Turkish Journal of Agriculture - Food Science and Technology, 12(4): 527-538, 2024
DOI: https://doi.org/10.24925/turjaf.v12i4.527-538.6663

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Silicon Improves Cold and Freezing Tolerance in Pea

Ufuk Akçay[1,a,*], Hande Nur Kumbul[1,b], İbrahim Ertan Erkan[1,c]

[1]*Department of Agricultural Biotechnology, Faculty of Agriculture, Isparta University of Applied Sciences, Isparta, Türkiye*
[*]*Corresponding author*

ARTICLE INFO

Research Article

Received : 10.01.2024
Accepted : 27.02.2024

Keywords:
Pisum sativum L.
Silicon
Cold stress
Freezing stress
TOP2

ABSTRACT

The most significant crop losses worldwide occur due to unfavorable temperatures such as heat, drought, cold, and freezing. Minerals like silicon can play important roles in the growth, development, and stress responses of plants. In this study, changes in stem/root length, dry weight, relative water content and silicon content, of peas under cold and freezing stress, as well as antioxidant system indicators such as proline, malondialdehyde, hydrogen peroxide, and chlorophyll levels, ion leakage, and the expressions of genes coding for the topoisomerase *TOP2* and DNA helicase *PDH47* enzymes, which play important roles in the replication, transcription, and repair of DNA molecules, were examined in root and stem tissues in the presence of two different concentrations of silicon. The results of the study showed that silicon application under cold and freezing stresses has induced various changes in pea metabolism, including increases in cell membrane integrity parameters and superoxide dismutase enzyme activity, as well as increase in the expressions of *TOP2* and *PDH47* genes. These changes have been found to have positive effects on the pea cold and freezing tolerance.

[a] *ufukakcay@isparta.edu.tr* *https://orcid.org/0000-0003-1260-3813*
[b] *handenurkuru@gmail.com* *https://orcid.org/0000-0002-8851-1686*
[c] *ibrahimertanerkan@gmail.com* *https://orcid.org/0000-0002-2815-412X*

Introduction

The abiotic stresses such as high and low temperatures and drought are major factors worldwide causing significant crop losses in agricultural plants (Jahed et al., 2023; Jan et al., 2023). Pea (*Pisum sativum* L.) cultivation involves seed planting in late winter or early spring, exposing plants to cold and freezing stresses. When these stresses coincide with drought during the seed formation period, it can lead to the loss of nearly the entire crop (Larran et al., 2023). Peas, a significant source of protein and carbohydrates, enrich the soil through the *Rhizobium* symbiosis specific to legumes, and due to their ability to fix nitrogen. They are annual leguminous plants that provide the highest yield per unit area under suitable weather conditions and play essential roles in sustainable agriculture worldwide (Stagnari et al., 2017). The most limiting factors for cultivation on larger expanses globally are the cold and drought stresses frequently encountered by the plant (Ding & Yang, 2022).

Although cold and freezing stresses fundamentally operate through similar mechanisms, they involve distinct components. Cold stress leads to various adverse effects, including a decrease in enzyme activities, stiffening of cell membranes, destabilization of protein complexes, stabilization of RNA secondary structures, accumulation of reactive oxygen species, retardation of photosynthesis, and leakage in the cell membrane. In advanced stages, cold stress can result in cell death (Manasa et al., 2022). The primary cause of freezing stress is the formation of ice within tissues. For instance, structures with very low water content, such as seeds, can maintain their vitality at low temperatures without showing signs of damage. Freezing stress primarily manifests its harmful effects through damage caused by advanced dehydration in cell and organelle membranes. The major genetic responses triggered during freezing stress involve an increase in the expression of *CBF* genes, which initiate transcriptional regulation, and mRNA degradation regulated by small RNAs through precursor mRNA cleavage, a post-transcriptional regulatory mechanism (Chinnusamy et al., 2007; Sunkar et al., 2007).

In the conducted studies, the biochemical and genetic responses of peas to cold and freezing stress have been particularly focused on the plant's photosynthetic efficiency and carbon or phosphate compound metabolism (Georgieva & Lichtenthaler, 1999; Streb et al., 2003; Stupnikova, 2006; Srivastava et al., 2006; Leterrier et al.,

2007). Recent research has examined and characterized some genes with increased expression in the pea genome under cold stress conditions (Lucau-Danila et al., 2012). Among these genes, *TOP2*, a topoisomerase involved in changing the topology of DNA in all processes associated with DNA metabolism such as replication, transcription, recombination, and chromosome segregation, and *PDH47*, a helicase enzyme with functions in DNA replication, repair, recombination, transcription, ribosome biogenesis, and translation initiation, have been identified (Hettiarachchi et al., 2005; Vashisht & Tuteja, 2006).

Among DNA topoisomerases, plant topoisomerases constitute the least studied enzyme group when compared to bacteria, yeast, and animal systems (Singh et al., 2004). Among plants *TOP2* gene sequences are known only in tobacco, pea and *Arabidopsis thaliana* (thale cress), which is a model plant in Brassicaceae family, for studying plant biology and genetics (Hacker et al., 2022). Recent studies have revealed that, in addition to its classical functions, the enzyme also plays a role in the relationship between DNA replication/cell cycle and abiotic stresses. It has been demonstrated that gene expression is regulated through light signal pathways mediated by phytochrome (Hettiarachchi et al., 2003). In another study, the expression of the *TOP2* gene in peas was increased under cold and salt stresses, as well as with applications of the phytohormones salicylic acid and ABA (Hettiarachchi et al., 2005).

Helicases, like topoisomerases, also play crucial roles in DNA metabolism. Most helicases belong to the DEAD-box protein family and are involved in fundamental cellular processes such as DNA replication, repair, recombination, transcription, ribosome biogenesis, and translation initiation (Vashisht & Tuteja, 2006). Compared to organisms like humans, flies, worms, and yeast, with sequenced genomes, the Arabidopsis plant boasts the highest number of DEAD-box RNA helicase genes, exceeding 50 (Boudet et al., 2001). In plants, the presence of DEAD-box helicase genes whose expression is regulated by cold and their involvement in stress signal transduction were first reported in *Arabidopsis* (Seki et al., 2001).

In peas, a DEAD-box DNA helicase gene named *PDH47* has been characterized, showing 93% similarity to the translation initiation factor eIF4A protein in tobacco. The exact function of helicases under stress conditions is not fully known, but it is believed that they regulate some stress-induced metabolic pathways by activating transcription factors, interact with topoisomerases, play regulatory roles in transcription and translation after being phosphorylated by protein kinases, and may have functions in the repair of damaged DNA/RNA molecules (Vashisht & Tuteja, 2006). The expression of the pea DEAD-box DNA helicase 47 gene has been found to significantly increase in stems and roots under salt and cold stress, but the gene did not respond to heat stress (Vashisht et al., 2005).

In recent years, silicon has been identified as one of the substances that have positive effects on the development of tolerance in plants under stress conditions (Tayade et al., 2022). Silicon ranks second among the most abundant elements in the Earth's crust (Raza et al., 2023). Plants obtain silicon from the soil through their roots in the form of monomeric molecule silicic acid [$Si(OH)_4$] when the pH is below 9 (Mandlik et al., 2020). Transportation of B from soil to plant roots takes place via passive diffusion as well as boron transporters NIP5;1 and BOR1, in addition to aquaporin proteins, which take role in transport of B across the plasma membranes (Takano et al. 2005; Fitzpatrick and Reid 2009). Silicon is not listed among the essential elements for plant development, and it is not present in nutrient media used in plant tissue cultures. This is because plants do not require this element for growth under optimum conditions (Epstein, 2009). However, recent studies have revealed the vital roles of silicon in the presence of biotic and abiotic stresses (Thakur et al., 2023; Shanmugaiah et al., 2023).

For example, the accumulation of silicon in roots reduces apoplastic bypass flow and provides binding sites for metals (Mostafa et al., 2021). Silicon reduces the uptake of toxic metals and salts into cells and their transmission from roots to stems, by competing selectively with transporter proteins (Mir et al., 2022). Silicon accumulation in leaves and stems helps strengthen cell walls and has a hardening effect, which protects the plants against strong winds and heavy rain (Collin et al., 2014; Zargar et al., 2019). A recent study used RNA sequencing analysis to reveal that silicon regulates the biosynthesis of alkaloids and flavonoids, helps maintenance of cellular redox homeostasis and osmotic adjustments, and promotes the deposition of complex carbohydrates in the cell wall (Biju et al., 2023). Additionally, silicon accumulation in leaves was shown to decrease transpiration in the cuticle, thereby enhancing resistance to low and high temperatures, drought stress, radiation, and UV stress in plants (Islam et al., 2020). The beneficial effects of silicon are found more pronounced in stem tissues (Ma & Yamaji, 2006).

