International Journal of Scientific Research in Biological Sciences
Vol.**11**, Issue.**4**, pp.**25-29**, August **2024**
E-ISSN: 2347-7520
Available online at: www.isroset.org

Research Article

Antioxidant Enzyme Activities Estimation In *vicia faba* Leaves

Zahraa A.N. Al-Yassiry[1*]

[1]Dept. of biology /Faculty of science, University of Babylon, Babel, Iraq

**Corresponding Author: zahraa.isam@science.uoqasim.edu.iq*

Received: 29/Jun/2024; **Accepted**: 31/Jul/2024; **Published**: 31/Aug/2024

Abstract— Plant cells have the inherent ability to produce reactive oxygen species and oxygen radicals as secondary products during various physiological processes associated with abiotic stress. The disruption of the equilibrium between the generation of reactive oxygen species and the defense systems against oxidative harm is a significant consequence of abiotic stress in plants. As a consequence, the accumulation of reactive oxygen species occurs, hence triggering the onset of oxidative stress. Jasmonates represent a class of plant hormones that exert significant regulatory influence over several physiological processes. These processes include gene and metabolic regulation, immune mechanisms, responses to stress, reproductive activities, and interaction between cells, among other roles. The presence of oxidative stress induces the synthesis of metabolites possessing antioxidant characteristics and augments the efficacy of antioxidant enzymes, hence conferring a safeguarding impact on plant tissues. The aim of this study was to investigate the effects of methyl jasmonate (JAME) administration on reactive oxygen species and the activities of H2O2 scavenging enzymes, namely superoxide dismutase, catalase and glutathione in vicia faba leaves. After implementing a therapy strategy with JAME, there was an elevation in the activity levels of the enzyme superoxide dismutase , catalase , and glutathione.

Keywords— MJ, OVG, *vicia faba , SOD, Catalase and* glutathione

1. Introduction

Plants, like mammals, face a variety of challenges, including the regulation of metabolic processes and reproductive functions, as well as the necessity to effectively respond to possible dangers. Plants exhibit adaptive responses to diverse environmental stressors, including but not limited to high temperatures, water scarcity, high salt levels, exposure to UV radiation or ozone, and infections caused by pathogenic organisms. The responses outlined above involve the activation of antioxidant defense enzymes, which play a crucial role in mitigating subsequent damage to the plants [1-3].

The functional component of the methyl chemical molecule is its methyl group. Gasmons, as fatty derivatives, play an important function as a reference to molecules in mediation in a wide range of plant responses to different types of stress. The influence of cellular organizations on a variety of developmental processes has been widely demonstrated in scientific literature. These activities include germination, root growth, leaf activity, gravity erosion, fertility, embryo development, sex identification, fruit maturity and fruit reduction [4].

Moreover, it is well recognized that gas plants play an important role in regulating the plant's evolution, evolution and responses to biological and non-biological pressures. Moreover, it has been well established in scientific research that Gazmons has the potential to promote defensive reactions of plants When faced with herbivores, nematodes and pathogens [4]. According to Korkmaz, [5], gasmons play an important role in the delivery of many important plant hormones, including oxin, gerrylic acid and salicylic acid. It is generally assumed that this association affects the evolution of the plant by altering many signal systems[6] .

Antioxidant enzymes include SOD and CAT. SOD converts superoxide roots into hydrogen peroxide and dioxygen through a process known as asymmetry, while CAT removes hydrogen peroxide. The three categories of superoxide dismutase are determined by the specific metal auxiliary factor found in its active location, which is manganese, iron, copper, and zinc. Prescribed enzymes are of great importance due to their crucial role in protecting against oxidative stress. Catalases are protein complexes with enzymatic properties, comprising four identical subunits known as tetrameric homoproteins [7] .

The process of catalase complex production is facilitated by several isozymes, which are encoded by genes situated within the cellular nucleus. The majority of these entities are mostly situated within peroxisomes and glyoxisomes [8]. When comparing peroxidase to catalase, it is evident that catalase has a significant degree of efficacy in the removal of hydrogen peroxide and does not rely on a reducing substrate for its catalytic activity. Catalase isozymes exhibit variations in their biochemical characteristics and specialized development, maybe associated with the germination process. However, it is likely that their primary role involves the enzymatic conversion of fatty acids. Moreover, Kumari et al. [9] have asserted that some isozymes have a role in the phenomena of lignification, photorespiration, and the aging process. The utilization of glutathione as a reliable indicator of oxidative stress in plants is a common practice, despite its involvement in various aspects of plant metabolism [10].

