IJPSR (2025), Volume 16, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 22 October 2024; received in revised form, 06 November 2024; accepted, 14 November 2024; published 01 March 2025

MODULATION OF ANTIOXIDANT ENZYME ACTIVITY IN *VIGNA RADIATA* BY POLYETHYLENE GLYCOL (PEG): INSIGHTS INTO CELLULAR DEFENSE MECHANISM

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Keywords:

PEG, Antioxidant enzymes, Vigna, proline, Polyphenol oxidase, Peroxidase, Water stress

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ABSTRACT: The physiological and biochemical changes in plants are affected by water deficiency caused by polyethylene glycol (PEG). It is a synthetic, water-soluble polymer and induces water deficit conditions. Its application is useful to induce drought stress in different plant varieties. The purpose of this study is to understand the effects of different concentrations of polyethylene glycol on the antioxidant system of *Vigna radiata* seeds. The polyphenol oxidase, peroxidase and proline content increased with the increment of water stress induced by polyethylene glycol. Enhanced activities of antioxidants could either be a response to the cellular damage induced by the stressors or a mechanism towards the damages. Obtained results of this research indicated that the activities of PPO, POD and proline had significant increase in the plants under the treatment of PEG.

INTRODUCTION: Abiotic stress is one of the most important environmental issues that reduce growth, development and yields for most major crop plants. Drought is a major abiotic environmental stress, which affects almost every aspect of plant life and significantly reduces crop yield in affected areas. Polyethylene glycol, widely used to induce water stress in plants, is a synthetic and water-soluble polymer which is not expected to penetrate into cells [1]. PEG caused a decrease in cell water potential, germination percentage, seedling vigour in crop plants [2]. PEG is considered as abiotic stress inducer and an osmotic agent in various studies. It is widely used in food, cosmetics and pharma industry. In recent years the role of PEG has been noticed in agriculture, especially in seedling development.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.16(3).786-90

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.16(3).786-90

Therefore, in drought stress investigation PEG treatment in plants is considered as an effective model [3-6]. When plants are subjected to stress conditions, the production of activated oxygen species occurs. Chloroplasts, mitochondria and peroxisomes are important intracellular generators of ROS (reactive oxygen species) responsible for causing stress induced cellular damages [7]. In order to minimize the damaging effects of ROS, the living organisms developed non-enzymatic defense systems and enzymatic protection mechanisms. In response to ROS, activity of antioxidant enzymes increases. Peroxidases decompose H_2O_2 by oxidation of co-substrates such as phenolic compounds or antioxidants.

It is a ubiquitous enzyme and catalyzes peroxide reduction and generates reactive oxygen species. It is one of the important enzymes in the enzymatic defense system [8]. Peroxidases find applications in bioremediation, textile synthetic dyes decolorization, polymer synthesis, paper pulp industry in development of biosensor diagnosis kits *etc* [9]. Polyphenol oxidase is an enzyme which

oxidizes some phenol to chinone. This enzyme is related to wounding, enzymatic browning and biosynthesis of alkaloids under biotic and abiotic stress conditions [10]. A better understanding of plant response under water stress may help in programs whose objective is to improve the drought tolerance of crops. During the duration of this stress active solute accumulation of compatible solutes such as proline and the activities of POD and PPO enzymes are claimed to be an effective stress tolerance mechanism. Therefore, the aim of this study was to evaluate the effects of PEG on antioxidant enzyme activity and proline of *Vigna radiata* under water stress conditions.

MATERIALS AND METHODS: Healthy Vigna seeds of uniform size were selected. Seeds were surface sterilized with teepol. The experiment was conducted in laboratory conditions. Three different concentrations (2%, 4% and 6%) of PEG solutions were prepared. The seeds were then soaked separately in solutions for 24 hours. The control group was soaked in double distilled water. Seeds of various treatment groups were placed in petri dishes on moistened cotton. POD and PPO activities were measured by Karo and Mishra [11] method with minor modifications. Proline activity measured as explained by Bates *et al*.[12].

RESULT AND DISCUSSION:

TABLE 1: ENZYME ACTIVITY IN DIFFERENT SAMPLES

S. no.	Concentration of PEG	Peroxidase (mol/min/gm)	PPO (mol/min/gm)	Proline (µM/gm)
1	Control	0.032	0.016	2.08
2	2%	0.064	0.468	5.19
3	4%	0.128	0.476	6.06
4	6%	0.160	0.528	6.75

