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Common bean seedlings show increased tolerance to cool temperatures when treated with progesterone, β-oestradiol, abscisic acid, and salicylic acid

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Abstract

Cold stress has a significant effect on plant physiology inducing growth hindrance and reduced yields. This study was aimed to evaluate the effect of mammalian sex hormones (progesterone and β-estradiol), abscisic acid (ABA), and salicylic acid (SA) on plant growth, chlorophyll, malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and the activities of anti-oxidative and nitrate assimilation enzymes in common bean (*Phaseolus vulgaris* L.) seedlings under cold stress. For this purpose, cold stress was conducted to 11-day-old seedlings in a climate chamber at 9/5°C (day/night), but for control, the temperature was set at 25/20°C (day/night). Progesterone (Pro) 10^{-9} M, β-oestradiol (ES) 10^{-6} M, ABA 10^{-4} M, and SA 10^{-4} M were applied to 11-day-old seedlings using a handheld sprayer. The results of this experiment showed that cold stress significantly decreased plant growth, and it was followed by an increase in MDA and H_2O_2 content in common bean seedlings. However, the exogenous application of Pro, ES, ABA, and SA mitigated the adverse effect of cold stress in common bean seedlings. In this respect, cold stress decreased the activities of nitrate reductase (NR), nitrite reductase (NiR), and glutamine synthetase (GS) by 41.03%, 35.20%, and 47.54%, respectively, compared to control. However, under cold stress, Pro, ES, ABA, and SA treatments significantly increased NR, NiR, and GS activities by 41.87%, 14.80%, 33.87%, and 64.82 % for NR, 3.4%, 30.10%, 12.75%, and 33.12% for NiR, and 83.00%, 50.10%, 83.14%, and 9.34% for GS, respectively, compared to the plants treated with cold stress. These results suggest that exogenous application of Pro 10^{-9} M, ES 10^{-6} M, ABA 10^{-4} M, and SA 10^{-4} M after three days mitigate the unfavourable impact of cold stress in common bean seedlings by enhancing nitrate metabolism and antioxidant enzyme activities; their spraying on the leaves is beneficial for plant recovery and growth under cold stress.

Keywords: cold stress, enzyme activities, nitrate metabolism, *Phaseolus vulgaris*.

Introduction

In nature, plants are daily exposed to many environmental stresses such as drought and cold stress, extreme heat, and excessive acidity (pH) (Boubakri et al., 2021; Shams, Yildirim, 2021). Nonetheless, cold stress can be considered as one of the most significant limiting agents for the cultivation, productivity, and durability of plants, as it drastically modifies various biological and molecular mechanisms (Janská et al., 2010; Tiryaki et al., 2019).

Low (0–15°C) temperature is a serious threat to the sustainability of crop yields, and the agricultural loss induced by low temperature runs hundreds of billions of dollars worldwide every year. However, the injury to crops largely occurs in physiological and biochemical systems of plants through a disruption in the cytoplasmic membrane and the injury to the structure and activity of protective enzymes. Also, by limiting electron transport and enzyme activities, it can decrease photosynthesis rates and modify chloroplasts (Janská et al., 2010; Sharma, 2000; Ding et al., 2019; Araz et al., 2021). In addition, cold stress targets the cell wall. It causes a significant reduction in pectin levels and enzyme activity (Bilska-Kos et al., 2018; Soliman et al., 2018). Cold stress induces thickening of the cell wall in the leaf, which is linked with modifications in the content of specific cell wall sugars (Lanna et al., 2018; Azarfam et al., 2021). It is correlated with a rapid collapse of photosynthetic pigments such as chlorophylls, which consequently causes a reduction in photosynthetic rates. Also, cold stress can induce over-accumulation of reactive oxygen species (ROS), and this increased production promotes the oxidation and decomposition of lipids, proteins, pigments, and DNA as well as the inactivation of the enzymes of the photosystems; consequently, it leads to cell death (Liu et al., 2019; Pasbani et al., 2020; Saleem et al., 2020; Yu et al., 2020).

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One of the main components in plant regulatory system is phytohormones, which perform a crucial role in controlling the growth and development of plants and actively cooperates in protective and adaptive reactions (Ding et al., 2019). Exogenous application of phytohormones such as salicylic and abscisic acids can increase plant tolerance and mitigate the toxic impacts of abiotic stress such as stresses of heavy metals and salinity on the germination of seedlings and the growth and development of plants (Saleem et al., 2020; Yu et al., 2020). It was shown that salicylic acid can decrease the permeability of the cell membrane, enhance proline concentration to maintain cell water content, decrease lipid peroxidation (MDA content), and significantly increase the cold tolerance of crops. In addition, salicylic acid can help the crops to eliminate the accumulation of reactive oxygen species by enhancing the activity of protective enzymes (Mutlu et al., 2016; Ghanbari, Sayyari, 2018; He et al., 2020). Many studies have shown that exogenous treatment of abscisic acid enhanced soluble sugar and proline content and decreased the malondialdehyde content (Kim et al., 2016; Huang et al., 2017; Yu et al., 2020).