The sequestration of xenobiotics and heavy metals, as well as its vital function as a constituent of the cellular antioxidative defense mechanism, renders glutathione highly crucial. The cellular defense system, as elucidated by Noctor and Foyer in their 1998 study, is responsible for the regulation of reactive oxygen species within the cellular environment. The significant impact of ant oxidative defense systems and redox processes on the adaptation of plants to their environment cannot be overstated. Glutathione has been recognized as a potentially effective stress marker in light of its suitability within this specific context [11,13].

The objective of this study was to investigate the correlation between the buildup of reactive oxygen species and the performance of antioxidant enzymes in vicia faba plant seeds subsequent to the application of methyl jasmonate and olvera gel (OVG).

2. Materials And Methods

2.1 Enzyme extraction

In order to isolate the enzyme from the plant sample, a 1 gram portion of the plant sample was finely ground using a manual pestle and mortar. The experiment was conducted using a solution consisting of 10 milliliters of phosphate-buffered saline (PBS) and 0.3 grams of polyvinylpyrrolidone (PVP). The temperature was meticulously regulated through the utilization of ice. The sample underwent centrifuged at a rate of 10,000 revolutions per minute for a period of 15 minutes, keeping an internal temperature of four Celsius. The aqueous component of the solution was collected and designated as the crude enzyme extract[14] .

2.2 CAT Activity Determination

The assessment of CAT activity was carried out utilizing the methods described by Misra and Gupta (15)

2.3 SOD activity determination

the evaluation of superoxide dismutase activity was conducted by assessing its capacity to hinder pyragallol oxidation, following the protocols outlined by Marklund in [16]. The empty specimen was exclusively prepared utilizing distilled water, while the estimation of efficiency was conducted employing the algorithm outlined by Frary et al. ([17].

SOD activity (units) =

2.4 Glutathione Content Determination

The Tietze [18] method was employed to determine the concentration of glutathione.

3. Results

The findings of the present study reveal significant disparities among the different therapeutic approaches, as seen by the data provided in Table 4.

The research conducted in this study revealed a significant rise in the concentrations of various antioxidants, such as SOD, CAT, and glutathione, among all the experimental groups as compared to the control group (p>0.05). Reactive oxygen species are frequently generated as a byproduct of metabolic processes taking place within chloroplasts, mitochondria, and peroxisomes in plants. However, it is important to note that plants have the ability to experience oxidative stress when they are subjected to specific stress circumstances, as previously mentioned.

The application of methyl jasmonate treatment induces the production of secondary metabolites in plant cell cultures through a wide range of regulatory mechanisms. The implementation of this therapeutic intervention leads to the activation of crucial genes, which in turn enhances cellular functioning at the biochemical and molecular levels. This process is facilitated by the participation of signaling molecules [19].

Table (1) : Antioxidant response in leaves of Fenugreek plants after seed treatment with biotic and abiotic agents. Data are means ± standard errors of four measurements.

Treatment	SOD (U/ml)	Catalase (Ku/l)	glutathione (Ku/l)
Control	7.33±0.88	22.33±1.20	0.83±0.12
MeJA	8.33±0.66	23.50±0.66	0.83±0.16
AVG	36.66±16.33	50.00±5.77	1.66±0.33
AVG+ MeJA	52.33±16.34	68.33±16.54	1.76±0.58
P= value	0.003	0.0001	0.032
LSD	**4.47**	**9.39**	1.36

4. Discussion

The present investigation documented a significant elevation in the concentrations of antioxidants, namely superoxide dismutase , catalase and glutathione, throughout all experimental cohorts in comparison to the control cohort (p>0.05). Reactive oxygen species are frequently generated endogenously within plant cells due to metabolic processes occurring within the chloroplasts, mitochondria, and peroxisomes. However, in specific stress-induced conditions, as previously explained, the plant may experience oxidative stress. It is crucial to recognize that plants can also undergo an oxidative stress response due to their exposure to heavy metals [20].