It has been suggested that silicon stimulates the formation of defense components under stress and has affinities with some organic compounds (Bakhat et al., 2018). The resistance-enhancing effects of silicon under various stresses have also been demonstrated in various plants in recent studies. For example, in the powdery mildew-infected *Arabidopsis* plants, silicon induced the differential expression of various genes, while its presence did not affect gene expression in non-infected plants (Fauteux et al., 2006). In pea plants, silicon increased the production of chitinase and glucanase enzymes in tissues infected with leaf spot pathogenic fungi and accumulated significantly in the leaves (Dann & Muir, 2002).

Silicon has also shown its positive effects in cucumber, tomato, and canola plants under salt stress, reducing tissue reactive oxygen levels and increasing antioxidant system activity (Zhu et al., 2004; Al-Aghabary et al., 2005; Hasanuzzaman et al., 2018). Under cold stress, wheat, maize, and barley responded to silicon applications mainly by activating enzymatic and non-enzymatic antioxidant system components, increasing tissue water content, and significantly enhancing cold resistance (Liang et al., 2008; Moradtalab et al., 2018; Joudmand & Hajiboland, 2019). In a study where the Lsi1 gene responsible for transporting silicon to roots was overexpressed, rice plants showed cold resistance by maintaining osmotic balance, increasing calcium storage, and proline production (Xie et al., 2022).

Studies on silicon applications in pea plants mostly focused on the development and responses of peas under salinity and heavy metal stress. In these studies, silicon application has been found to enhance the main enzymatic and non-enzymatic antioxidant defense systems in peas, providing stress tolerance (Batool et al., 2022; El-Okkiah et al., 2022; Ismail et al., 2022; Oliveira et al., 2020; Rahman et al., 2017; Cruzado-Tafur et al., 2023; Salman et al., 2023).

To our knowledge, there is no information on the damage to the cell membrane in peas under cold stress and silicon application, as well as chlorophyll content, hydrogen peroxide (H_2O_2), and proline levels, and the enzymatic defense initiated by the superoxide dismutase (SOD) enzyme activity, which converts superoxide radicals to H_2O_2. In the scope of this study, various physiological and genetic responses of peas under cold and freezing stress were examined comparatively. Parameters such as stem/root length, dry/fresh weight, relative water content, proline, malondialdehyde, hydrogen peroxide contents, chlorophyll levels, ion leakage, and activities of the SOD enzyme, indicators of the antioxidant system, were investigated. Furthermore, gene expressions of a topoisomerase *TOP2*, which plays important roles in the replication, transcription, and repair of DNA, and *PDH47*, a DEAD-box DNA helicase gene, were examined in leaf tissues under cold and freezing stress in the presence of silicon. Thereby, the physiological and genetic responses of peas under cold and freezing stresses were analyzed comparatively, potential tolerance mechanisms were identified, and the role of silicon in regulating gene expressions and interacting with proline and SOD metabolic pathways were evaluated for potential agricultural applications.

Materials and Methods

In this study, the pea variety Emerald, developed and registered by Istanbul Seed Company was used.

Cold Stress Applications

Pea seeds were sterilized with a 2% sodium hypochlorite solution to prevent seed derived contamination in hydroponics solution. The lowest sodium hypochlorite concentration that ensured sterility without reducing germination rate was determined as 2%, as a result of our preliminary experiments on pea. The seeds were germinated in 200 ml polypropylene containers containing sterile perlite, with three seeds each, and irrigated with ½ Hoagland solution (Hoagland & Arnon, 1950) every three days. The seedling development continued for 15 days in a plant growth chamber (Nuve GC 400, Turkey) with a 16-hour light/8-hour dark cycle, at 23°C, and 50% humidity, to imitate long days of late spring and early summer. Cold stress was applied by lowering the temperature to 4°C after 15 days, and samples for analysis were collected on the 1st and 4th days of cold stress (24 hours and 96 hours after the initiation of cold stress). Freezing stress was induced by lowering the temperature to -0.5°C, and samples were collected during the same periods as cold stress. Plants treated with silicon dioxide (at concentrations of 1 and 2 mM) were germinated by irrigating with ½ Hoagland solution containing silicon dioxide of the specified concentrations as soon as they were placed on perlite. The concentration of silicon dioxide was determined based on previous hydroponic studies in plant-silicon literature (Parveen & Ashraf 2010; Zhang et al., 2011). Cold and freezing stresses, as well as sample collection periods, were applied as described above. Control plants kept under normal conditions spent an additional 1 and 4 days in the same environment after the 15-day germination period, ensuring that they experienced the same duration as the stressed plants in the culture environments.

All treatments were performed in triplicate, with each replicate consisting of four polypropylene containers containing three seeds each. This resulted in 12 pea seedlings prepared per treatment, and with replicates, there were a total of 36 seedlings for each treatment.

Determination of Stem/Root Length and Relative Water Content

After completing 15 days of development, plants subjected to stress with or without the presence of silicon dioxide, along with control plants, were uprooted from perlite, washed under tap water, and root and stem lengths were determined. Root and stem tissues were separated, weighed, and after drying at 60°C for 48 hours, weighed again to determine dry weights. Relative water content (RWC) was calculated according to the formula RWC(%) = (Fresh weight - Dry weight) / (Turgid weight - Dry weight) X 100, from Smart and Bingham (1974). Turgid weight was calculated by soaking tissues in distilled water at room temperature for 24 hours.

Determination of Proline Content

The determination of proline content started with the homogenization of 0.3 g leaf samples in liquid nitrogen, followed by dissolution in 1 ml of 3% sulfosalicylic acid (Bates et al., 1973). Then, 0.1 ml of the sample, after centrifugation, was mixed with 0.2 ml acid ninhydrin, 0.2 ml of 96% acetic acid, and 0.1 ml of 3% sulfosalicylic acid. The mixtures were held at 96°C for 1 hour, and after centrifugation and mixing with 1 ml of toluene, the absorbance of the upper phase was read at 520 nm.

Determination of Hydrogen Peroxide Content

The amounts to be determined according to Bergmeyer (2012) started with the liquid nitrogen homogenization of 0.5 g leaf tissue and dissolution in 1.5 ml of 100 mM potassium phosphate buffer (pH 6.8). In the samples where 0.25 ml of supernatant was collected after centrifugation, the enzymatic reaction was initiated by mixing with 1.25 ml peroxidase solution (83 mM potassium phosphate buffer, pH 7.0, 0.005% (w/v) o-dianisidine, 40 µg peroxidase/ml) at 30°C. After 10 minutes, the reaction was stopped by adding 0.25 ml of 1 N perchloric acid, and the absorbance of the supernatant was measured at 436 nm after centrifugation.

Determination of Malondialdehyde (MDA) Content

The determination of MDA content for assessing cell membrane damage followed the Ohkawa et al. (1979) method. The homogenization of 0.2 g leaf tissue in liquid nitrogen was followed by adding 1 ml of 5% trichloroacetic acid (TCA). After centrifugation, the same volume of 20% TCA containing 0.5% thiobarbituric acid (TBA) was added,

and the mixture was kept at 96°C for 25 minutes. The samples were cooled on ice, and the absorbance was read at 532 nm. Non-specific absorbance at 600 nm was determined and subtracted from the initial absorbance value.

Ion Leakage

Ion leakage, determined according to the method of Nanjo et al. (1999), involved shaking six leaves in 15 ml tubes containing 5 ml of 0.4 M mannitol. Electrical conductivity was recorded as C1 using a Mettler Toledo MPC 227 conductivity meter. After boiling for 15 minutes and cooling the samples to room temperature, C2 readings were taken, and the leakage-related conductivity was calculated using the formula [(C1/C2) X 100].

Determination of Chlorophyll Content

Chlorophyll amounts were determined using the method of Lichtenthaler and Wellburn (1983). This involved the liquid nitrogen homogenization of 3 g leaf tissue, centrifugation, and determination of the supernatant at different absorbance values. Chlorophyll a (mg/L) and chlorophyll b (mg/L) were calculated using the following formulas:

- Chlorophyll a (mg/L) = 15.65 Abs666 – 7.340 Abs653
- Chlorophyll b (mg/L) = 27.05 Abs653 – 11.21 Abs666

Determination of Superoxide Dismutase (SOD) Enzyme Activities

Enzyme activities were determined according to the method of Beauchamp and Fridovich (1971). The homogenization of 0.2 g leaf samples in a glass-glass homogenizer mixed with homogenization buffer on ice was followed by centrifugation, and the supernatant was stored at -80°C until use. The protein content of the extracts was determined by the Bradford (1976) method. A native polyacrylamide gel consisting of a separation part and stacking part with a 30% (29:1) acrylamide-bis solution was prepared for use in a Bio-Rad midi gel apparatus. After loading the samples, electrophoresis was conducted at 8 V/cm, monitoring the tracking dye. Subsequently, the gel apparatus was disassembled, and for the determination of different isozymes of the SOD enzyme, first KCN and hydrogen peroxide were applied, and then negative activity staining with NBT was performed to visualize the enzyme isozyme bands.