Nonetheless, progesterone, oestrogen (oestrone, oestriol and oestradiol), and androsterone belong to mammalian sex hormones (MSHs). They naturally exist in the roots, leaves, and flowers of plants and have a certain impact on the differentiation, growth, and homeostasis of higher eukaryotes. Exogenous application of oestradiol significantly mitigated the genotoxicity of 2,4-dichlorophenoxyacetic acid (2,4-D) in the common bean genome (Aydin, Nalbantoğlu, 2011). Genisel et al. (2013) found that progesterone treatment protected chickpea seedlings against the hazardous effect of cold stress and decreased the content of malondialdehyde and hydrogen peroxide. In addition, the positive impact of MSHs on the growth and development of plants under non-stress conditions was demonstrated in maize by Erdal and Genisel (2016). However, their effect on common bean seedlings under cold stress are not clear.

Common bean (*Phaseolus vulgaris* L.) belongs to the Fabaceae family; its growth is seriously hindered by temperature below about 20°C and stops altogether at a temperature around 10°C. Turkey has the highest common bean production after the USA and China, but due to cold stress in some parts of Turkey such as Erzurum its cultivation is limited (Tekin, Ceyhan, 2020).

Therefore, in the present study, the effect of progesterone, β-oestradiol, abscisic acid, and salicylic acid on the physiological and biochemical response of common bean seedlings under cold stress conditions was investigated.

Materials and methods

Plant growth and treatment conditions. The experiment was carried out in 2020 at Ataturk University using a completely randomized design with four replications. Firstly, the common bean (*Phaseolus vulgaris* L.) seeds were rinsed with 96% alcohol, then exposed to surface sterilisation in 5% NaClO (sodium hypochlorite) for 5 min. Later, the seeds were rinsed with distilled water, left to swell in distilled water at room conditions, and planted into the prepared sand-filled pots (2 L, 13 × 13 × 16.5 cm). For seedling growth, a climate chamber was prepared under control conditions: 24/20°C temperature (day/night), 14/10-hour light-dark period, 20,000 lux, and 70% humidity.

Eleven-day-old common bean seedlings, in which third leave from the base of plants appeared, were classified into ten groups: (1) control, (2) cold, (3) progesterone (Pro) 10^{-9} M, (4) β-oestradiol (ES) 10^{-6} M, (5) abscisic acid (ABA) 10^{-4} M, (6) salicylic acid (SA) 10^{-4} M, (7) cold + Pro, (8) cold + ES, (9) cold + ABA, and (10) cold + SA. At the age of eleven days, distilled water was sprayed on the seedlings of groups 1 and 2, while the seedlings of other groups were sprayed with Pro, ES, ABA, and SA with a handheld sprayer, and then the seedlings were transferred to the climate chamber. In the climate chamber, seedlings of groups 1, 3, 4, 5, and 6 were grown at 25/20°C (day/night), and seedlings of other groups (2 and 7–10) were grown at 9/5°C (day/night) temperatures. The seedlings were irrigated daily with an equal amount of half-strength Hoagland solution until the day of sampling. For physiological and biochemical analysis, sampling was done at the age of 14 days.

Dry weight. To determine root and shoot dry weight, the common bean seedlings were sampled at 14-day old, then the samples were placed in a hot-air oven at 65°C temperature until a constant weight was reached.

Detection of malondialdehyde (MDA), chlorophyll (Chl) and hydrogen peroxide (H_2O_2) content. The level of MDA was determined by using the 2-thiobarbituric acid (TBA) test. Chl pigments were determined from the leaves in 80% acetone and colorimetrically using a spectrophotometer (Turk et al., 2019; 2020). In leaves, determination of H_2O_2 was done according to Shams et al. (2020). First, leaf samples (0.3 g) were ground in liquid nitrogen, and homogenisation was done in 3 mL of 5% (w/v) TCA (trichloroacetic acid) solution. After centrifugation of the samples at 12 000 × g for 15 min, supernatants were collected and H_2O_2 content was determined by using a reaction mixture: 10 mM potassium phosphate buffer, pH 7.0 and 1 ml 1 M potassium iodide. Sample absorbance was read at 390 nm with a spectrophotometer Multiskan™ GO Microplate (Thermo Scientific, Japan).

Assay of antioxidant enzyme activity. Leaf samples (0.2 g) were homogenised in line with the method of Shams et al. (2019) and Dadasoglu et al. (2021). The ascorbate peroxidase (APX) activity was determined by the reduction of ascorbate in the reaction mixture and measuring the decrease in absorbance at 290 nm for 1 min. The activity of peroxidase (POD) was calculated to base its capability to change guaiacol to tetra guaiacol at 436 nm. The superoxide dismutase (SOD) activity was measured by considering the restriction of the depletion of P-nitro-blue tetrazolium chloride (NBT) (Shams et al., 2016).