The involvement of methyl jasmonate in plant physiology has garnered significant interest, leading to extensive discussions on its diverse modes of action [21]. The objective of this study was to assess the efficacy of externally administered JAME on vicia faba leaves in mitigating stress-induced reactions associated with antioxidative enzymes and ROS.

It has been observed that low levels of reactive oxygen exercise regulatory control over a wide range of cellular processes. However, the excessive concentration of reactive oxygen types within the intracellular environment may have adverse and harmful effects. Thus, cells should strictly regulate reactive oxygen levels to avoid oxidative damage rather than completely eliminate it [22]. The use of external methyl gasmons in laboratory cells has been found as a new means of causing the accumulation of excessive secondary metabolites. Previous research indicated that the use of MJ in laboratory cultures enhanced the activity of antioxidant enzymes, regulated genes associated with defence mechanisms, and raised the synthesis of secondary metabolites [23].

Recently, there has been a significant increase in the use of MJ in scientific research including laboratory culture procedures such as cell suspension, accidental root, hairy root, and multiple buds transplant systems. Many of the research provided empirical data to support its conclusions about the usefulness of antioxidant enzymes. The potential use of induced treatment may have resulted in the generation of reactive oxygen types (ROS) as a result of stress induction. In response to the presence of reactive oxygen species, plant tissue activates antioxidant enzymes, increasing the synthesis of secondary metabolites [24]. Previous study revealed that external control of methyl plant gases has the potential to enhance their antioxidant capabilities, thus helping to reduce reactions [25-30].

Reactive Oxygen (ROS) provides defense against oxidative stress [31]. However, when methyl gasmons were applied in isolation from 0.05 micrometres, there was no statistically significant increase in these enzyme activities when tested under non-stress control conditions. Lack of progress can be linked to MeJA's reliance on free stress signals that activate and promote antioxidant enzyme activities, as noted by Huang et al. (32). Previous research has shown that increasing MeJA levels (2-3 mm) can harm plants, reducing growth and drought tolerance in wheat seedlings [33]. Furthermore, prior research has revealed that methyl gasmons (MeJA) have the capacity to increase the generation of reactive oxygen species inside photosynthetic tissue, hence exacerbating oxidative damage in the presence of asmotic stress.

(Myura and Tada, [34]. Study by Corkmaz et al. [35] revealed that the application of SA at doses ranging from 0.1 to 1 mm in melon seedlings has led to an increase in drought tolerance, and may offer significant protective advantages in mitigating the adverse consequences associated with high levels of reactive oxygen observed immediately after exposure. To Jami. Inspection of plant defense signals often requires the use of typical plants. However, different plant species are likely to show distinctive characteristics in their reactions. Hence, the importance of investigating plant defence signals, as highlighted by Soares et al., [36]

The research undertaken by Fracasso et al. [37] has provided evidence for the substantial role of jasmonic acids (JA) in the communication of antioxidant responses triggered by water stress, particularly through affecting ascorbate metabolism. According to the findings of Nafie et al. [38], the exogenous administration of jasmonic acid (JA) has exhibited notable effectiveness in mitigating the Oxidative stress induced by drought in plants. This objective is achieved through the augmentation of the efficacy of antioxidant enzymes. The application of methyl jasmonate (MeJA) has been observed to augment the capacity for reactive oxygen species scavenging through the promotion of secondary metabolite synthesis. Furthermore, a study conducted by Farooq et al. [39] has demonstrated that this specific chemical elicits a response in the antioxidant defense system, leading to a decrease in arsenic concentrations [40-41]. .

5. Conclusion and future scope

The findings of this study indicate a notable increase in the levels of antioxidants, including (SOD), catalase (CAT), and glutathione, across all of the experiment groups as compared to the control group ($p>0.05$).

Conflict of interest statement
The author declares that she has no conflict of interest in this work.

Data Availability
None.

Funding source
None.

Authors' Contributions
Zahraa A.N. Al-Yassiry conceived the idea and wrote the original manuscript draft, and the author reviewed and edited the final version.

