTTGTGG	
β-TUB (Beta-tubulin)	F	CCGTTGTGGAGCCTTACAAT	Eticha et al. (2010)
	R	GCTTGAGGGTCCTGAAACAA	

2.6. qRT-PCR analysis

Using CFX Connect Real-Time PCR System (Bio-Rad, Hercules, CA, United States), the quantitative Real-Time PCR analysis was employed. The reaction mix includes 1 µL of cDNA, 12.5 µL of RealQ Plus 2x Master Mix Green without ROX (Ampliqon, Odense, Denmark), 10 µL of RNase-free ddH_2O and 0.75 µL each of primers (10 µM). The qRT-PCR was employed as one cycle at 95°C for 15 min, followed by 40 cycles of 95°C for 15 s, 61°C for 45 s, and 72°C for 20 s. Afterward, using the CFX Maestro Software, Ct values were calculated. The accuracy of each amplicon was verified by performing melting curve analyses after the final PCR cycle. The samples were incrementally heated from 65 to 95°C during the analysis. To ensure accuracy, each sample was used as three technical duplicates. As a reference gene, *β-TUB* was used in this study (Eticha et al., 2010).

2.7. Statistical Analyses

According to the relative quantitative method ($2^{-\Delta\Delta Ct}$), the relative expression profiles were determined in root tissue subjected to control and Al treatments (Livak and Schmittgen, 2001). Analysis of variance (ANOVA) was done using the expression data. Using SAS statistical software, the mean values were compared according to Least significant difference (LSD) test at a significance level ($P<0.05$). Graphics were constructed in GraphPad Prism version 6.04.

3. Results and Discussion

Many biotic and abiotic stress factors have adversely affected common bean production (Şen et al., 2020; Palacıoğlu et al., 2021; Tóth et al., 2021; Çelik et al., 2023). When the common bean is exposed to stress, environmental and genetic factors influence the function and structure of the roots (Rangel et al., 2010). They enable them to sense and respond to environmental challenges. Therefore, it is important to protect roots from stresses such as Al toxicity, which can inhibit root function and growth. Understanding the genetic variation of local genotypes for Al stress is one of the most crucial tasks of a breeding program (Ambachew and Blair, 2021). In this study, root lengths of seedlings were measured using ruler before and after the Al exposure, and the relative root elongation of common bean genotypes/cultivars were determined (Figure 1).

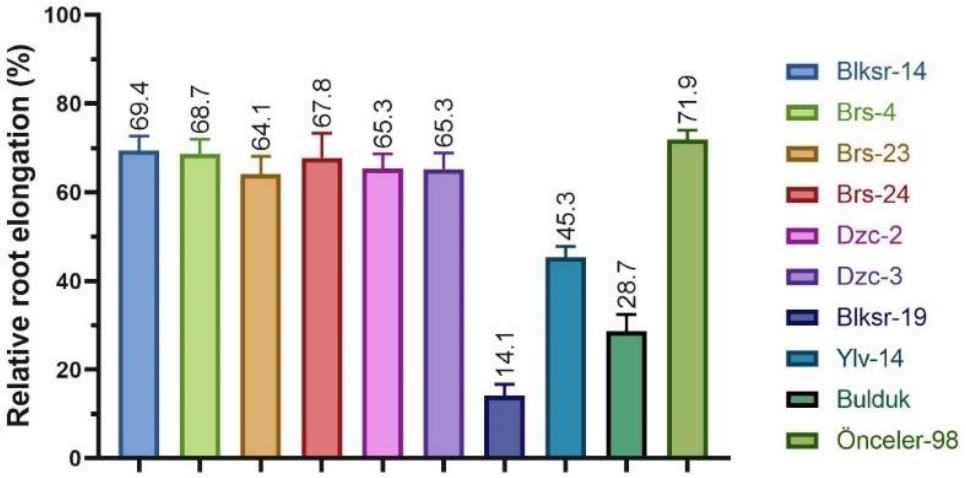

Figure 1. The relative root elongation values of common bean genotypes/cultivars under Al stress.