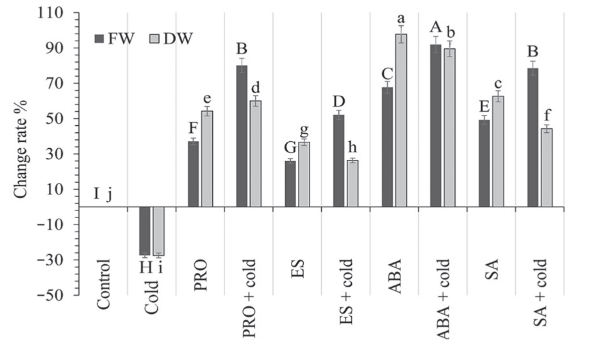
Nitrogen assimilation-related parameters. The activity of nitrate reductase (NR) was assayed in leaf extracts by measuring the production of nitrite. The production of 1 μmol h^{-1} of nitrite was represented as one unit of NR activity (Turk et al., 2020). The nitrite reductase (NiR) activity was also determined according to Choudhary and Agrawal (2014) method. The activity of glutamine synthetase (GS) was measured using the method of Kaya (2020). The production of 1 μmol min^{-1} of y-glutamyl hydroxamate was assumed as one unit of GS activity.

Statistical analysis. The experiment was conducted following a completely randomised design with three replications. The software package *SPSS Statistics*, version 20 (IBM Inc., USA) was used for the analysis of variance (ANOVA) and Duncan's multiple range test at 0.01 significance level.

Results

Fresh (FW) and dry (DW) weight. The effect of animal sex hormones, ABA and SA, on the FW and DW of common bean seedlings under cold stress is presented in Figure 1. Cold stress significantly decreased the FW and DW of the plants. However, application of ABA, SA, and sex hormones mitigated the adverse effect of cold stress in seedlings. In this regard, cold stress decreased the FW and DW of the seedlings by −27.37% and

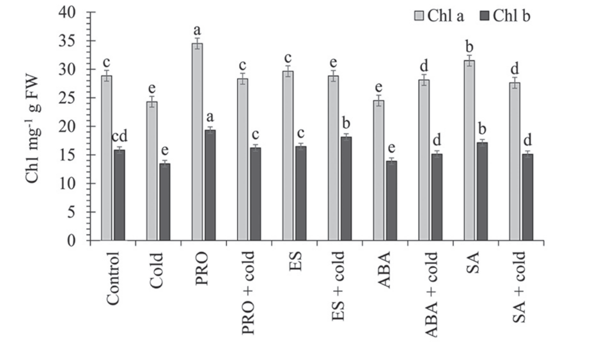
−27.48%, respectively, but the simultaneous application of Pro, ES, ABA, and SA with cold stress significantly increased FW by 80.01%, 52.09%, 91.88%, and 78.53% compared to the plants treated with cold stress; also, they increased DW by 60.00%, 26.31%, 89.47%, and 44.21%, respectively. Moreover, separate application of Pro, ES, ABA, and SA significantly increased the FW and DW of the seedlings compared to control.



Note. Letters above and below the bars indicate differences by Duncan multiple range test, $p < 0.01$ at each treatment; vertical bars indicate the mean ± standard error.

Figure 1. Effect of progesterone (Pro), β-oestradiol (ES), abscisic acid (ABA) and salicylic acid (SA) treatments on fold changes in fresh (FW) and dry (DW) weight of 14-day-old common bean seedlings under cold stress

Chlorophyll (Chl) content. As shown in Figure 2, cold stress significantly affected the Chl content in common bean seedlings, but the simultaneous application of Pro, ES, ABA, and SA with cold stress decreased the adverse effect of cold stress compared to the plants treated with cold alone. In this respect, cold stress decreased Chl *a* and *b* content by −15.77% and −15.03%, but the combined application of Pro, ES, ABA, and SA



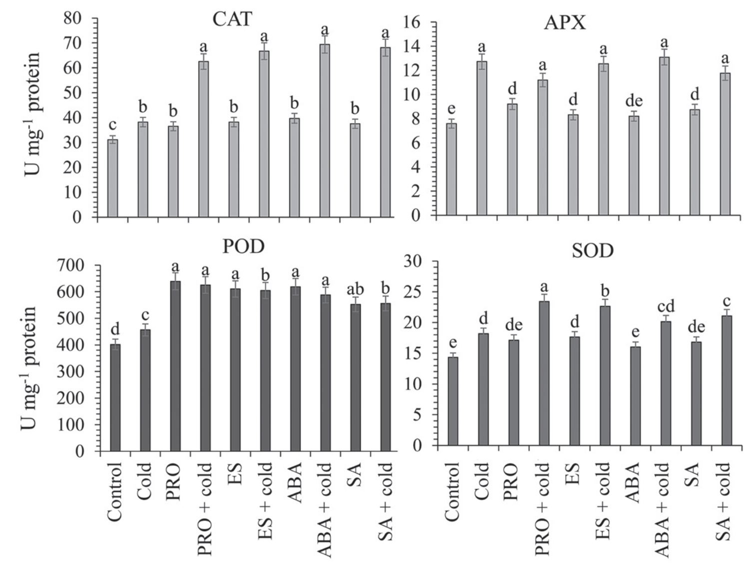
Note. Letters above the bars indicate differences by Duncan multiple range test, $p < 0.01$ at each treatment; vertical bars indicate the mean ± standard error.