ACKNOWLEDGEMENTS
The Department of Biology, College of Science, University of Babylon , is thanked by the authors for their assistance and for providing the facilities required for this work.

References

[1] Rella Berni *et al.*, “Reactive oxygen species and heavy metal stress in plants: Impact on the cell wall and secondary metabolism,” *Environmental and Experimental Botany*, vol. 161, pp. 98–106, May 2019. doi:10.1016/j.envexpbot.2018.10.017

[2] Uazam Azeem and Khalid Hakeem, *Jasmonates and plant defense*, Jul. 2024. doi:10.1201/9781003454243

[3] Mella Farooq *et al.*, "Methyl jasmonate regulates antioxidant defense and suppresses arsenic uptake in brassica napus L.," *Frontiers in Plant Science*, vol. 7, Apr. 2016. doi:10.3389/fpls.2016.00468

[4] Alessandra Fracasso, Lusia Trindade, and Stephani Amaducci, "Drought tolerance strategies highlighted by two sorghum bicolor races in a dry-down experiment," *Journal of Plant Physiology*, vol. 190, pp. 1–14, Jan. 2016. doi:10.1016/j.jplph.2015.10.009

[5] Anna Frary *et al.*, "Salt tolerance in solanum pennellii: Antioxidant response and related QTL," *BMC Plant Biology*, vol. 10, no. 1, Apr. 2010. doi:10.1186/1471-2229-10-58

[6] "Significance of glutathione to plant adaptation to the environment," *Plant Ecophysiology*, 2001. doi:10.1007/0-306-47644-4

[7] Zahd Hossain, Mella López-Climent, Vella Arbona, Rellq Pérez-Clemente, and Alla Gómez-Cadenas, "Modulation of the antioxidant system in citrus under waterlogging and subsequent drainage," *Journal of Plant Physiology*, vol. 166, no. 13, pp. 1391–1404, Sep. 2009. doi:10.1016/j.jplph.2009.02.012

[8] Hera Huang, Beren Liu, Lilia Liu, and Sella Song, "Jasmonate action in plant growth and development," *Journal of Experimental Botany*, vol. 68, no. 6, pp. 1349–1359, Feb. 2017. doi:10.1093/jxb/erw495

[9] Guzhoon Kang *et al.*, "Proteomics reveals the effects of salicylic acid on growth and tolerance to subsequent drought stress in wheat," *Journal of Proteome Research*, vol. 11, no. 12, pp. 6066–6079, Nov. 2012. doi:10.1021/pr300728y

[10] Ahmed Korkmaz, Murt Uzunlu, and Ali Demirkiran, "Treatment with acetyl salicylic acid protects muskmelon seedlings against drought stress," *Acta Physiologiae Plantarum*, vol. 29, no. 6, pp. 503–508, May 2007. doi:10.1007/s11738-007-0060-3

[11] Gellan Kumari *et al.*, "Jasmonic acid induced changes in protein pattern, antioxidative enzyme activities and peroxidase isozymes in peanut seedlings," *Biologia plantarum*, vol. 50, no. 2, pp. 219–226, Jun. 2006. doi:10.1007/s10535-006-0010-8

[12] Zunglen Ma and Kufa Gao, "Spiral breakage and photoinhibition of Arthrospira platensis (Cyanophyta) caused by accumulation of reactive oxygen species under solar radiation," *Environmental and Experimental Botany*, vol. 68, no. 2, pp. 208–213, Apr. 2010. doi:10.1016/j.envexpbot.2009.11.010 .

[13] Majid Nojavan-Asghar and Akbar Norastehnia, "A possible role for methyl jasmonate in effecting superoxide dismutase and catalase activities under PQ-induced oxidative stress in maize seedlings," *Journal of Biological Sciences*, vol. 6, no. 1, pp. 55–60, Dec. 2005. doi:10.3923/jbs.2006.55.60

[14] Stufan MARKLUND and Gurun MARKLUND, "Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase," *European Journal of Biochemistry*, vol. 47, no. 3, pp. 469–474, Sep. 1974. doi:10.1111/j.1432-1033.1974.tb03714.x