Determination of Gene Expressions for TOP2 and PDH47 Genes

The mRNA sequences of the *TOP2* and *PDH47* genes with GenBank accession numbers Y14559.1 and AY167670.1 were obtained from the National Center For Biotechnology Information (NCBI) database. The expression at the transcription level of these genes was investigated using the semi-quantitative reverse-transcription PCR (RT-PCR) technique. The pea actin gene with the GenBank accession number X68649.1 was used as an internal control.

RNA Isolation and Reverse Transcription PCR (RT-PCR)

RNA isolation from pea leaves was performed using Qiagen RNeasy plant mini kits based on guanidine-isothiocyanate lysis and silica-membrane purification methods. The quantity of the obtained total RNAs was determined spectrophotometrically, and their quality was assessed by separating and visualizing them with 2% agarose gel electrophoresis. cDNA libraries were created using the Thermo First Strand cDNA Synthesis Kit (Thermo, USA) from the obtained RNA molecules. From this library, the *TOP2* and *PDH47* genes with NCBI accession numbers Y14559.1 and AY167670.1 were amplified by PCR using primers designed with the PrimerPremier 5.0 program from CA, USA, providing the most suitable conditions for amplification. The obtained bands were separated on a 0.8% agarose gel and visualized using the Biolab UV Tech gel imaging system. The bands were analyzed numerically using the ImageJ software developed by the National Institute of Health (NIH, USA) to determine differences in gene expression levels.

Statistical Analyses

The data obtained in the study were evaluated using the SPSS 16.0 program. Differences between applications were determined by comparing means with the One Way Anova and Tukey Test.

Results and Discussion

Pea plants exposed to cold and freezing stresses exhibited different morphological features depending on the presence of silicon in the environment, and the water-holding capacity of leaves also changed (Table 1). The stem length of the plants decreased with the intensity of stress on the 4th day, while the presence of silicon did not help length recovery. Root lengths did not respond to the presence of stress or silicon, except showing significant increases upon silicon applications under normal growth conditions.

The only treatment altering the relative water content compared to control plants is the 4-day freezing stress application. Under this treatment, the leaf water holding capacity of control plants decreased; however, both concentrations of silicon significantly increased water holding capacity. The application that most notably affected water holding capacity was 2mM silicon, showing a 15% increase compared to the control. The dry weights of stems and roots varied between 0.27-0.32g and 0.19-0.25g, respectively, under different treatments, but none of the applications had a significant effect on tissue dry weights. Therefore, these results have not been included in the Table 1.

Low ion leakage levels, an indicator of cell integrity, demonstrated that tissues under silicon applications suffered less damage compared to the control in both root and stem tissues and at all applied temperature values (Table 2). While similar decreases were observed under all treatments, statistically significant decreases in ion leakage were determined as follows: in 1-day stem tissues under normal conditions with 1mM silicon application, in 4-day stem tissues under normal conditions with 2mM silicon application, in stem tissues after 1-day cold stress compared to the control under all applications, in stem tissues after 4-day freezing stress compared to the control under all applications, and in stem tissues under 1mM silicon application during 1-day freezing stress. A similar situation was observed in root tissues, and statistically significant decreases were determined in 1-day cold stress with 1mM silicon application, in 1-day freezing stress with 2mM silicon application, and 4-day freezing stress compared to the control under all silicon applications.

Table 1. Plant morphological responses and leaf relative water content (RWC).

Treatments	Relative Water Content (%)	Shoot Lenght (cm)	Root Lenght (cm)
1st day of stress			
C*	99 ± 0.7	5.74 ± 0.22	$8.36 \pm 0.44_a$
S1	99 ± 1.2	5.97 ± 0.40	$10.04 \pm 0.42_b$
S2	100 ± 0.7	6.51 ± 0.32	$11.14 \pm 0.69_b$
C st4**	99 ± 1.1	5.21 ± 0.24	8.69 ± 0.58
S1 st4	100 ± 0.3	6.50 ± 0.21	9.68 ± 0.37
S2 st4	96 ± 0.4	6.32 ± 0.51	9.71 ± 0.40
C st-	99 ± 0.7	4.40 ± 0.25	8.92 ± 0.48
S1 st-	100 ± 0.6	4.51 ± 0.23	8.91 ± 0.20
S2 st-	99 ± 1.3	4.91 ± 0.21	8.03 ± 0.37
4th day of stress			
C	95 ± 1.2	7.35 ± 0.46	$9.72 \pm 0.2_a$
S1	100 ± 0.7	7.72 ± 0.29	$10.77 \pm 0.27_b$
S2	100 ± 0.3	7.62 ± 0. 32	$11.00 \pm 0.31_b$
C st4	100 ± 0.5	5.32 ± 0.27	7.61 ± 0.34
S1 st4	97 ± 1.6	5.23 ± 0.16	8.41 ± 0.30
S2 st4	100 ± 0.9	5.20 ± 0.35	8.47 ± 0.46
C st-	$66 \pm 1.8_a$	4.88 ± 0.44	9.28 ± 0.51
S1 st-	$73 \pm 1.9_b$	5.46 ± 0.53	10.02 ± 0.64
S2 st-	$81 \pm 1.2_b$	4.61 ± 0.45	8.98 ± 0.39

*C, S1 and S2 represent plants irrigated with Hoagland solution containing 0mM, 1mM and 2mM silicon in nutrient media, respectively. ** The abbreviations next to the treatments; st4 represents cold stress applied at 4°C, and st- represents freezing stress applied at -0.5°C; The letters at the bottom right of the results indicate statistically significant differences ($p \leq 0.05$) compared to the respective control. No significant difference is present where no letter was indicated within the treatment groups (C. S1. S2).

Table 2. Ion leakage. malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) levels in silicone-applied pea tissues under cold and freezing stress and normal conditions.

Treatments	Ion Leakage (%)		Malondialdehyde Content (nmol/g)		H_2O_2 Content (nmol/g)	
1st day of stress						
	Shoot	Root	Shoot	Root	Shoot	Root
C*	$21.16 \pm 1.97_a$	40.07 ± 2.96	0.019 ± 0.001	$0.012 \pm 0{,}001_a$	$0.26 \pm 0.03_a$	$0.37 \pm 0.04_a$
S1	$15.03 \pm 0.56_b$	35.51 ± 2.67	0.018 ± 0.000	$0.009 \pm 0.000_a$	$0.10 \pm 0.02_b$	$0.31 \pm 0.04_b$
S2	$17.57 \pm 1.92_a$	32.30 ± 2.89	0.018 ± 0.002	$0.008 \pm 0.001_b$	$0.12 \pm 0.01_b$	$0.23 \pm 0.02_b$
C st4**	$19.58 \pm 1.26_a$	$31.36 \pm 3.30_a$	0.020 ± 0.002	0.014 ± 0.001	$0.46 \pm 0.07_a$	$0.49 \pm 0.04_a$
S1 st4	$15.6 \pm 0.97_b$	$23.14 \pm 1.32_b$	0.021 ± 0.002	0.013 ± 0.000	$0.21 \pm 0.03_b$	$0.29 \pm 0.01_b$
S2 st4	$14.26 \pm 1.08_b$	$27.15 \pm 2.86_a$	0.021 ± 0.001	0.013 ± 0.001	$0.24 \pm 0.03_b$	$0.22 \pm 0.00_b$
C st-	$17.34 \pm 1.94_a$	$16.88 \pm 1.40_a$	$0.018 \pm 0.001_a$	$0.018 \pm 0.002_a$	0.37 ± 0.02	$0.50 \pm 0.06_a$
S1 st-	$13.06 \pm 0.64_b$	$13.84 \pm 0.56_a$	$0.018 \pm 0.001_a$	$0.013 \pm 0.000_b$	0.30 ± 0.04	$0.30 \pm 0.04_b$
S2 st-	$13.81 \pm 1.73_a$	$10.90 \pm 0.83_b$	$0.012 \pm 0.002_b$	$0.012 \pm 0.001_b$	0.29 ± 0.02	$0.20 \pm 0.03_b$
4th day of stress						
	Shoot	Root	Shoot	Root	Shoot	Root
C	$25.66 \pm 3.21_a$	34.07 ± 3.79	0.018 ± 0.002	0.010 ± 0.000	$0.32 \pm 0.02_a$	$0.49 \pm 0.04_a$
S1	$22.82 \pm 2.75_a$	31.42 ± 2.76	0.015 ± 0.001	0.008 ± 0.000	$0.25 \pm 0.02_b$	$0.38 \pm 0.03_b$
S2	$18.73 \pm 2.06_b$	27.82 ± 2.16	0.015 ± 0.001	0.008 ± 0.000	$0.24 \pm 0.02_b$	$0.24 \pm 0.01_b$
C st4	23.45 ± 2.22	37.33 ± 2.73	0.021 ± 0.001	$0.018 \pm 0.001_a$	$0.45 \pm 0.03_a$	$0.51 \pm 0.03_a$
S1 st4	22.46 ± 1.78	37.3 ± 3.18	0.021 ± 0.002	$0.013 \pm 0.001_b$	$0.32 \pm 0.01_b$	$0.33 \pm 0.01_b$
S2 st4	23.26 ± 2.74	36.22 ± 2.41	0.019 ± 0.001	$0.013 \pm 0.000_b$	$0.18 \pm 0.02_b$	$0.39 \pm 0.03_b$
C st-	$20.12 \pm 1.79_a$	$24.20 \pm 1.46_a$	0.014 ± 0.000	$0.012 \pm 0.001_a$	$0.25 \pm 0.03_a$	$0.68 \pm 0.05_a$
S1 st-	$13.59 \pm 1.04_b$	$20.03 \pm 1.83_b$	0.012 ± 0.002	$0.012 \pm 0.001_a$	$0.20 \pm 0.00_b$	$0.47 \pm 0.02_b$
S2 st-	$11.75 \pm 1.00_b$	$17.25 \pm 0.69_b$	0.012 ± 0.001	$0.007 \pm 0.000_b$	$0.20 \pm 0.01_b$	$0.31 \pm 0.02_b$