Figure 2. Effect of progesterone (Pro), β-oestradiol (ES), abscisic acid (ABA), and salicylic acid (SA) treatments on the chlorophyll (Chl) content of 14-day-old common bean seedlings under cold stress

with cold stress increased Chl *b* content by 17.70%, 25.77%, 11.16%, and 11.04%, respectively, compared to the plants treated with cold stress. Regarding Chl *a* content, it was increased by 14.22%, 12.31%, 3.26%, and 4.21%, respectively. Furthermore, separate application of Pro and SA increased the Chl *a* and *b* content in seedlings compared to control, but ABA decreased their content.

Enzyme activity. Based on the results, SOD, catalase (CAT), and POD activities were significantly boosted by cold stress in common bean seedlings. However, the concomitant application of Pro, ES ABA, and SA with cold stress caused a re-increase in their activities compared to the plants treated with cold alone.

Regarding the APX activity, cold stress significantly increased its activity by 62.84%. As presented in Figure 3, under cold stress, the APX activity increased significantly in seedlings subjected to cold stress, but the combined treatment of Pro, ES, ABA, and SA with cold stress did not affect its activity. Also, their separate application had no significant effect. The POD activity was significantly affected by cold stress and phytohormones. In this respect, the CAT activity increased by 13.17% in cold-treated common bean seedlings. The CAT activity was impacted significantly by the simultaneous application of phytohormones with cold stress compared to the plants treated with cold alone. In this regard, Pro, ES, ABA, and SA application increased the CAT activity by 63.66%, 74.58%, 81.67%, and 78.32%, respectively, compared to the plants treated by cold alone. However, the combined effect of Pro, ES,

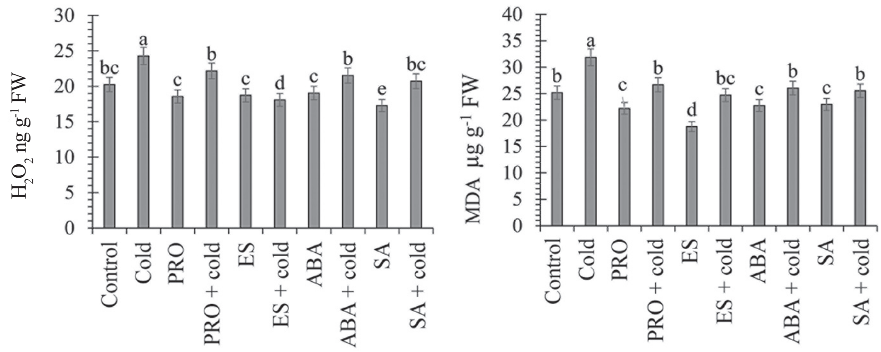


Explanation under Figure 2

Figure 3. Effect of progesterone (Pro), β-oestradiol (ES), abscisic acid (ABA), and salicylic acid (SA) treatments on the ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) activities of 14-day-old common bean seedlings under cold stress

ABA, and SA with cold stress significantly increased POD activity by 37.06%, 32.45%, 28.72%, and 21.71% compared to the plants exposed to cold alone. In this respect, by exposing the seedlings to cold stress, the SOD activity was increased by 26.95% compared to control, while the application of Pro, ES, ABA and SA on the cold-treated seedlings caused a re-increase in the SOD activity of 63.54%, 58.17%, 40.64%, and 47.06%, respectively, compared to the plants treated with cold alone.

Malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content. As presented in Figure 4, cold stress significantly increased the MDA and H_2O_2 content in common bean seedlings: an increase of 19.96% and 26.6% was identified in the MDA and H_2O_2 content, respectively, compared to control. However, the application of phytohormones significantly decreased their content in the plants treated with cold stress. In this regard, Pro, ES, ABA, and SA application on the plants treated with cold stress decreased the MDA content by −8.73%, −22.57%, −11.36%, and −14.66%, respectively, compared to the plants treated with cold alone. In the absence of cold stress, they also decreased the MDA content by −8.3%, −7.5%, −5.92%, and −14.62%, respectively, compared to the plants grown under control conditions.



Explanation under Figure 2

Figure 4. Effect of progesterone (Pro), β-oestradiol (ES), abscisic acid (ABA) and salicylic acid (SA) treatments on the malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content in 14-day old common bean seedlings under cold stress

In respect to the H_2O_2 content, cold stress significantly increased it by 26.60% compared to control. However, simultaneous application of Pro, ES, ABA, and SA with cold stress significantly decreased H_2O_2 content by −16.34%, −22.42%, −16.56%, and −20.54% compared to the plants treated with cold stress. Separate application of plant phytohormones also decreased the H_2O_2 content compared to control.

Nitrogen assimilation-related parameters. The activities of NR, NiR, GS, and cold stress-induced substantial declines in their activity are shown in the Table. The cold stress decreased the activities of NR, NiR, and GS by 41.03%, 35.20%, and 47.54%, respectively, compared to control. However, simultaneous application of Pro, ES, ABA, and SA with cold stress significantly mitigated the adverse effect of cold stress. In this respect, application of Pro on the plants treated with cold stress resulted in an increase of 41.78%, 32.99%, and 82.35% in NR, NiR and GS activities, respectively, in common bean seedlings.