[15] Medasr Mir, Gella Sirhindi, Mella Alyemeni, P. Alam, and P. Ahmad, "Jasmonic acid improves growth performance of soybean under nickel toxicity by regulating nickel uptake, redox balance, and oxidative stress metabolism," *Journal of Plant Growth Regulation*, vol. 37, no. 4, pp. 1195–1209, May 2018. doi:10.1007/s00344-018-9814-y

[16] Neta Misra and Alhha Gupta, "Effect of salinity and different nitrogen sources on the activity of antioxidant enzymes and indole alkaloid content in catharanthus roseus seedlings," *Journal of Plant Physiology*, vol. 163, no. 1, pp. 11–18, Jan. 2006. doi:10.1016/j.jplph.2005.02.011

[17] Kenji Miura and Yosami Tada, "Regulation of water, salinity, and cold stress responses by salicylic acid," *Frontiers in Plant Science*, vol. 5, 2014. doi:10.3389/fpls.2014.00004

[18] Hella. Murthy, E.-J. Lee, and Kella Paek, "Production of secondary metabolites from cell and organ cultures: Strategies and approaches for biomass improvement and metabolite accumulation," *Plant Cell, Tissue and Organ Culture (PCTOC)*, vol. 118, no. 1, pp. 1–16, Mar. 2014. doi:10.1007/s11240-014-0467-7

[19] Eetzaz Nafie, Tahany Hathout, and Al Al Mokadem, "Jasmonic acid elicits oxidative defense and detoxification systems in Cucumis melo L. cells," *Brazilian Journal of Plant Physiology*, vol. 23, no. 2, pp. 161–174, 2011. doi:10.1590/s1677-04202011000200008

[20] Gela Noctor and Cella Foyer, "Ascorbate and glutathione: Keeping active oxygen under control," *Annual Review of Plant Physiology and Plant Molecular Biology*, vol. 49, no. 1, pp. 249–279, Jun. 1998. doi:10.1146/annurev.arplant.49.1.249

[21] Koop-Y. Paek, H ella Murthy, E.-J. Hahn, and J.-J. Zhong, "Large scale culture of ginseng adventitious roots for production of ginsenosides," *Biotechnology in China I*, pp. 151–176, 2009. doi:10.1007/10_2008_31

[22] Jeen Pilz, Iella Meineke, and Cella Gleiter, "Measurement of free and bound malondialdehyde in plasma by high-performance liquid chromatography as the 2,4-dinitrophenylhydrazine derivative," *Journal of Chromatography B: Biomedical Sciences and Applications*, vol. 742, no. 2, pp. 315–325, Jun. 2000. doi:10.1016/s0378-4347(00)00174-2

[23] Pella Sharma, Alla . Jha, Rella . Dubey, and M. Pessarakli, "Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions," *Journal of Botany*, vol. 2012, pp. 1–26, Apr. 2012. doi:10.1155/2012/217037

[24] Gella Tanou, Aella Molassiotis, and Gora Diamantidis, "Induction of reactive oxygen species and necrotic death-like destruction in strawberry leaves by salinity," *Environmental and Experimental Botany*, vol. 65, no. 2–3, pp. 270–281, Mar. 2009. doi:10.1016/j.envexpbot.2008.09.005

[25] Charels Crowley, Beran Gillham, and Mechiael Thorn, "A direct enzymic method for the determination of reduced glutathione in blood and other tissues," *Biochemical Medicine*, vol. 13, no. 3, pp. 287–292, Jul. 1975. doi:10.1016/0006-2944(75)90087-3

[26] Calus Wasternack, "Action of jasmonates in plant stress responses and development — applied aspects," *Biotechnology Advances*, vol. 32, no. 1, pp. 31–39, Jan. 2014. doi:10.1016/j.biotechadv.2013.09.009

[27] Jjom Xia, Hi Zhao, W. Liu, Lea. Li, and Yi. He, "Role of cytokinin and salicylic acid in plant growth at low temperatures," *Plant Growth Regulation*, vol. 57, no. 3, pp. 211–221, Oct. 2008. doi:10.1007/s10725-008-9338-8