*C, S1 and S2 represent plants irrigated with Hoagland solution containing 0mM, 1mM and 2mM silicon in nutrient media, respectively. ** The abbreviations next to the treatments; st4 represents cold stress applied at 4°C, and st- represents freezing stress applied at -0.5°C; The letters at the bottom right of the results indicate statistically significant differences ($p \leq 0.05$) compared to the respective control. No significant difference is present where no letter was indicated within the treatment groups (C. S1. S2).

MDA levels, an indicator of cell membrane damage, showed a decrease in stem tissues on the 1st day of freezing stress with 2mM silicon application compared to the control. MDA levels, which remained the same under all treatments under normal conditions, exhibited significant decreases, especially in root tissues, with silicon application under cold stress. When root tissues were examined, it was observed that even under normal conditions, the 2mM silicon application reduced tissue MDA levels, and under 4-day cold stress, silicon

applications effectively reduced MDA levels. Under freezing stress, a 1-day application with two different silicon concentrations and a 4-day application with 2mM silicon reduced MDA levels in root tissues.

Even without any stress application, silicon concentrations on the 1st day in pea tissues kept hydrogen peroxide levels lower than in other tissues. During the 4-day culture stage, tissue hydrogen peroxide levels remained low at all silicon concentrations. Under cold stress, all silicon concentrations effectively reduced hydrogen peroxide levels in both root and stem tissues. Under freezing stress, silicon applications had a level-reducing effect on stem tissues for the 4-day duration, while in root tissues, silicon concentrations reduced hydrogen peroxide levels compared to other applications under all stress durations.

On the first day of cold stress, chlorophyll levels increased compared to applications without cold stress, while under freezing stress, they decreased both under normal conditions and compared to cold stress (Table 4). Silicon applications did not alter chlorophyll levels in tissues under any stress condition. Osmoprotectant proline levels, which play a role in stress resistance, remained lower in stem tissues even under 1-day normal conditions with silicon applications compared to the control, with no significant differences observed among applications in root tissues. Both in root and stem tissues, silicon concentrations under 1 and 4 days of cold stress caused a significant decrease in proline levels. Under freezing stress, silicon applications reduced proline levels in both stem and root tissues.

Table 3. Chlorophyll a, chlorophyll b and proline levels in silicone-applied pea tissues under cold and freezing stress and normal conditions.

Treatments	Chlorophyll Content (µg/g)		Proline Content (nmol/g)	
	1st day of stress			
	Ca	Cb	Shoot	Root
C*	15.17 ± 1.87	9.05 ± 1.02	$188.66 \pm 13.21_a$	73.20 ± 6.71
S1	14.70 ± 1.65	9.03 ± 0.08	$138.32 \pm 13.75_b$	74.74 ± 6.64
S2	15.86 ± 1.24	9.31 ± 0.07	$147.42 \pm 12.02_b$	81.70 ± 7.20
C st4**	18.68 ± 1.89	11.50 ± 1.23	$463.40 \pm 16.21_a$	$102.31 \pm 9.89_a$
S1 st4	19.59 ± 2.22	11.71 ± 1.12	$189.00 \pm 13.89_b$	$75.26 \pm 7.23_b$
S2 st4	18.95 ± 1.98	11.60 ± 1.06	$173.71 \pm 14.15_b$	$62.11 \pm 5.36_b$
K st-	8.22 ± 1.12	7.83 ± 0.07	$208.59 \pm 14.33_a$	$149.14 \pm 13.15_a$
S1 st-	8.23 ± 0.08	7.47 ± 0.06	$194.59 \pm 12.86_a$	$82.47 \pm 13.11_b$
S2 st-	7.99 ± 0.09	7.21 ± 0.08	$132.47 \pm 13.42_b$	$85.05 \pm 12.78_b$
	4th day of stress			
	Ca	Cb	Shoot	Root
C	19.35 ± 1.43	11.80 ± 1.78	145.53 ± 12.99	73.54 ± 9.29
S1	19.15 ± 2.13	11.45 ± 0.87	142.84 ± 13.21	70.10 ± 6.14
S2	20.27 ± 1.29	11.02 ± 1.03	140.52 ± 12.93	69.93 ± 5.96
C st4	19.32 ± 2.06	11.31 ± 1.33	$418.30 \pm 17.32_a$	$106.96 \pm 11.69_a$
S1 st4	19.62 ± 1.92	11.58 ± 1.85	$248.97 \pm 14.66_b$	$76.80 \pm 5.12_b$
S2 st4	20.69 ± 1.69	12.56 ± 1.98	$202.58 \pm 13.51_b$	$85.05 \pm 6.60_b$
C st-	8.34 ± 0.34	8.69 ± 0.60	$274.23 \pm 14.47_a$	99.48 ± 8.34
S1 st-	9.01 ± 1.08	7.64 ± 0.71	$163.40 \pm 13.95_b$	87.29 ± 8.03
S2 st-	8.68 ± 0.44	7.70 ± 0.63	$192.78 \pm 12.38_b$	86.08 ± 7.72

*C, S1 and S2 represent plants irrigated with Hoagland solution containing 0mM, 1mM and 2mM silicon in nutrient media, respectively. ** The abbreviations next to the treatments; st4 represents cold stress applied at 4°C, and st- represents freezing stress applied at -0.5°C; The letters at the bottom right of the results indicate statistically significant differences ($p \leq 0.05$) compared to the respective control. No significant difference is present where no letter was indicated within the treatment groups (C. S1. S2).

Table 4. Silicon accumulation in stem and root tissues under normal conditions with cold and freezing stress.

Treatments	Amount of Silicon (mg/kg dry weight)			
	1st day of stress		4th day of stress	
	Shoot	Root	Shoot	Root
S1	$257.5 \pm 7.52_a$	$264.8 \pm 2.85_a$	$327.2 \pm 1.86_{a,\,b}$	$1430 \pm 35.8_a$
S2	$359.9 \pm 4.17_b$	$550.2 \pm 36.42_c$	$470.9 \pm 14.61_c$	$1455 \pm 24_a$
S1 st4*	$282.7 \pm 7.15_a$	$633 \pm 21.22_d$	$346.1 \pm 10.49_{a,\,b}$	$1782 \pm 21.54_b$
S2 st4	$339.5 \pm 32.75_b$	$809 \pm 17.19_e$	$523.8 \pm 12.50_d$	$1809 \pm 39.41_b$
S1 st-	$279.2 \pm 12.77_a$	$408.1 \pm 6.98_b$	$320.8 \pm 0.58_a$	$916.1 \pm 40.86_c$
S2 st-	$338.2 \pm 15.38_b$	$573.8 \pm 12.25_c$	$347.4 \pm 7.05_b$	$1258 \pm 40.6_d$

*C, S1 and S2 represent plants irrigated with Hoagland solution containing 0mM, 1mM and 2mM silicon in nutrient media, respectively. ** The abbreviations next to the treatments; st4 represents cold stress applied at 4°C, and st- represents freezing stress applied at -0.5°C; Different letters at the bottom right of the results indicate statistically significant differences ($p \leq 0.05$) compared to the respective control in the same column.