Blksr-14, Dzc-2, Dzc-3, Brs-4, Brs-23, Brs-24 and Önceler-98 revealed high relative root elongation values. On the other hand, Blksr-19, Ylv-14 and Bulduk had low relative root elongation values of 14.1%, 45.3% and %28.7, respectively. The highest relative root elongation was obtained in the Önceler-98 cultivar (71.9%), while the lowest relative root elongation was determined in Blksr-19. It was reported that the root is the most sensitive plant organ, and the first symptom of Al toxicity is decreased root growth (Delhaize et al., 1993; Tóth et al., 2021). Rangel et al. (2007) examined the short- and medium-term effects of Al treatment (20 µM) on root growth and Al accumulation in Al-resistant and Al-sensitive common bean genotypes. They reported that root elongation in both genotypes was significantly inhibited within the initial 3-4 hours of Al treatment. Ambachew and Blair (2021) assessed 227 common bean genotypes to explore their tolerance to Al toxicity (50 µM) and to determine candidate genes linked to Al tolerance. It was observed that the number of root forks, number of links, root surface area, number of root tips, total root length and root volume decreased under Al toxicity treatment. Very recently, Ambachew et al. (2023) investigated the relationship between root characteristics and genetic variation among 262 common bean genotypes under different Al treatments. They found that the 50 µM Al treatment was sufficient to elucidate genotypic differences in the studied root traits. Under Al-toxicity treatment, a decrease in all root traits compared to the control group was observed.

The histochemical procedure has an advantage over the biochemical procedure. This procedure shows the localization of the Al-enhanced peroxidation of lipids in situ on the root surface with high sensitivity. The roots of common bean genotypes and cultivars were dyed with Schiff's reagent and Evan's Blue to determine the impact of Al toxicity. Positive results were detected in Al-treated roots when compared to controls (Figure 2). As a result of staining the roots with Schiff's reagent dye, the cracks at the root tip of the Blksr-19 genotype, which has the lowest relative root elongation, especially under Al stress, were seen in Figure 2.