Moreover, by application of ES on the plants treated with cold stress, an increase of 16.66%, 30.1%, and 50.46%, and regarding ABA application an increase of 33.33%, 12.75%, and 84.11%, and regarding SA application an increase of 66.66%, 33.12%, and 8.43% was observed in the NR, NiR and GS activities, respectively, in common bean seedlings treated with cold stress.

Table. Effect of progesterone, β-oestradiol, abscisic acid, and salicylic acid on nitrate reductase (NR), nitrite reductase (NiR), and glutamine synthetase (GS) activities in 14-day-old common bean seedlings under cold stress

Treatment	NR µmol NO_2 g^{-1} FW	NiR µmol NO_2 g^{-1} FW	GS µmol y-glutamyl hydroxamate min^{-1} mg^{-1} FW
Control	31.29 ± 0.35 a	24.55 ± 0.42a	204.63 ± 0.32 a
Cold stress	18.45 ± 0.31 f	15.91 ± 0.29 e	107.89 ± 0.28 f
Progesterone (Pro)	26.90 ± 0.28 c	18.94 ± 0.36 c	190.60 ± 0.26 b
β-estradiol (ES)	25.14 ± 0.19 d	21.85 ± 0.43 b	151.90 ± 0.25 d
Abscisic acid (ABA)	29.97 ± 0.38 b	20.69 ± 0.26 b	194.72 ± 0.35 b
Salicylic acid (SA)	26.46 ± 0.24 c	20.31 ± 0.15 b	197.74 ± 0.35 b
Pro + cold	26.16 ± 0.35 c	16.45 ± 0.21 de	196.74 ± 0.33 b
ES + cold	21.18 ± 0.25 e	20.84 ± 0.38 b	161.52 ± 0.29 a
ABA + cold	24.70 ± 0.18 d	17.94 ± 0.25 d	197.91 ± 0.36 b
SA + cold	30.41 ± 0.32 a	21.18 ± 0.43 b	116.99 ± 0.32 e

Note. FW – fresh weight; data in columns followed by different letters are significantly different ($p \leq 0.01$) by Duncan's multiple range test; values are means ± standard deviation.

Discussion

Cold stress is generally known for imposing a reduction in crop productivity, largely by extreme disturbances in plant growth, chlorophyll biosynthesis, photosynthesis and enzyme activities. In the present experiment, cold stress negatively impacted the overall performance of common bean seedlings. As identified during the experiment, a decrease in growth due to cold stress has clearly been shown in many plant species at various developmental stages (Esim, Atici, 2015; Turk et al., 2019; 2020). However, in this experiment, exogenous application of sexual hormones and ABA as well as SA significantly alleviated the adverse effect of cold stress by improving the growth and physiology of the plants. Results of our experiment have been consistent with the earlier studies on Pro, ES ABA and SA induced alleviation of the inhibitory impact of cold stress on plants (Gharib, Hegazi, 2010; Erdal, Genisel, 2016; Leng et al., 2021).

The enzyme activities, MDA and H_2O_2 content in plant tissues are essential markers to assess the adverse effect of cold stress on plants. In this experiment, cold stress markedly increased the MDA and H_2O_2 content, but the exogenous application of Pro, ES ABA and SA significantly decreased the MDA and H_2O_2 content in common bean seedlings treated by cold stress. The decrease in MDA and H_2O_2 content can be associated with the stimulation of enzymatic activity by spraying phytohormones on the plants; therefore, results of our experiment have been consistent with the earlier findings as a decrease in oxidative stress under colds stress by exogenous treatment of ABA and SA (Ignatenko et al., 2019; Yu et al., 2020). However, this is the first report on the effect of Pro and ES in reducing the adverse effect of cold stress in common bean seedlings. Nonetheless, by comparing the effect of hormones on cold tolerance, it was concluded that ES had a strong effect in reducing oxidative stress.

In higher plants, NR catalyses NO_3^- to NO_2^- and many environmental factors as well as endogenous factors such as metabolites and plant phytohormones can regulate NR activity. NiR catalyses NO_2^- to NH_4^+ and subsequently NH_4^+ is incorporated into carbon skeletons by rendering glutamate via the glutamine synthetase/glutamine oxoglutarate aminotransferase or glutamate synthase (Sharma, Shanker Dubey, 2005; Sanz-Luque et al., 2015). In this experiment, lower levels of NR, NiR and GS were observed in the cold-treated common bean seedlings. Results of our experiment have been consistent with the earlier findings as a reduction in the activity of nitrate assimilation enzymes (Aydin, Nalbantoğlu, 2011; Zhao et al., 2021). It seems that cold stress significantly affected the enzymes of the nitrate assimilation pathway, and this can be associated with a reduction in nitrate availability in plants under cold stress, which subsequently could inhibit NR and NiR gene transcription and decrease the mRNA stability of NR and NiR (Sharma, Shanker Dubey, 2005).

However, exogenous application of Pro, ES ABA and SA participated in an increase in NR, NiR and GS activities in common bean seedlings under cold stress. This can be related to the influence of phytohormones in improving cold tolerance in seedlings suggesting that those phytohormones can stimulate biochemical and physiological mechanisms to withstand hazardous effect of cold stress.