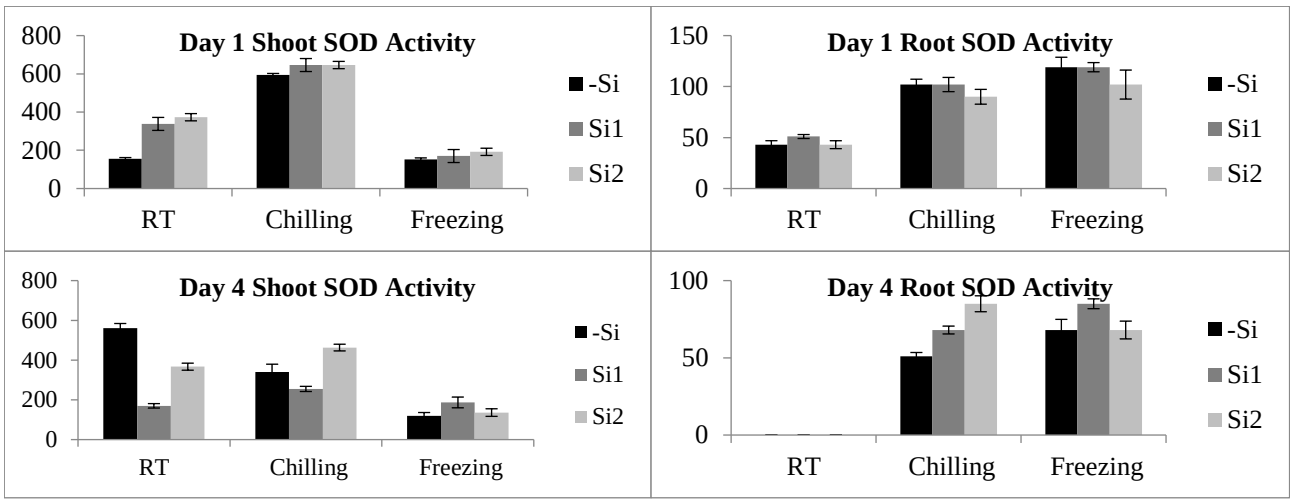

Figure 1. Total SOD enzyme activity of shoot and root tissues under different silicon and stress applications

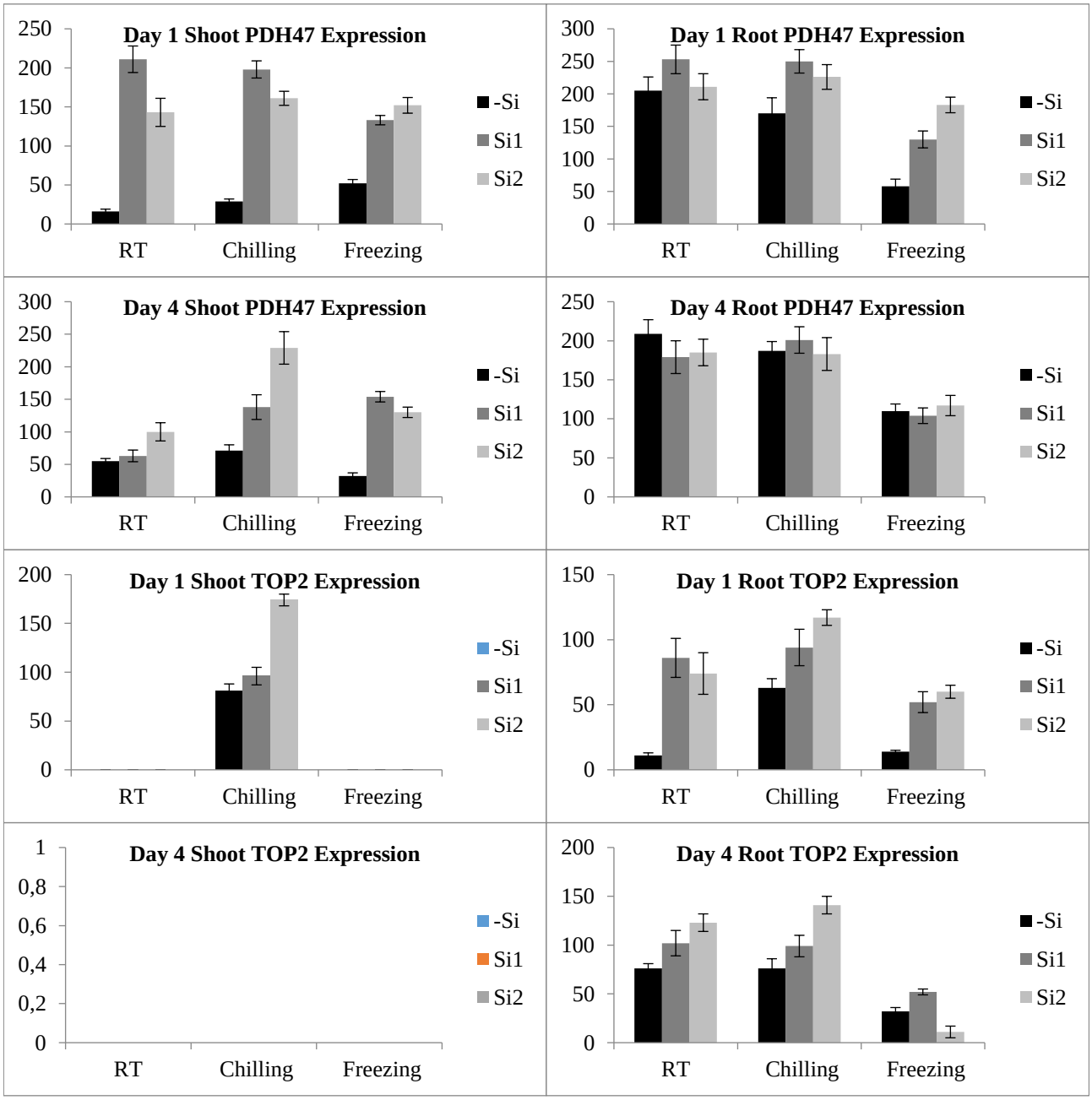

Figure 2. The colors of Si treatments should be black, dark gray and light gray as other graphs

SOD isoenzyme activity was examined using the non-denaturing PAGE method. While three different isoenzyme activities were observed in leaves, one or two different isoenzyme activities were observed in roots under different treatments. The isoenzyme types were determined to be Cu/ZnSOD and MnSOD using KCN and H_2O_2. Isoenzyme activities were determined by examining band thicknesses formed under different treatments, and it was observed that different isoenzyme activities could appear in different tissues under different temperature applications. For example, MnSOD and CuZnSOD1 activities were observed in roots only under freezing stress, CuZnSOD2 isoenzyme activity was observed on the 1st day under all temperature applications, and on the 4th day, it was only evident in root tissues under cold stress. In stem tissues, the activities of all three SOD isoenzymes were observed under every condition. In root tissues, the application of 1mM silicon on the 4th day of cold and freezing stress increased the activity of the SOD enzyme (CuZnSOD2).

In stem tissues, on the 1st day under normal conditions, SOD activity belonging to three different isoenzymes increased in all applications compared to the control, while under normal conditions on the 4th day, the same applications decreased all isoenzyme activities compared to the control (Figure 1). Under cold stress on the 1st day, two different silicon concentrations increased SOD enzyme activity in stem tissues. On the fourth day of cold stress, under 2mM silicon applications, the activity in stem tissues increased. Under freezing stress, all applications increased total SOD activity in the form of CuZnSOD2 in the stem on both the 1st and 4th stress days, and 1mM silicon increased all isoenzyme activities on the 4th stress day.

Among the genes examined for expression levels under stress conditions and various substance applications in the study, *TOP2*, exhibited expression only in stem tissues under 1-day cold stress, and its expression increased with silicon applications (Figure 2). The *TOP2* gene activity was observed in root tissues for every application, and its expression intensity increased in the presence of silicon in all applications, including normal conditions on the 1st day, and on the 4th stress day, both under normal conditions and cold stress.

In the absence of any treatments, the *PDH47* gene expression in pea roots was more intense compared to stems (Figure 2). *PDH47* gene expression increased in all treatments, with an increase in all concentrations of silicon present in the environment. Although the expression of *PDH47* in root tissues was not as intense as in stem tissues, it increased in response to all silicon applications except for the 4th freezing stress day. The expression of the *PDH47* gene in stem tissues increased under cold stress, with a decrease observed only on the 4th day under freezing stress compared to the control. *PDH47* gene expression decreased in root tissues under both cold and freezing stress. Actin expression decreased under both cold and freezing stresses in both root and stem tissues, except for the stem tissues on the 4th day under cold stress.

Elemental silicon analysis in pea tissues was conducted using the Perkin Elmer OPTIMA 5300 DY ICP OES device, following the EPA 6010 method. As expected, silicon uptake into tissues from environments containing 2mM silicon was greater than those containing 1mM silicon (Table 4). The obtained data revealed that silicon accumulation in root tissues showed increases up to fivefold compared to the stem. These data suggest that silicon uptake into cells via silicon carriers is an efficient mechanism, but it is not transmitted to the stem at the same rate as vascular tissues. The most notable finding from elemental analysis was the higher concentration of silicon in root tissues under cold stress compared to the control. This condition persisted on the 1st day under freezing stress, but on the 4th day of freezing stress, silicon uptake into cells did not continue at the same pace.