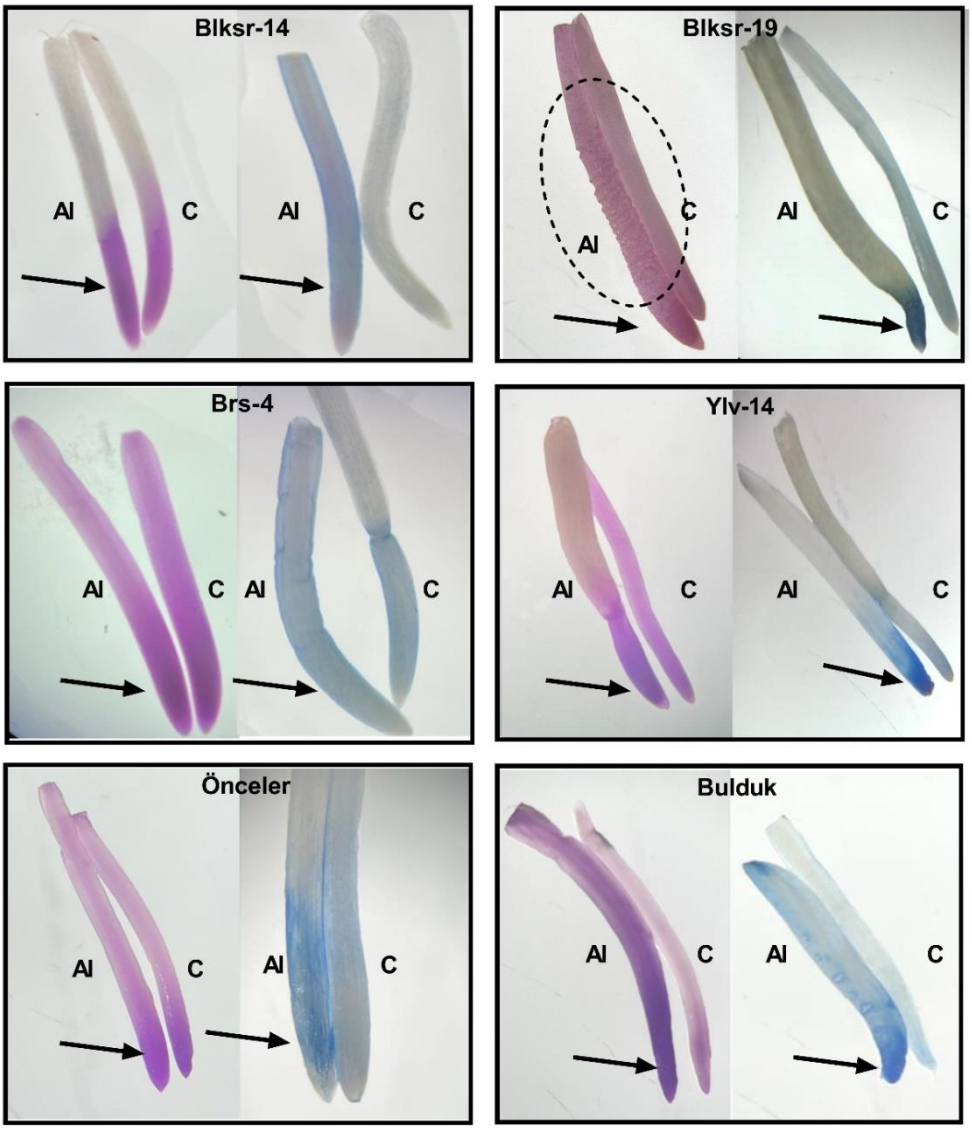

Figure 2. Histochemical detection of the Al effect in common bean roots. The roots were stained with Schiff's reagent (pink color) and Evan's blue (Blue color). Al= Al treated roots, C= control roots.

Yamamoto et al. (2001) investigated the response of pea roots to Al stress using histochemical and biochemical techniques. They reported that the cracks in the pea roots under Al stress are caused by differential cell expansion created by root elongation inhibition. As a result of dyeing the roots with low relative root elongation under Al stress with Evan's blue, it was seen that the color intensity is quite high at the tips of the roots (Blksr-19, Ylv-14 and Bulduk) when compared to controls. On the other hand, there was low color intensity at the tips of the roots in Önceler-98, Brs-4 and Blksr-14 having high root elongation. Al sensitivity is concentrated at the tip of the root, particularly in the transition zone located 1-2 mm behind the root (Rangel et al., 2007; Butare et al., 2012). This leads to changes in the cell wall and plasma membranes, which affect the mechanical characteristics of the cell wall. These changes play a significant role in inhibiting root elongation caused by Al (Yang et al., 2011). Our phenotypic evaluation results were supported by the histochemical analyses.

The expression levels of the *PvGST* and *PvPOD* genes were determined in root tissues of Önceler-98 and Blksr-19 at different time points (0 and 24 h). As seen in Figure 3, the Al stress findings showed that both genes were upregulated in the Blksr-19 (Al-sensitive) root tissue. This genotype had higher expression levels than the tolerant cultivar (Önceler-98) for both genes. The expression levels of the *PvGST* gene in Önceler-98 showed no statistical differences compared to Al stress treatment at 24 h. On the other hand, Blksr-19 revealed a 2.49-fold increase at 24 h compared to the control. When comparing Al-treated plants to the control, significant differences in gene expression for the *PvPOD* gene were detected. The transcript level of the *PvPOD* gene was significantly decreased at 24 h in Önceler-98, while this level was significantly increased 3.76-fold at 24 h in Blksr-19. Dmitriev et al. (2016) reported that exposure to Al leads to the production of ROS and lipid peroxidation in plants. Plants facing oxidative stress, improve their ability to scavenge ROS by improving the enzymatic activity of antioxidants such as glutathione S-transferase, as well as peroxidase, superoxide dismutase and catalase (Mhamdi et al., 2010; Nanda et al., 2010). It has been observed that there is an enhancement in GST expression under Al stress in both sensitive and resistant maize lines (Cançado et al., 2005), *Arabidopsis* (Ezaki et al., 2004), and pea roots (Panda and Matsumoto, 2010). This indicates the participation of GST in the response of plants to Al stress. On the other hand, differential transcript level of *PvPOD* gene under Al treatment was detected in common bean genotype/cultivar. In previous study, Eticha et al. (2010) reported that peroxidases may assist in the plant's resistance to Al by detoxifying ROS, which are produced as a result of oxidative stress induced by Al. Tóth et al. (2021) observed differential POD activity under Al treatment in common bean. They found that POD activity was significantly higher in 12 cultivars and lower in others. The *PvPOD* gene was expressed differentially during the *Sclerotinia sclerotiorum* infection and BCMV infection in common bean (Oliveira et al., 2015; Yeken et al., 2024). Al tolerance in common bean is a complex process that involves the coordination of different genes (Ambachew and Blair, 2021). The effect of Al on plant growth and development varies based on factors such as concentration, species, genotype, cultivar, and duration of exposure (Kopittke et al., 2016). We suppose that *PvGST* and *PvPOD* genes might be involved in the defense mechanism against Al stress in the root tissue of common bean. However, further research on this subject, including different Al doses and different time intervals, and a better understanding of the molecular mechanisms are required.