[28] Kwa Zhao *et al.*, "Maize Rhizosphere in Sichuan, China, hosts plant growth promoting burkholderia cepacia with phosphate solubilizing and antifungal abilities," *Microbiological Research*, vol. 169, no. 1, pp. 76–82, Jan. 2014. doi:10.1016/j.micres.2013.07.003

[29] Aean Iqbal *et al.*, "Biodiversity in the sorghum (sorghum bicolor L. Moench) germplasm of Pakistan," *Genetics and Molecular Research*, vol. 9, no. 2, pp. 756–764, 2010. doi:10.4238/vol9-2gmr741

[31] Govarthanan Mealn., "Genetic variability among coleus sp. studied by RAPD banding pattern analysis," *International Journal for Biotechnology and Molecular Biology Research*, vol. 2, no. 12, Dec. 2011. doi:10.5897/ijbmbr11.030

[32] Husham Enshasy *et al.*, "The edible mushroom pleurotus spp.: I. Biodiversity and nutritional values," *International Journal of Biotechnology for Wellness Industries*, vol. 4, no. 2, pp. 67–83, Jul. 2015. doi:10.6000/1927-3037.2015.04.02.4

[33] Maheshwari, "Assessment of genetic diversity among capsicum annuum L. genotypes using RAPD markers," *AFRICAN JOURNAL OF BIOTECHNOLOGY*, vol. 10, no. 76, Nov. 2011. doi:10.5897/ajb11.497

[34] Nella Menolli, *et al.*,, "The genus pleurotus in Brazil: A molecular and taxonomic overview," *Mycoscience*, vol. 55, no. 5, pp. 378–389, Sep. 2014. doi:10.1016/j.myc.2013.12.001 .

[35] Young-Jin Park, "Genetic diversity analysis of ganoderma species and development of a specific marker for identification of medicinal mushroom ganoderma lucidum," *African Journal of Microbiology Research*, vol. 6, no. 25, Jul. 2012. doi:10.5897/ajmr12.846

[35] Young-Jin Park, "Genetic diversity analysis of ganoderma species and development of a specific marker for identification of

medicinal mushroom ganoderma lucidum," *African Journal of Microbiology Research*, vol. 6, no. 25, Jul. 2012. doi:10.5897/ajmr12.846

[36] Aela. Pawlik, Gelan. Janusz, Jegery. Koszerny, Welaf. Małek, and Jena . Rogalski, "Genetic diversity of the edible mushroom pleurotus sp. by amplified fragment length polymorphism," *Current Microbiology*, vol. 65, no. 4, pp. 438–445, Jul. 2012. doi:10.1007/s00284-012-0175-7 .

[37] Zahraa Isam Jameel , "MicroRNA Biogenesis, Mechanisms of Function, Circulation and Application Role in Human Diseases," International Journal of Scientific Research in Biological Sciences, Vol.10, Issue.5, pp.71-80, 2023.

[38] Zahraa Isam Jameel , "Bioinformatics Usage, Application and Challenges to Detect Human Genetic Diseases (Mini Review)," *International Journal of Scientific Research in Biological Sciences*, Vol.10, Issue.5, pp.59-67, 2023.

[39] Zahraa Jameel, Zahraa Lawi, Naval Al-Dujaili -Investigation of SOD2 Gene Polymorphism in the Patients with Type Two Diabetes Disease in Babylon Province Biochem Cell Arch, 2019|; vol.10,no.06,pp.70-75

[40] Shiv Kumar Sharma, Teena Gupta, "A Novel Approach for Plant Environment," International Journal of Biological Sciences, Vol.4, Issue.12, pp.1-5, 2014.

[41] Reena Solanki, "A Proposed New Approach for Cell Biology," In the Proceedings of the 2016 International Conference of Medical Sciences, India, pp.542-545, 2016.

AUTHORS PROFILE

Zahraa A.N. Al-Yassiry earned her B.Sc.and M.Sc. from the University of Babylon, Iraq. She is a lecturer and research fellow in the Department of Biology at Babylon University. She has published more than 22 research papers in reputed international journals including Thomson Reuters (SCI & Web of Science) and conferences including IEEE and it's also available online. Her main research work focuses on plant genetics. She has 15 years of teaching experience and 10 years of research experience.