In summary, under cold and freezing stress, the dry weights of pea roots and stems remained unchanged independent of cold stress and silicon applications. Different temperature applications did not have a significant effect on root lengths on the 1st and 4th day of stress. Shoot tissue was also not affected on the 1st day of stresses, however on the 4th day, stems under cold and freezing stress were shorter than those developing under normal conditions. Sogarwal et al. (2023) demonstrated that exogenous Si application in wheat increased cuticle and epidermal layer thickness due to accumulated silicon, preventing the harmful effects of drought, heat, and cold stress. In the context of this study, root lengths were observed to be extended in the presence of silicon under normal growth conditions compared to the control; however, no significant effect of silicon presence were observed in tissue weights and lengths under the investigated stress conditions, likely due to stress intensity and difference in plant response used in this particular study.

When all indicators of oxidative stress levels were compared, it was determined that silicon played an important role in preserving cell membrane integrity, thereby reducing ion leakage and lipid peroxidation while increasing water retention capacity. Additionally, silicon significantly reduced hydrogen peroxide levels in tissues, lowering the levels of accumulated reactive oxygen species in cells. In stressed tissues, especially under cold stress, proline levels increased significantly; however, silicon did not further increase proline levels under stress conditions. Instead the levels decreased under stress upon silicon application. Studies conducted on turfgrass and corn plants have shown that silicon application increases tissue proline levels under cold stress (He et al., 2010; Moradtalab et al., 2018). In our study, silicon likely contributed to a decrease in stress levels by reducing internal reactive oxygen species and, consequently, proline levels. This observation suggests that different physiological responses may be obtained in different plants depending on stress duration and dosage.

The findings of this study indicated that SOD enzyme activity, especially on the 1st day of cold stress, showed significant increases compared to plants under normal conditions, but as stress conditions prolonged or intensified in the form of freezing stress, activities in stems decreased. Pea seedlings adapted to short periods of low-intensity cold stress in stem tissues through enzymatic antioxidant defense proteins, but they could not withstand prolonged severe cold stress likely due to possible structural and functional impairments in defense system components. However, SOD enzyme activities continued intensively in root tissues under both cold and freezing stress, with

significant increases observed in the presence of silicon at different enzyme isoform levels. Similar protective effects of silicon on SOD enzyme and other enzymatic antioxidants have also been observed in studies on paspalum turfgrass, rice, corn, and barley (He et al., 2010; Azeem et al., 2016; Moradtalab et al., 2018; Joudmand & Hajiboland, 2019).

Under cold and freezing stresses, our examinations with silicon applications revealed that *TOP2*, *PDH47*, and actin gene expressions were activated by stress, indicating a response to the presence of silicon in the environment. The obtained data suggest that *TOP2*, which is one of the topoisomerase enzyme genes involved in DNA metabolism processes such as replication, transcription, recombination, and chromosome segregation, starts expressing on the first day of cold stress, despite not being expressed under normal conditions. This implies that *TOP2* begins to alter the topology of DNA, potentially affecting various stress-related genes and transcription factors involved in replication, transcription, recombination, and chromosome segregation.

The presence of silicon in the environment significantly increased the expression of *TOP2*. Hettiarachchi et al. (2005) also demonstrated an increase in *TOP2* gene expression under cold and salt stresses, as well as with applications of salicylic acid and ABA from phytohormones. The *PDH47* gene showed significant increases in expression in stem and root tissues under salinity and cold stress in peas (Vashisht et al., 2005). When the *PDH47* gene was transferred to indica rice, the plant exhibited resistance features under drought stress, as evidenced by increased relative water content with proline and reduced internal hydrogen peroxide levels (Singha et al., 2020; Singha et al., 2017). Although the reason for the increase in *TOP2* gene expression under stress, when DNA replication and cellular activities decrease, has not been fully explained, it has been suggested that it may be related to chromatin modeling and the necessity for DNA to adopt an appropriate topology for the expression of stress-regulated genes.

The data obtained in this study also suggest that the expressions of genes involved in ribosome biogenesis, transcription, translation, and repair of damaged DNA/RNA molecules, which are considered to have functions in stress tolerance, can be enhanced by silicon. These increases are believed to have positive effects on the antioxidative defense system. Silicon applications in pea stem tissues also increased the expression of the actin gene, which is investigated as an internal control under different temperatures. Changes in the expression of the actin protein, which structures the intracellular skeleton system, indicate that silicon may have protective functions related to the skeletal system.

The results of elemental silicon analysis have revealed an increase in the amount of silicon taken up by tissues at high silicon concentrations. It has also been found that roots contain a higher amount of silicon compared to stems, and the transport of silicon into the tissues increases under cold stress conditions. These data may explain the observed increase in root lengths and unaffected stem lengths under normal conditions with silicon application, as well as the decrease in malondialdehyde levels, which was only observed in roots under cold stress conditions with silicon applications.

Conclusions

This study has demonstrated that the applications of silicon to pea plants, when externally added to the environment in which they are cultivated, have positive effects on the cold resistance of plants. The obtained data have shown that silicon increases the expressions of *TOP2* and *PDH47* genes, which can alter the structure of the DNA molecule and facilitate the transcription of genes encoding defense enzymes, under both cold and freezing stresses. SOD enzyme activity, one of the most important enzymes used in combating reactive oxygen species, by converting superoxide radicals to hydrogen peroxide, increased under stress conditions and in the presence of silicon. The observed decrease in tissue hydrogen peroxide levels also indicates the active involvement of other components of the enzymatic defense system. Therefore, within the scope of this study, it can be concluded that silicon application under cold and freezing stresses has induced various changes in pea metabolism, including, an increase in the expressions of *TOP2* and *PDH47* genes and an increase in SOD enzyme activity, which in turn decreased tissue hydrogen peroxide levels and increased cell membrane integrity. Overall the changes have been found to have positive effects on the plants cold and freezing stress responses and silicon was determined as a potential soil amendment to be used in agricultural production.

Acknowledgment

This study was funded by TÜBİTAK with the project number 102O367.

References

Al-Aghabary, K., Zhu, Z., & Shi, Q. (2005). Influence of Silicon Supply on Chlorophyll Content, Chlorophyll Fluorescence, and Antioxidative Enzyme Activities in Tomato Plants Under Salt Stress. *Journal of Plant Nutrition, 27*(11), 2101–2115. https://doi.org/10.1080/01904160500315818

Azeem, S., Li, Z., & Zheng, H. (2016). Quantitative proteomics study on Lsi1 in regulation of rice (*Oryza sativa L.*) cold resistance. *Plant Growth Regulation, 78*(3), 307–323. https://doi.org/10.1007/s10725-015-0094-2

Bakhat, H. F., Bibi, N., Zia, Z., Abbas, S., Hammad, H. M., Fahad, S., Ashraf, M. R., Shah, G. M., Rabbani, F. & Saeed, S. (2018) Silicon mitigates biotic stresses in crop plants: A review. Crop Protection, *104*, 21-34. https://doi.org/10.1016/j.cropro.2017.10.008

Bates, L. S., Waldren, R. P., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil, 39*(1), 205–207. https://doi.org/10.1007/BF00018060

Batool, T., Javied, S., Ashraf, K., Sultan, K., Zaman, Q. U., & Haider, F. U. (2022). Alleviation of Cadmium Stress by Silicon Supplementation in Peas by the Modulation of Morpho-Physio-Biochemical Variables and Health Risk Assessment. *Life, 12*(10), 1479. https://doi.org/10.3390/life12101479

Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry, 44*(1), 151–155. https://doi.org/10.1016/0003-2697(71)90370-8

Bergmeyer, H.U. (2012). *Methods of Enzymatic Analysis*. Elsevier.

Biju, S., Fuentes, S., & Gupta, D. (2023) Novel insights into the mechanism(s) of silicon-induced drought stress tolerance in lentil plants revealed by RNA sequencing analysis. *BMC Plant Biol*, *23*(1), 498. https://10.1186/s12870-023-04492-5.