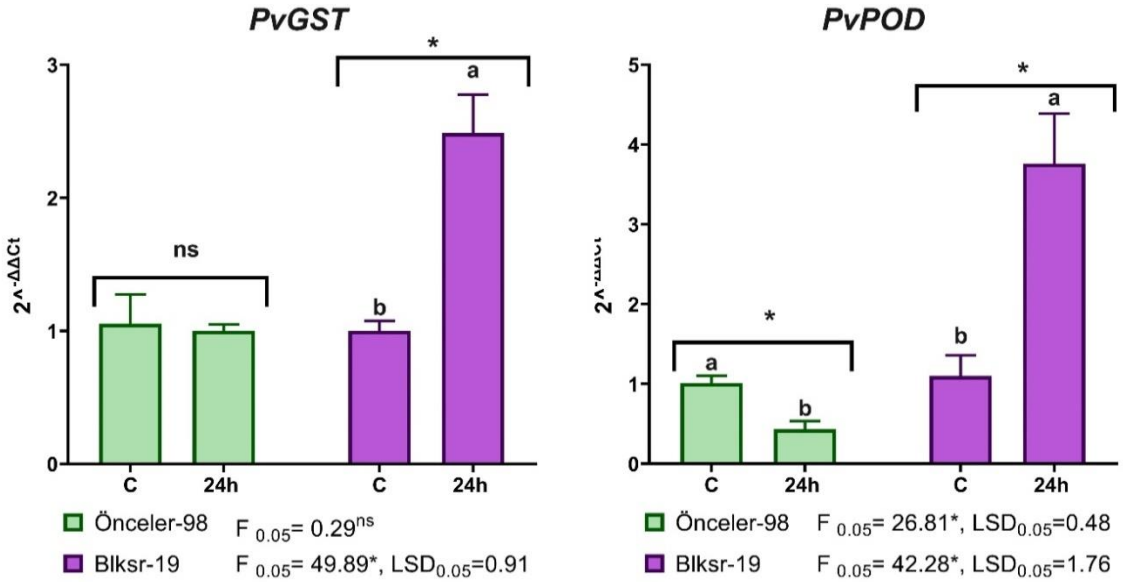

Figure 3. The differences in expression levels of *PvGST* and *PvPOD* genes in roots subjected to Al stress. * significant (P<0.05), ns: non-significant, C= control.

4. Conclusion

This study evaluated the variation of relative root elongation under Al stress and the impact of Al toxicity using different histochemical dyes in common bean genotypes/cultivars. Moreover, the expression levels of *PvGST* and *PvPOD* genes against Al stress in the root tissue of the common bean were investigated for the first time. The obtained results in this study will enhance the knowledge of the literature on the *PvGST* and *PvPOD* genes in response to the Al stress in common bean. The use of the genetic resources discovered herein will hopefully allow the production of common bean cultivars resistant to Al toxicity. These sources might be used in Al tolerance breeding projects in the near future.

