The Quarterly Scientific Journal of Applied Biology

Vol.36, No.2, Sering. 76, Summer 2024, p. 14-16.

Journal homepage: https://jab.alzahra.ac.ir

10.22051/jab.2022.40942.1497

Effect of freezing stress on some physiological and enzymatic responses of Ornamental plant, Viola (*Viola* × *wittrockiana*) and Snapdragon (*Antirrhinum majus*)

Behrooz Salehi-Eskandari [1*], Zohre Nasirian Jazi[1], Jalil Abbaspour[2]

[1*]Department of Biology, Payame Noor University, Tehran, Iran

[2]Graduated from PhD in Plant Physiology, University of Isfahan

E-mail of the corresponding author:[*]Behsalehi@pnu.ac.ir

Accepted: 2023.02.12 **Received: 2022.08.19**

Introduction

Plants can experience cold abiotic stress when they are exposed to low temperatures for a long time. Freezing stress is very deleterious to plants, especially when plants are in the growth phase. (Nievola *et al.,* 2017; Hajihashemi *et al.,* 2020). Cold stress causes altered varus morphological, cellular, physiological and other biochemical processes of plants, which results in reduced growth and crops (Yadav, 2010).

Cold stress, including chilling (0–15°C) and freezing (< 0°C), is an abiotic stress that adversely affects the growth and agricultural productivity of plants (Guo *et al*., 2018). Chilling stress usually restricts plant growth and development. Chilling stress affects membrane stability in plant cells, (Orvar *et al*., 2000) and disturbs the stability of proteins or protein complexes and reduces the activities of enzymes such as ROS scavenging enzymes. which results in photo-inhibition and impaired photosynthesis (Siddiqui and Cavicchioli, 2006; Ruelland *et al.,* 2009). Cold stress will affect the synthesis of proteins by affecting gene expression (Ding et al. 2019).

The growing of ornamental flowers of the Viola (*Viola* × *wittrockiana*) and Snapdragon (*Antirrhinum majus***)** in the mountainous city of Fereydounshahr at the end of November prompted us to investigate the resistance mechanism to freezing stress in these plants. This research leads to a better understanding of the tolerance to freezing of these plants after their adaptation to cold and finding the difference between the two species by evaluating growth changes and measuring some antioxidant enzymes at different temperatures.

Material and methods

After 50 days, plants growing in plastic pots were placed in the outdoor environment of the greenhouse (Isfahan City) with a temperature of 8.4 ± 2.6 °C to adapt to the cold stress (at the end of December) and finally, the 70-day-old seedlings were planted in early January. For 15 days in three different places, including the environment of the greenhouse (25 ± 5 °C), the farm near the greenhouse (Isfahan City with an altitude of 1550 m) and the farm located in Fereydoun Shahr (with an altitude of 2490 m) were placed the average day and night

temperature in these three places was 25±5, 5.6±5.9 and 0.7±4.5 °C, respectively, and the minimum temperature recorded for these places during this period was 20, -3 and -11 °C.

After harvesting, plants were separated to root and shoot and the length of the stem was determined (cm) for calculating plant growth. Pigments were extracted in 80% (v/v) acetone according to the method of Arnon (1949). Total phenolic contents and Water-Soluble Content (WSC) were determined according to the method of Velioglu et al. (1998) and Dubois et al., respectively. H_2O_2 was assessed using the method of Sergiev et al. (1997). The homogenate supernatant of leaf samples in potassium phosphate buffer (pH 7.8) is used to evaluate the antioxidant enzyme activity. Catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (POD) activities were determined using the methods of Aebi (1984), Asada (1987) and Plewa et al. (1991), respectively.

Results and Discussion

The results showed that the length stem of both plants significantly decreased in low temperatures (-3, -11°C) in comparison with the control group (20 °C). Low temperatures reduced the growth of root and stem by inhibiting cell growth (Pouramir-Dashtmian et al., 2014). In order to reach the cold acclimation required, plants inhibited growth during the cold acclimation period with frost. The distance between nodes is reduced, then the leaves become rosettes such as winter varieties of crop plants (Rashed Mohassel et al. 2009). The chlorophyll a/b ratio increased in Snapdragon at -3°C. However, it decreased in Viola at -3, -11°C. The reduction in the ratio may indicate which chlorophyll a is more sensitive to frost in the Viola, while the increase in the ratio probably shows photoinhibition photosystem I (PSI) to freezing stress.

Total phenolic content increased with decreasing temperature in both species. Similar observations have been reported for *Haberlea rhodopensis* (Georgieva et al., 2021). Then these compounds are added to the cell wall as suberin or lignin which enhanced the resistance of plants versus low temperatures (Chalker-Scott and Fuchigami, 2018).

Freezing temperatures (-3, -11°C) induced a significant increase in soluble carbohydrates in the two plants. Soluble sugars contribute to the osmotic adjustment in abiotic stress, which related to water deficiency such as freezing stress. Soluble sugars contribute to osmolytes, signaling molecules, carbon skeletons and energy reserves in plant tissues in winter (Couée et al., 2006; Guy et al., 2008).

In summary, the results of this study suggest that freezing stress causes H_2O_2 accumulation in both plants with the highest H_2O_2 was observed in the lowest temperature treatment (-11°C). It has also been suggested that hydrogen peroxide has a double effect because in low concentrations it acts as to induce the synthesis of ROS inhibiting enzymes, and on the other hand, it in high concentrations can harm cellular components (Quan et al., 2008). Then the H_2O_2 accumulation at freezing temperatures due to the increase in the production of ROS, which is more than the antioxidant capacity of the plants

Catalase activity significantly increased in both plants under freezing temperatures, while the increase in ascorbate peroxidase activity was significant only in Snapdragon at -11 °C. The comparison of catalase activity among different genotypes of a species has confirmed the role of this enzyme in creating cold resistance in resistant genotypes. The comparison of catalase activity among different genotypes of a species has confirmed the role of this enzyme in creating tolerance cold in resistant genotypes (Janda *et al.*, 2007).

Conclusion

The growth is reduced under freezing stress in both plants, but the chlorophyll a/b ratio was different at -3°C. A similar increasing elevation pattern in the content of phenolic compounds and soluble carbohydrates was shown with decreasing temperatures in both plants. The phenolic compounds are antioxidant and reduce growth with the lignification cell wall. Accumulation of sugars helps maintain turgor. The highest H_2O_2 concentration was observed at -11°C treatment, which showed the creation of ROS is more than the antioxidant capacity of both plants. The stress-reducing antioxidant enzyme in both plants is catalase and the activity of APX dependent was variable.

Keywords: *Antioxidants, Freezing stress, Ornamental plants, Osmoticum*

Acknowledgment: The current research has been financially supported by Esfahan Payame Noor University.

Declaration of conflict of interest: The authors declare that they have no conflicts of interest.

Statement on ethics:

The authors declare that this work has not been published elsewhere and has not been submitted to another publication at the same time.