Boudet, N., Aubourg, S., Toffano-Nioche, C., Kreis, M., & Lecharny, A. (2001). Evolution of intron/exon structure of DEAD helicase family genes in *Arabidopsis*, *Caenorhabditis*, and *Drosophila*. *Genome Research*, *11*(12), 2101–2114. https://doi.org/10.1101/gr.204001

Bradford, M. M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1-2), 248–254. https://doi.org/10.1016/0003-

Chinnusamy, V., Zhu, J., & Zhu, J. K. (2007). Cold stress regulation of gene expression in plants. *Plant Science*, *173*(6), 540–548. https://doi.org/10.1016/j.plantsci.2007.09.014

Collin, B., Doelsch, E., & Keller, C. (2014). Evidence of sulfur-bound reduced copper in bamboo exposed to high silicon and copper concentrations. *Environmental Pollution*, *187*, 22–30. https://doi.org/10.1016/j.envpol.2013.12.024

Cruzado-Tafur, E., Orzol, A., & Golebiowski, A. (2023). Metal tolerance and Cd phytoremoval ability in *Pisum sativum* grown in spiked nutrient solution. *Journal of Plant Research*, *136*(6), 931–945. https://doi.org/10.1007/s10265-023-01493-1

Dann, E. K., & Muir, S. (2002). Peas grown in media with elevated plant-available silicon levels have higher activities of chitinase and Beta-1,3-glucanase, are less susceptible to a fungal leaf spot pathogen and accumulate more foliar silicon. *Australasian Plant Pathology*, *31*(1), 9–13. https://doi.org/10.1071/AP01076

Ding, Y., & Yang, S. (2022) Surviving and thriving: How plants perceive and respond to temperature stress. *Dev Cell*, *57*(8), 947-958. https://doi.org/10.1016/j.devcel.2022.03.010

El-Okkiah, S. A. F., El-Tahan, A. M., Ibrahim, O. M., Taha, M. A., Korany, S. M., Alsherif, E. A., & Sharaf-Eldin, M. A. (2022). Under cadmium stress, silicon has a defensive effect on the morphology, physiology, and anatomy of pea (*Pisum sativum* L.) plants. *Frontiers in Plant Science*, *13*, 997475. https://doi.org/10.3389/fpls.2022.997475

Epstein, E. (2009). Plant nutrition, plant stress, and plant silicon. *Comparative Biochemistry and Physiology A-Molecular and Integrative Physiology*, *153*(2), 185-186. https://doi.org/ 10.1016/j.cbpa.2009.04.405

Fauteux, F., Chain, F., Belzile, F., Menzies, J. G., & Belanger, R. R. (2006). The protective role of silicon in the *Arabidopsis*-powdery mildew pathosystem. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(47), 17554–17559. https://doi.org/10.1073/pnas.0606330103

Fitzpatrick, K. L., & Reid, R. J. (2009). The involvement of aquaglyceroporins in transport of boron in barley roots. *Plant Cell Environ*, *32*, 1357–1365. https://10.1111/j.1365-3040.2009.02003.x

Georgieva, K., & Lichtenthaler, H. K. (1999). Photosynthetic activity and acclimation ability of pea plants to low and high-temperature treatment as studied by means of chlorophyll fluorescence. *Journal of Plant Physiology*, *155*, 416–423. https://doi.org/10.1016/S0176-1617(99)80014-7

Hacker, L., Dorn, A. Enderle, J., & Puchta, H. (2022) The repair of topoisomerase 2 cleavage complexes in *Arabidopsis*. *The Plant Cell*, *34*, 287–301. https://doi.org/10.1093/plcell/koab228

Hasanuzzaman, M., Nahar, K., & Rohman, M. M. (2018). Exogenous silicon protects *Brassica napus* plants from salinity-induced oxidative stress through the modulation of AsA-GSH pathway, thiol-dependent antioxidant enzymes and glyoxalase systems. *Gesunde Pflanz*, *70*, 185–194. https://doi.org/10.1007/s10343-018-0430-3

He, Y., Xiao, H., & Wang, H. (2010). Effect of silicon on chilling-induced changes of solutes, antioxidants, and membrane stability in seashore paspalum turfgrass. *Acta Physiol Plant*, *32*, 487–494. https://doi.org/10.1007/s11738-009-0425-x

Hettiarachchi, G. H. C. M., Reddy, M. K., Sopory, S. K., & Chattopadhyay, S. (2005). Regulation of TOP2 by Various Abiotic Stresses Including Cold and Salinity in Pea and Transgenic Tobacco Plants. *Plant Cell Physiology*, *46*, 1154–1160. https://doi.org/10.1093/pcp/pci125

Hettiarachchi, G. H. C. M., Yadav, V., Reddy, M. K., Chattopadhyay, S., & Sopory, S. K. (2003). Light-mediated regulation defines a minimal promoter region of TOP2. *Nucleic Acids Res.*, *31*, 5256–5265. https://doi.org/10.1093/nar/gkg733

Hoagland, D. R., & Arnon, D. I. (1950). The water-culture method for growing plants without soil. *Cal Agric Exp Sta Circ*, *347*, 1–32.

Islam, W., Tayyab, M., & Khalil, F. (2020). Silicon-mediated plant defense against pathogens and insect pests. *Pestic Biochem Physiol*, *168*, 104641. https://doi.org/10.1016/j.pestbp.2020.104641

Ismail, L. M., Soliman, M. I., Abd El-Aziz, M. H., & Abdel-Aziz, H. M. M. (2022). Impact of Silica Ions and Nano Silica on Growth and Productivity of Pea Plants under Salinity Stress. *Plants (Basel)*, *11*(4), 494. https://doi.org/10.3390/plants11040494

Jahed, K. R., Saini, A. K., & Sherif, S. M. (2023) Coping with the cold: unveiling cryoprotectants, molecular signaling pathways, and strategies for cold stress resilience. *Front Plant Sci*, *14*, 1246093. https://doi.org/10.3389/fpls.2023.1246093

Jan, S., Rustgi, S., Barmukh, R., Shikari, A. B., Leske, B., Bekuma, A., Sharma, D., Ma, W., Kumar, U., Kumar, U., Bohra, A., Varshney, R. K., & Mir, R. R. (2023) Advances and opportunities in unraveling cold-tolerance mechanisms in the world's primary staple food crops. *Plant Genome*, *13*, e20402. https://doi.org/10.1002/tpg2.20402

Joudmand, A., & Hajiboland, R. (2019). Silicon mitigates cold stress in barley plants via modifying the activity of apoplasmic enzymes and concentration of metabolites. *Acta Physiol Plant*, *41*, 1–13. https://doi.org/10.1007/s11738-019-2817-x

Larran AS, Pajoro A, & Questa JI. (2023) Is winter coming? Impact of the changing climate on plant responses to cold temperature. *Plant Cell Environ*, *46*(11), 3175-3193. https://doi.org/10.1111/pce.14669

Leterrier, M., Del Rio, L. A., & Corpas, F. J. (2007). Cytosolic NADP-isocitrate dehydrogenase of pea plants: Genomic clone characterization and functional analysis under abiotic stress conditions. *Free Radical Research*, *41*, 191–199. https://doi.org/10.1080/10715760601034055

Liang, Y., Zhu, J., Li, Z., Chu, G., Ding, Y., Zhang, J., & Sun, W. (2008). Role of silicon in enhancing resistance to freezing stress in two contrasting winter wheat cultivars. *Environmental and Experimental Botany*, *64*, 286–294. https://doi.org/10.1016/j.envexpbot.2008.06.005

Lichtenthaler, H. K., & Wellburn, A. R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. In: Portland Press Ltd.

Lucau-Danila, A., Toitot, C., Goulas, E., Blervacq, A.S., Hot, D., Bahrman, N., Sellier, H., Lejeune-Henaut, I., & Delbreil, B. (2012). Transcriptome analysis in pea allows to distinguish chilling and acclimation mechanisms. *Plant Physiol Biochem*, *58*, 236-244. https://doi.org/ 10.1016/j.plaphy.2012.07.012.

Ma, J. F., & Yamaji, N. (2006). Silicon uptake and accumulation in higher plants. *Plant Science*, *11*(5). https://doi.org/ 10.1016/j.tplants.2006.06.007

Manasa, S. L., Panigrahy, M., & Panigrahi, K. C. S. (2022). Overview of Cold Stress Regulation in Plants. *Bot. Rev*, *88*, 359–387. https://doi.org/10.1007/s12229-021-09267-x

Mandlik, R., Thakral, V., Raturi, G., Shinde, S., Nikolic, M., Tripathi, D. K., Sonah, H., & Deshmukh, R. (2020). Significance of silicon uptake, transport, and deposition in plants. *J Exp Bot, 71*(21), 6703-6718. https://doi.org/10.1093/jxb/eraa301

Mir, R. A., Bhat, B. A., & Yousuf, H. (2022). Multidimensional role of silicon to activate resilient plant growth and to mitigate abiotic stress. *Frontiers in Plant Science, 13*, 819658. https://doi.org/10.3389/fpls.2022.819658

Moradtalab, N., Weinmann, M., Walker, F., Höglinger, B., Ludewig, U., & Neumann, G. (2018). Silicon improves chilling tolerance during early growth of maize by effects on micronutrient homeostasis and hormonal balances. *Frontiers in Plant Science, 9*, 420. https://doi.org/10.3389/fpls.2018.00420

Mostafa, M. G., Rahman, M. M., Ansary, M. M. U., Keya, S. S., Abdelrahman, M., Miah, M. G., & Tran, L. S. P. (2021). Silicon in mitigation of abiotic stress-induced oxidative damage in plants. *Critical Reviews in Biotechnology, 41*, 918–934. https://doi.org/10.1080/07388551.2021.1892582

Nanjo, T., Kobayashi, M. Y., Yoshiba, Y., Kakubar, K., Yamaguchi-Shinozaki, Y., & Shinozaki, K. (1999). Antisense suppression of proline degradation improves tolerance to freezing and salinity in *Arabidopsis thaliana*. *FEBS Letters, 461*, 205–210.