Author Contributions

The percentage of the author(s) contributions is presented below. The author reviewed and approved the final version of the manuscript.

	M.Z.Y.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans. The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to.

References

Abdul Kayum M, Nath UK, Park JI, Biswas MK, Choi EK, Song JY, Kim HT, Nou IS. 2018. Genome-wide identification, characterization, and expression profiling of glutathione S-transferase (GST) family in pumpkin reveals likely role in cold-stress tolerance. Genes, 9(2): 84.

Ambachew D, Asfaw A, Blair MW. 2023. Phenotypic variability for root traits in andean common beans grown with and without aluminum stress conditions. Agronomy, 13: 619.

Ambachew D, Blair MW. 2021. Genome wide association mapping of root traits in the andean gene pool of common bean (*Phaseolus vulgaris* L.) grown with and without aluminum toxicity. Front Plant Sci, 12: 628687.

Baloch FS, Nadeem MA. 2022. Unlocking the genomic regions associated with seed protein contents in Turkish common bean germplasm through genome-wide association study. Turk J Agric For, 46(1): 113-128.

Bartoli G, Sanità di Toppi L, Andreucci A, Ruffini Castiglione, M. 2017. Aluminum effects on embryo suspensor polytene chromosomes of *Phaseolus coccineus* L. Plant Biosys, 152(4): 880-888

Butare L, Rao IM, Lepoivre P, Cajiao C, Polania J, Cuasquer J, Beebe S. 2012. Phenotypic evaluation of interspecific recombinant inbred lines (RILs) of Phaseolus species for aluminium resistance and shoot and root growth response to aluminium-toxic acid soil. Euphytica, 186: 715-730.

Cançado GMA, DeRosa VE, Fernandez JH, Maron LG, Jorge RA, Menossi M. 2005. Glutathione S-transferase and aluminum toxicity in maize. Funct Plant Biol, 32: 1045-1055.

Çelik A, Emiralioğlu O, Yeken MZ, Çiftçi V, Özer G, Kim Y, Baloch FS, Chung YS. 2023. A novel study on bean common mosaic virus accumulation shows disease resistance at the initial stage of infection in *Phaseolus vulgaris*. Front Genet, 14: 1136794.

Delhaize E, Ryan PR, Randall P. 1993. Aluminium tolerance in wheat (*Triticum aestivum* L.) II. Aluminium-stimulated excretion of malic acid from root apices. Plant Physiol, 103: 695-702.

Dmitriev AA, Krasnov GS, Rozhmina TA, Kishlyan NV, Zyablitsin AV, Sadritdinova AF, Snezhkina AV, Fedorova MS, Yurkevich OY, Muravenko OV, Bolsheva NL, Kudryavtseva AV, Melnikova NV. 2016. Glutathione S-transferases and UDP-glycosyltransferases are involved in response to aluminum stress in flax. Front Plant Sci, 7: 1920.

dos Santos Neto J, Delfini J, Willian ST, Akihide HA, Marcos NJ, Simões Azeredo Gonçalves L, Moda-Cirino V. 2020 Response of common bean cultivars and lines to aluminum toxicity. Agronomy, 10: 296

Eticha D, Zahn M, Bremer M, Yang Z, Rangel AF, Rao IM, Horst WJ. 2010. Transcriptomic analysis reveals differential gene expression in response to aluminium in common bean (*Phaseolus vulgaris*) genotypes. Ann Bot, 105(7): 1119-1128.

Ezaki B, Suzuki M, Motoda H, Kawamura M, Nakashima S, Matsumoto H. 2004. Mechanism of gene expression of Arabidopsis glutathione S-transferase, *AtGST*1, and *AtGST*11 in response to aluminum stress. Plant Physiol, 134: 1672-1682.

FAO. 2023. Food and Agriculture Statistics. Retrieved May 20, 2023 from: https://www.fao.org/faostat/en/#home (accessed date: June 15, 2023).

Ikegawa H, Yamamoto Y, Matsumoto H. 2000. Responses to aluminium of suspension-cultured tobacco cells in a simple calcium solution. Soil Science and Plant Nutrition, 46(2): 503-514.