Conclusion

The findings of this experiment revealed that cold stress markedly decreased plant growth and enzymes activities and increased the malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content. However, exogenous application of progesterone (Pro), β-oestradiol (ES), abscisic acid (ABA) and salicylic acid (SA) enhanced tolerance of common bean seedlings to cold stress by increasing the growth, antioxidative enzyme activities, chlorophyll (Chl) content and activities of nitrate (NR) assimilation enzymes. Therefore, it can be concluded that mammalian sex hormones (Pro and ES) same as ABA and SA mitigated the adverse effect of cold stress in common bean seedlings, and their spraying on the leaves is beneficial for plant recovery and growth under cold stress. However, their effect on nitrate metabolism is unknown and needs to be studied further to find their significance in enhancing the NR and nitrite reductase (NiR) activities under cold stress.

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References

Araz O., Ekinci M., Yuce M., Shams M., Agar G., Yildirim E. 2021. Low-temperature modified DNA methylation level, genome template stability, enzyme activity, and proline content in pepper (*Capsicum annuum* L.) genotypes. Scientia Horticulturae, 294: 110761. https://doi.org/10.1016/j.scienta.2021.110761

Aydin B., Nalbantoğlu B. 2011. Effects of cold and salicylic acid treatments on nitrate reductase activity in spinach leaves. Turkish Journal of Biology, 35 (4): 443–448.

Azarfam S. P., Nadian Ghomsheh H., Moezzi A., Gholami A. 2021. The effect of different levels of silicon from a source of silicic acid on *Aloe vera* L. growth traits under cold stress. Iranian Journal of Soil and Water Research, 51 (12): 3103–3113. https://doi.org/10.22059/ijswr.2020.301196.668582

Bilska-Kos A., Panek P., Szulc-Głaz A., Ochodzki P., Cisło A., Zebrowski J. 2018. Chilling-induced physiological, anatomical and biochemical responses in the leaves of *Miscanthus* × *giganteus* and maize (*Zea mays* L.). Journal of Plant Physiology, 228: 178–188. https://doi.org/10.1016/j.jplph.2018.05.012

Boubakri H., Chihaoui S.-A., Najjar E., Gargouri M., Barhoumi F., Jebara M. 2021. Genome-wide analysis and expression profiling of H-type Trx family in *Phaseolus vulgaris* revealed distinctive isoforms associated with symbiotic N_2-fixing peformance and abiotic stress response. Journal of Plant Physiology, 260: 153410. https://doi.org/10.1016/j.jplph.2021.153410

Choudhary K. K., Agrawal S. B. 2014. Ultraviolet-B induced changes in morphological, physiological and biochemical parameters of two cultivars of pea (*Pisum sativum* L.). Ecotoxicology and Environmental Safety, 100: 178–187. https://doi.org/10.1016/j.ecoenv.2013.10.032

Dadasoglu E., Ekinci M., Kul R., Shams M., Turan M., Yildirim E. 2021. Nitric oxide enhances salt tolerance through regulating antioxidant enzyme activity and nutrient uptake in pea. Legume Research, 44 (1): 41–45. https://doi.org/10.18805/LR-540

Ding Y., Shi Y., Yang S. 2019. Advances and challenges in uncovering cold tolerance regulatory mechanisms in plants. New Phytologist, 222 (4): 1690–1704. https://doi.org/10.1111/nph.15696

Erdal S., Genisel M. 2016. The property of progesterone to mitigate cold stress in maize is linked to a modulation of the mitochondrial respiratory pathway. Theoretical and Experimental Plant Physiology, 28 (4): 385–393. https://doi.org/10.1007/s40626-016-0076-4

Esim N., Atici Ö. 2015. Effects of exogenous nitric oxide and salicylic acid on chilling-induced oxidative stress in wheat (*Triticum aestivum*). Frontiers in Life Science, 8 (2): 124–130. https://doi.org/10.1080/21553769.2014.998299

Genisel M., Turk H., Erdal S. 2013. Exogenous progesterone application protects chickpea seedlings against chilling-induced oxidative stress. Acta Physiologiae Plantarum, 35 (1): 241–251. https://doi.org/10.1007/s11738-012-1070-3

Ghanbari F., Sayyari M. 2018. Controlled drought stress affects the chilling-hardening capacity of tomato seedlings as indicated by changes in phenol metabolisms, antioxidant enzymes activity, osmolytes concentration and abscisic acid accumulation. Scientia Horticulturae, 229: 167–174. https://doi.org/10.1016/j.scienta.2017.10.009

Gharib F., Hegazi A. 2010. Salicylic acid ameliorates germination, seedling growth, phytohormone and enzymes activity in bean (*Phaseolus vulgaris* L.) under cold stress. Journal of American Science, 6 (10): 675–683.