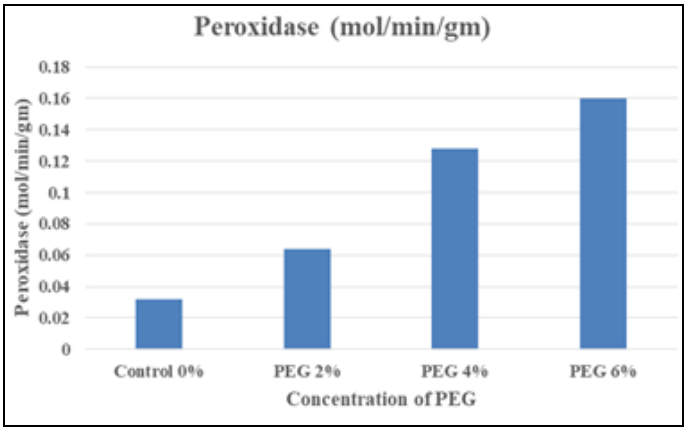

FIG. 1: EFFECT OF PEG ON PEROXIDASE

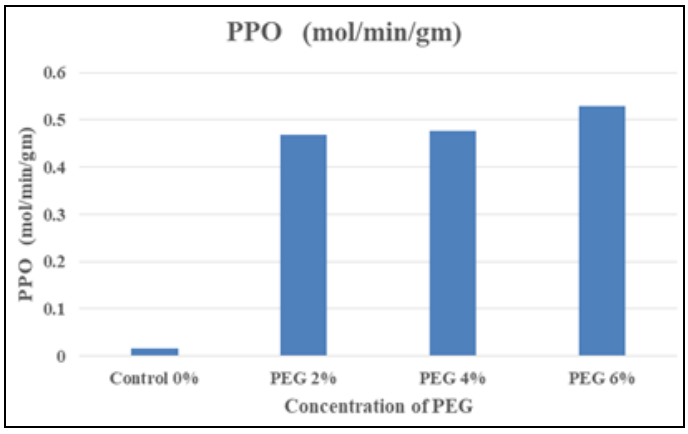

FIG. 2: EFFECT OF PEG ON POLYPHENOL OXIDASE

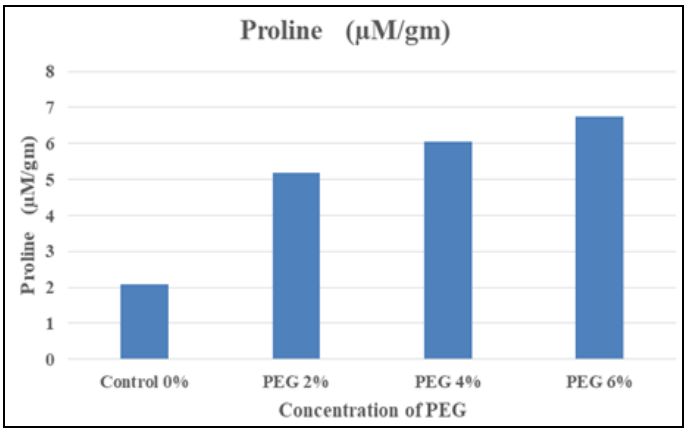

FIG. 3: EFFECT OF PEG ON PROLINE

Peroxidase: In the present investigation, the highest peroxidase activity is recorded in twigs treated with 6% of polyethylene glycol concentration and lowest peroxidase activity

occurred in twigs treated with 0.4% of polyethylene glycol concentration **Fig. 1**. The results so obtained are in correlation with the similar findings of work done by Hajihashemi and Sofo [13] in *Stevia rebaudiana*, Liang and Yang [14] in Olive, Hossain *et al* [15] in Tartary Buckwheat.

The activities of antioxidants enzyme peroxidase increased progressively with increase of heavy metal concentration, which may represent a secondary defensive mechanism against oxidative stress and with increasing peroxidase hydrogen peroxideis utilized [16-17]. This increase may occur owing to cellular damage brought about by stressors or mechanisms to control the damage resulting due to lipid peroxidation and increased peroxidise [18].

Polyphenol Oxidase (PPO): In this study, the highest PPO occurred in twigs treated with 6% of polyethylene glycol concentration and the lowest PPO content occurred in twigs treated with 2% of polyethylene glycol concentration **Fig. 2**. Similar results have been obtained by Hajihashemi and Sofo [13] in *Stevia rebaudiana*, Sharma *et al.* [19] in barley, Kakar *et al.* [20] in *Zea mays* and Hossain *et al.* [15] in Tartary Buckwheat.

Many enzymatic protection mechanisms (superoxide dismutase, catalase, and peroxidases) and non-enzymatic defence systems (ascorbic acid, reduced glutathione, tocopherols etc.) have beendeveloped by the living organisms in order to minimize the detrimental effects of ROS. H_2O_2 is metabolized into water and oxygen by catalase and peroxidise [20]. The level of reactive compounds can be kept under permanent control by the effective regulation of these protective enzymes [21].

Proline: In this study it was found that the highest proline content occurred in the twigs treated with 6% of polyethylene glycol concentration and lowest proline content occurred in twigs treated with 2% polyethylene glycol. The proline content is shown to increase with increasing concentration of polyethylene glycol **Fig. 3**. Similar results have been shown in works of Kisk *et al.*[22] on Grand Nain banana plants, Bansod *et al.* [23] on *Trigonellafoenum-graecum* L. and Dharshini *et al.* [24] in greengram. Proline is known to act as a compatible osmolyte and its enhanced production confirms osmo-tolerance in plants, which protects plants from excessive stress and can function either as an osmoregulator at the cellular level or as a protector of certain enzymes, so as to stabilize the structure of macromolecules and organelles [25]. According to Abdel Latef [26] plants develop self defence mechanism by producing antioxidant enzymes and a continued increase in these enzyme activities indicates detoxification of hydrogen peroxide under stress condition. The synthesis of proline in larger quantities under heavy metal stress could be an adaptive mechanism to regulate NAD^+ to NADH $^+H^+$ ratio, as the level of NADH (H+) increases due to suppression in mitochondrial electron transport [27]. The increase inprolinecontent supposedly plays important role in osmoregulation, protecting enzyme denaturation and scavenging the increased peroxide content.

ACKNOWLEDGEMENT: The authors are grateful to the DST CURIE LAB (Reference No. DST/CURIE-PG/2022/39 (G)).

CONFLICTS OF INTEREST: Authors declared no conflict of interest.

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How to cite this article:
Srivastava R, Rathore A, Kumawat S and Chauhan S: Modulation of antioxidant enzyme activity in *Vigna radiata* by polyethylene glycol (PEG): insights into cellular defense mechanism. Int J Pharm Sci & Res 2025; 16(3): 786-90. doi: 10.13040/IJPSR.0975-8232.16(3).786-90.