Jiang HW, Liu MJ, Chen C, Huang CH, Chao LY, Hsieh HL. 2010. A glutathione S-transferase regulated by light and hormones participates in the modulation of *Arabidopsis* seedling development. Plant Physiol, 154: 1646-1658.

Kariya K, Sameeullah M, Sasaki T, Yamamoto Y. 2017. Overexpression of the sucrose transporter gene NtSUT1 alleviates aluminum-induced inhibition of root elongation in tobacco (*Nicotiana tabacum* L.). Soil Sci Plant Nutr, 63(1): 45-54.

Kopittke PM, Menzies NW, Wang P, Blamey FPC. 2016. Kinetic

and nature of aluminium rhizotoxic effects: A review. J Exp Bot, 67: 4451-4467.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. Methods, 25: 402-408.

Mhamdi A, Hager J, Chaouch S, Queval G, Han Y, Taconnat L, Saindrenan P, Gouia H, Issakidis-Bourguet E, Renou JP, Noctor G. 2010. Arabidopsis glutathione reductase1 plays a crucial role in leaf responses to intracellular hydrogen peroxide and in ensuring appropriate gene expression through both salicylic acid and jasmonic acid signaling pathways. Plant Physiol, 153: 1144-1160.

Nanda AK, Andrio E, Marino D, Pauly N, Dunand C. 2010. Reactive oxygen species during plant-microorganism early interactions. J Integr Plant Biol, 52: 195-204.

Oliveira MB, de Andrade RV, Grossi-de-Sá MF, Petrofeza S. 2015. Analysis of genes that are differentially expressed during the *Sclerotinia sclerotiorum–Phaseolus vulgaris* interaction. Front Microbiol, 6: 1162.

Palacıoğlu G, Özer G, Yeken MZ, Ciftci, V, Bayraktar H. 2021. Resistance sources and reactions of common bean (*Phaseolus vulgaris* L.) cultivars in Turkey to anthracnose disease. Genet Resour Crop Evol, 68(8): 3373-3381.

Panda SK, Matsumoto H. 2010. Changes in antioxidant gene expression and induction of oxidative stress in pea (*Pisum sativum* L.) under Al stress. Biometals, 23: 753-762.

Rangel AF, Rao IM, Braun HP, Horst WJ. 2010. Aluminum resistance in common bean (*Phaseolus vulgaris*) involves induction and maintenance of citrate exudation from root apices. Physiol Plant, 138: 176-190.

Rangel AF, Rao IM, Horst WJ. 2007. Spatial aluminium sensitivity of root apices of two common bean (*Phaseolus vulgaris* L.) genotypes with contrasting aluminium resistance. J Exp Bot, 58: 3895-3904.

Şen K, Koca AS, Kaçar G. 2020. Fasulye tohum böceği *Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae)'un önemi, biyolojisi, zararı ve mücadelesi. JIST, 10(3): 1518-1527.

Tóth B, Moloi MJ, Szőke L, Danter M, Grusak MA. 2021. Cultivar differences in the biochemical and physiological responses of common beans to aluminum stress. Plants, 10(10): 2097.

Yamamoto Y, Kobayashi Y, Matsumoto H. 2001. Lipid peroxidation is an early symptom triggered by aluminum, but not the primary cause of elongation inhibition in pea roots. Plant Physiol, 125(1): 199-208.

Yang ZB, Eticha D, Rotter B, Rao IM, Horst WJ. 2011. Physiological and molecular analysis of polyethylene glycol-induced reduction of aluminium accumulation in the root tips of common bean (*Phaseolus vulgaris*). New Phytol, 192(1): 99-113.

Yeken MZ. 2023. Investigation of genotype × environment interactions for the seed mineral composition in *Phaseolus vulgaris* L. J Food Compost Anal, 124: 105657.

Yeken MZ, Çelik A, Emiralioğlu O, Çiftçi V, Baloch FS, Özer G. 2024. Exploring differentially expressed genes in *Phaseolus vulgaris* L. during BCMV infection. Physiol Mol Plant Pathol, 130: 102238.