He X., Liu T., Ren K., Chen J., Zhao G., Hu B., Xu A., Jin Y., Zhu Y., Zou C. 2020. Salicylic acid effects on flue-cured tobacco quality and curing characteristics during harvesting and curing in cold-stressed fields. Frontiers in Plant Science, 11: 580597. https://doi.org/10.3389/fpls.2020.580597

Huang X., Shi H., Hu Z., Liu A., Amombo E., Chen L., Fu J. 2017. ABA is involved in regulation of cold stress response in bermudagrass. Frontiers in Plant Science, 8: 1613. https://doi.org/10.3389/fpls.2017.01613

Ignatenko A., Talanova V., Repkina N., Titov A. 2019. Exogenous salicylic acid treatment induces cold tolerance in wheat through promotion of antioxidant enzyme activity and proline accumulation. Acta Physiologiae Plantarum, 41: 80. https://doi.org/10.1007/s11738-019-2872-3

Janská A., Maršík P., Zelenková S., Ovesná J. 2010. Cold stress and acclimation – what is important for metabolic adjustment? Plant Biology, 12 (3): 395–405. https://doi.org/10.1111/j.1438-8677.2009.00299.x

Kaya C. 2020. Nitrate reductase is required for salicylic acid-induced water stress tolerance of pepper by upraising the AsA-GSH pathway and glyoxalase system. Physiologia Plantarum, 172 (2): 351–370. https://doi.org/10.1111/ppl.13153

Kim Y.-H., Choi K.-I., Khan A. L., Waqas M., Lee I.-J. 2016. Exogenous application of abscisic acid regulates endogenous gibberellins homeostasis and enhances resistance of oriental melon (*Cucumis melo* var. L.) against low temperature. Scientia Horticulturae, 207: 41–47. https://doi.org/10.1016/j.scienta.2016.05.009

Lanna A. C., Silva R. A., Ferraresi T. M., Mendonça J. A., Costa Coelho G. R., Souza Moreira A., Ribeiro Valdisser P. A. M., Brondani C., Pereira Vianello R. 2018. Physiological characterization of common bean (*Phaseolus vulgaris* L.) under abiotic stresses for breeding purposes. Environmental Science and Pollution Research, 25: 31149–31164. https://doi.org/10.1007/s11356-018-3012-0

Leng Y., Li Y., Ma Y.-H., He L.-F., Li S.-W. 2021. Abscisic acid modulates differential physiological and biochemical responses of roots, stems, and leaves in mung bean seedlings to cadmium stress. Environmental Science and Pollution Research, 28: 6030–6043. https://doi.org/10.1007/s11356-020-10843-8

Liu C., Yang X., Yan Z., Fan Y., Feng G., Liu D. 2019. Analysis of differential gene expression in cold-tolerant vs. Cold-sensitive varieties of snap bean (*Phaseolus vulgaris* L.) in response to low temperature stress. Genes and Genomics, 41: 1445–1455. https://doi.org/10.1007/s13258-019-00870-2

Mutlu S., Atıcı Ö., Nalbantoğlu B., Mete E. 2016. Exogenous salicylic acid alleviates cold damage by regulating antioxidative system in two barley (*Hordeum vulgare* L.) cultivars. Frontiers in Life Science, 9 (2): 99–109. https://doi.org/10.1080/21553769.2015.1115430

Pasbani B., Salimi A., Aliasgharzad N., Hajiboland R. 2020. Colonization with arbuscular mycorrhizal fungi mitigates cold stress through improvement of antioxidant defense and accumulation of protecting molecules in eggplants. Scientia Horticulturae, 272: 109575. https://doi.org/10.1016/j.scienta.2020.109575

Saleem M., Fariduddin Q., Janda T. 2020. Multifaceted role of salicylic acid in combating cold stress in plants: a review. Journal of Plant Growth Regulation, 40: 464–485. https://doi.org/10.1007/s00344-020-10152-x

Sanz-Luque E., Chamizo-Ampudia A., Llamas A., Galvan A., Fernandez E. 2015. Understanding nitrate assimilation and its regulation in microalgae. Frontiers in Plant Science, 6: 899. https://doi.org/10.3389/fpls.2015.00899

Shams M., Yildirim E. 2021. Variations in response of *CaPAO* and *CaATG8c* genes, hormone, photosynthesis and antioxidative system in pepper genotypes under salinity stress. Scientia Horticulturae, 282: 110041. https://doi.org/10.1016/j.scienta.2021.110041

Shams M., Yildirim E., Ekinci M., Turan M., Dursun A., Parlakova F., Kul R. 2016. Exogenously applied glycine betaine regulates some chemical characteristics and antioxidative defence systems in lettuce under salt stress. Horticulture, Environment, and Biotechnology, 57: 225–231. https://doi.org/10.1007/s13580-016-0021-0

Shams M., Ekinci M., Ors S., Turan M., Agar G., Kul R., Yildirim E. 2019. Nitric oxide mitigates salt stress effects of pepper seedlings by altering nutrient uptake, enzyme activity and osmolyte accumulation. Physiology and Molecular Biology of Plants, 25: 1149–1161. https://doi.org/10.1007/s12298-019-00692-2

Shams M., Yildirim E., Arslan E., Agar G. 2020. Salinity induced alteration in DNA methylation pattern, enzyme activity, nutrient uptake and H_2O_2 content in pepper (*Capsicum annuum* L.) cultivars. Acta Physiologiae Plantarum, 42: 59. https://doi.org/10.1007/s11738-020-03053-9

Sharma K. C. 2000. Influence of integrated nutrient management on yield and economics in broccoli (*Brassica oleracea* L. var. *Italica*) plenck under cold temperate conditions. Vegetable Science, 27 (1): 62–63.