Ohkawa, H., Ohishi, N., & Yagi, Y. (1979). Assay of lipid peroxides in animal tissue by thiobarbituric acid reaction. *Analytical Biochemistry, 95*, 51–358.

Oliveira, K. R., Souza Junior, J. P., Bennett, S. J., Checchio, M. V., Alves, R. de C., Felisberto, G., Prado, R. de M., Gratao, P. L. (2020). Exogenous silicon and salicylic acid applications improve tolerance to boron toxicity in field pea cultivars by intensifying antioxidant defense systems. *Ecotoxicology and Environmental Safety, 201*, 110778. https://doi.org/10.1016/j.ecoenv.2020.110778

Parveen, N. & Ashraf, M. (2010). Role of silicon in mitigating the adverse effects of salt stress on growth and photosynthetic attributes of two maize (*Zea mays* l.) cultivars grown hydroponically. *Pak. J. Bot., 42*(3), 1675-1684.

Rahman, M. F., Ghosal, A., Alam, M. F., Kabir, A. H., & Kabir, A. H. (2017). Remediation of cadmium toxicity in field peas (*Pisum sativum* L.) through exogenous silicon. *Ecotoxicology and Environmental Safety, 135*, 165–172. https://doi.org/10.1016/j.ecoenv.2016.09.019

Raza, T., Abbas, M., Imran, S., Khan, M. Y., Rebi, A., Rafie-Rad, Z., & Eash, N. S. (2023) Impact of Silicon on Plant Nutrition and Significance of Silicon Mobilizing Bacteria in Agronomic Practices. *Silicon, 15*(9), 3797–817. doi: 10.1007/s12633-023-02302-z.

Salman, S., Shahzad, L., Khan, W.-U. D., Riaz, U., Sharif, F., Tahir, A., & Saeed, M. T. (2023). Influence of silicon doped biochar on germination and defense mechanisms of pea (*Pisum sativum* L.) under copper and salinity stresses. *Journal of Plant Nutrition, 46*(16), 3933–3953. https://doi.org/10.1080/01904167.2023.2220720

Seki, M., Narusaka, M., Abe, H., Kasuga, M., Yamaguchi-Shinozaki, K., Carninci, P., & Shinozaki, K. (2001). Monitoring the expression pattern of 1300 *Arabidopsis* genes under drought and cold stresses by using a full-length cDNA microarray. *Plant Cell, 13*, 61–72. https://doi.org/10.1105/tpc.13.1.61

Shanmugaiah, V., Gauba, A., & Hari, S. K. (2023). Effect of silicon micronutrient on plant's cellular signaling cascades in stimulating plant growth by mitigating the environmental stressors. *Plant Growth Regulation, 100*, 391–408. https://doi.org/10.1007/s10725-023-00982-6

Singh, B. N., Sopory, S. K., & Reddy, M. K. (2004). Plant DNA Topoisomerases: Structure, Function, and Cellular Roles in Plant Development. *Critical Reviews in Plant Sciences, 23*, 251–269. https://doi.org/10.1080/07352680490452816

Singha, D. L., Sarma, S., & Singh, S. (2020). Understanding the mode of regulation of proline biosynthesis for drought tolerance in transgenic rice overexpressing PDH47 gene. *Indian Journal of Biotechnology, 19*, 73-81.

Singha, D. L., Tuteja, N., Boro, D., Hazarika, G. N., & Singh, S. (2017). Heterologous expression of PDH47 confers drought tolerance in indica rice. *Plant Cell, Tissue and Organ Culture (PCTOC), 130*, 577-589. https://doi.org/10.1007/s11240-017-1248-x

Smart, R. E., & Bingham, G. E. (1974). Rapid estimates of relative water content. *Plant Physiology, 53*, 258–260.

Sogarwal, A., Kumari, N., & Sharma, V. (2023). Role of silicon in abiotic stress tolerance in wheat. *Cereal Research Communications, 51*, 809–819. https://doi.org/10.1007/s42976-022-00346-2

Srivastava, S., Rahman, M. H., Shah, S., & Kav, N. N. V. (2006). Constitutive expression of the pea ABA-responsive 17 (ABR17) cDNA confers multiple stress tolerance in *Arabidopsis thaliana*. *Plant Biotechnology Journal, 4*, 529–549. https://doi.org/10.1111/j.1467-7652.2006.00201.x

Stagnari, F., Maggio, A., & Galieni, A. (2017) Multiple benefits of legumes for agriculture sustainability: an overview. *Chem. Biol. Technol. Agric. 4*, 2. https://doi.org/10.1186/s40538-016-0085-1

Streb, P., Aubert, S., Gout, E., & Bligny, R. (2003). Cold- and light-induced changes of metabolite and antioxidant levels in two high mountain plant species *Soldanella alpina* and *Ranunculus glacialis* and a lowland species *Pisum sativum*. *Physiologia Plantarum, 118*, 96–104. https://doi.org/10.1034/j.1399-3054.2003.00099.x

Stupnikova, I., Benamar, A., Tolleter, D., Grelet, J., Borovskii, G., Dorne, A. J., & Macherel, D. (2006). Pea seed mitochondria are endowed with a remarkable tolerance to extreme physiological temperatures. *Plant Physiology, 140*, 326–335. https://doi.org/10.1104/pp.105.073015

Sunkar, R., Chinnusamy, V., Zhu, J., & Zhu, J.K. (2007). Small RNAs as big players in plant abiotic stress responses and nutrient deprivation. *Trends in Plant Science, 12*(7), 301–309. https://doi.org/10.1016/j.tplants.2007.05.001

Takano, J., Miwa, K., Yuan, L.X., von Wiren N., & Fujiwara, T. (2005). Endocytosis and degradation of BOR1, a boron transporter of *Arabidopsis thaliana*, regulated by boron availability. *P Natl Acad Sci, 102*, 12276–12281. doi:10.1073/pnas.0502060102

Tayade, R., Ghimire, A., Khan, W., Lay, L., Attipoe, J.Q., & Kim, Y. (2022). Silicon as a Smart Fertilizer for Sustainability and Crop Improvement. *Biomolecules, 12*(8), 1027. https://doi.org/ 10.3390/biom12081027.

Thakur, A., Singh, A., & Tandon, A. (2023). Insights into the molecular mechanisms of uptake, phytohormone interactions, and stress alleviation by silicon: a beneficial but non-essential nutrient for plants. *Plant Growth Regulation, 101*(1), 1–13. https://doi.org/10.1007/s10725-023-01002-3

Vashisht, A. A., & Tuteja, N. (2006). Stress responsive DEAD-box helicases: A new pathway to engineer plant stress tolerance. *Journal of Photochemistry and Photobiology Biology, 84*, 150–160. https://doi.org/10.1016/j.jphotobiol.2006.02.010

Vashisht, A. A., Pradhan, A., Tuteja, R., & Tuteja, N. (2005). Cold- and salinity stress-induced bipolar pea DNA helicase 47 is involved in protein synthesis and stimulated by phosphorylation with protein kinase C. *The Plant Journal, 44*, 76–87. https://doi.org/10.1111/j.1365-313X.2005.02511.x

Xie, Y., Waqas, M., & Khan, M. U. (2022). Overexpression of the rice gene Lsi1 (low silicon gene 1) enhances plant–microbe interactions that result in improved chilling tolerance. *Plant Growth Regulation, 98*, 525–538. https://doi.org/10.1007/s10725-022-00890-1

Zargar, S. M., Mahajan, R., & Bhat, J. A. (2019). Role of silicon in plant stress tolerance: opportunities to achieve a sustainable cropping system. *3 Biotech*, *9*, 73. https://doi.org/10.1007/s13205-019-1613-z

Zhang, J., Liu, H., Zhao, Q. Z. Du, Y. X., Chang, Q. X. & Lu, Q. L. (2011). Effects of ATP production on silicon uptake by roots of rice seedlings. *Plant Biosystems*, *145*, 866-872. https://doi.org/ 10.1080/11263504.2011.601771

Zhu, Z., Wei, G., Li, J., Qian, Q., & Yu, J. (2004). Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (*Cucumis sativus* L.). *Plant Science*, *167*, 527–533. https://doi.org/10.1016/j.plantsci.2004.04.020