Sharma P., Shanker Dubey R. 2005. Modulation of nitrate reductase activity in rice seedlings under aluminium toxicity and water stress: role of osmolytes as enzyme protectant. Journal of Plant Physiology, 162 (8): 854–864. https://doi.org/10.1016/j.jplph.2004.09.011

Soliman M. H., Alayafi A. A., El Kelish A. A., Abu-Elsaoud A. M. 2018. Acetylsalicylic acid enhance tolerance of *Phaseolus vulgaris* L. to chilling stress, improving photosynthesis, antioxidants and expression of cold stress responsive genes. Botanical Studies, 59: 6. https://doi.org/10.1186/s40529-018-0222-1

Tekin N. B., Ceyhan E. 2020. Effects of cold stress on enzyme activities in peas. Turkish Journal of Agricultural and Natural Sciences, 7 (1): 170–177. https://doi.org/10.30910/turkjans.680055

Tiryaki D., Aydın İ., Atıcı Ö. 2019. Psychrotolerant bacteria isolated from the leaf apoplast of cold-adapted wild plants improve the cold resistance of bean (*Phaseolus vulgaris* L.) under low temperature. Cryobiology, 86: 111–119. https://doi.org/10.1016/j.cryobiol.2018.11.001

Turk H., Erdal S., Dumlupinar R. 2019. Exogenous carnitine application augments transport of fatty acids into mitochondria and stimulates mitochondrial respiration in maize seedlings grown under normal and cold conditions. Cryobiology, 91: 97–103. https://doi.org/10.1016/j.cryobiol.2019.10.003

Turk H., Erdal S., Dumlupinar R. 2020. Carnitine-induced physio-biochemical and molecular alterations in maize seedlings in response to cold stress. Archives of Agronomy and Soil Science, 66: 925–941. https://doi.org/10.1080/03650340.2019.1647336

Yu J., Cang J., Lu Q., Fan B., Xu Q., Li W., Wang X. 2020. ABA enhanced cold tolerance of wheat 'dn1' via increasing ROS scavenging system. Plant Signaling and Behavior, 15 (8): 1780403. https://doi.org/10.1080/15592324.2020.1780403

Zhao S., Zhang Y., Ou X., Wu C., Ma L., Yue X., Zhao Z. 2021. Comparison with Arabidopsis reveals optimal nitrogen allocation strategy and mechanism in *Chorispora bungeana*, a cryophyte with strong freezing tolerance. Journal of Plant Physiology, 256: 153311. https://doi.org/10.1016/j.jplph.2020.15331121

Progesteronu, β-estradioliu, abscizo ir salicilo rūgštimis apdoroti pupelių daigai yra atsparesni vėsioms temperatūroms

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Santrauka

Vėsios temperatūros (0–15° C) arba šaltis (<0° C) sukelia augalų įtampą, lėtina augimą ir mažina derlių. Tyrimo tikslas – nustatyti, kaip abscizo rūgštis (ABA) bei salicilo rūgštis (SA) ir žinduolių lytiniai hormonai (progesteronas ir β-estradiolis) veikia vėsiose temperatūrose auginamų paprastosios pupelės (*Phaseolus vulgaris* L.) daigų masę, chlorofilo, malondialdehido (MDA) bei vandenilio peroksido (H_2O_2) koncentracijas ir antioksidacinių bei nitratų asimiliacijos fermentų aktyvumą. Šalčio įtampa klimato kamerose buvo sukurta temperatūrą mažinant iki 9/5° C (diena/naktis) ir lyginant su įprastinėje temperatūroje 25/20° C (diena/naktis) augintais daigais. Rankiniu purkštuvu 11 dienų amžiaus daigai buvo apipurkšti progesteronu (Pro) 10^{-9} M, β-estradioliu (ES) 10^{-6} M, ABA 10^{-4} M ir SA 10^{-4} M. Vienuolikos dienų daigus išoriškai apdorojus Pro, ES, ABA ir SA, po trijų dienų vėsių temperatūrų nepalankus poveikis sumažėjo. Vėsi temperatūra nitratų reduktazės (NR), nitritų reduktazės (NiR) ir glutamino sintezės (GS) aktyvumą sumažino atitinkamai 41,03, 35,20 ir 47,54 %. Tačiau vėsiose temperatūrose augintų daigų apdorojimas Pro, ES, ABA ir SA reikšmingai padidino NR, NiR ir GS aktyvumą: NR – 41,87, 14,80, 33,87 ir 64,82 %, NiR – 3,4, 30,10, 12,75 ir 33,12 %, GS – 83,00, 50,10, 83,14 ir 9,34 %, lyginant su nepurkštais vėsioje temperatūroje augintais daigais. Taigi, daigų purškimas Pro 10^{-9} M, ES 10^{-6} M, ABA 10^{-4} M ir SA 10^{-4} M tirpalais po trijų dienų sušvelnina nepalankų vėsių temperatūrų poveikį pupelių daigams, nes pagerėja nitratų apykaita ir antioksidacinių fermentų aktyvumas.

Reikšminiai žodžiai: šalčio stresas, fermentų aktyvumas, nitratų apykaita, *Phaseolus vulgaris*.