Effects of Exogenous Calcium and Magnesium on Physio-Hormonal Attributes of Trigonella Foenum-Graecum L: Under Polyethylene Glycol (PEG) Induce Drought Stress

Syed Hidayat Yar[1], Hussan Ara Begum[1], Naseen Rafiq[2], Syed Maqsood Ali[1], Behram Ali[1], Ilhanuddin[1]

[1]Department of Botany, Abdul Wali Khan University Mardan, Khyber Pakhtunkhwa, Pakistan
[2]Department of Zoology, Abdul Wali Khan University Mardan, Khyber Pakhtunkhwa, Pakistan

*__Correspondence__: hussanara.begum@gmail.com

Citation | Yar. S. H, Begum. H. A, Rafiq. N, Ali. S. M, Ali. B, Ilhanuddin, "Effects of Exogenous Calcium and Magnesium on Physio-Hormonal Attributes of Trigonella Foenum-Graecum l. Under Polyethylene Glycol (Peg) Induce Drought Stress", IJIST, Vol. 7 Issue. 1 pp 523-533, March 2025

Received| Jan 30, 2025 **Revised|** Feb 28, 2025 **Accepted|** March 6, 2025 **Published|** March 9, 2025.

Ddrought stress is one of the abiotic stresses that adversely affect the plant growth parameters and physio-hormonal attributes. In the current work, we study the adverse effects of induced PEG drought stress in Trigonella foenum-graecum L. in the presence of calcium and magnesium concentration. The experiment was conducted in the botanical garden of Abdul Wali Khan University Mardan in a completely random design. There are eight treatments and one control having each of the trees replicated. The nutrients of calcium and magnesium ratio (4, 2, and 0.18) were added to the plant after 30 days adding the polyethylene glycol of concentration of (0.6 Mpa and 0.2 MPa) for 8 days. The results show that drought stress induced by PEG had a significant effect on the growth and physio-hormonal indices of the plant. It was found that calcium and magnesium both reduce the adverse effects of polyethylene glycol. All treatments helped ascorbic acid, salicylic acid, and auxins to give plant possible growth and development in due time reducing the effects of PEG. Similarly in enzymatic activities, the maximum lipid peroxidase contents at $p > 0.05$ are found in calcium and magnesium ratio 0.18 and polyethylene glycol 0.2 Mpa. The maximum ascorbic acid peroxidase was found at $p > 0.05$ in Ca/Mg ratio 4. It is concluded from the study that the calcium and magnesium ratio mitigated the adverse effects of polyethylene glycol on Trigonella foenum-graecum L. growth by promoting hormones and enzymatic activities under PEG-induced drought stress.

Keywords: Stress; Physio-Hormonal Attributes; Calcium; Magnesium; Poly Ethylene Glycol

Introduction:

Trigonella foenum-graecum (Fabaceae), which contains calcium, proteins, lipids, dietary fiber, vitamins A and C, and minerals, has commercial value as a spice and green leafy vegetable [1]. It is a medicinal plant that has been utilized for its numerous therapeutic properties as well as a food condiment since ancient times [2]. Additionally, it is applied as organic manure to help fix nitrogen [3]. The seeds have been discovered to be useful against cancer, inflammation, diabetes, and hypercholesterolemia in addition to their nutritional benefits [4].

Drought is a significant abiotic stress that inhibits plant development and productivity [5][6]. Drought severity is unpredictable since it depends on a variety of variables, including the amount and distribution of rainfall, evaporative needs, and the soil's ability to store moisture [7].

According to estimates, 4.9 million acres, or one-fourth of Pakistan's entire arable land are susceptible to drought as things continue to deteriorate [8]. Since the previous few decades, all of Pakistan's provinces, particularly the largest portions of Sindh and Baluchistan, have been suffering from water shortages due to irregular rainfall and declining river flows [9]. The main causes of desertification in vast areas are a lack of water and inadequate rainfall [10]. Water levels in reservoirs have also fallen to dead levels, and the capacity of the reservoirs has decreased owing to siltation. Pakistan is also sucking out groundwater at a rate that is alarmingly higher than what is necessary to replace "fossil" groundwater, which could result in a catastrophe [11]. Drought reduced transpiration rate and stomatal conductance [12]. The accumulation of soluble carbohydrates, sucrose, glucose, and fructose in leaf tissues under water stress during the vegetative and reproductive stages [13].

Ca is a necessary mineral that affects plant development and metabolism via a variety of physiological and biochemical processes. Ca is necessary for plant growth, cell wall thickness, restoration, and plant tissue formation [14][15]. Ca^{2+} supplementation improved tomato growth, fruit production, and quality. Under acidic soil conditions, calcium, as an essential element, plays a critical role in regulating the transport of other nutrients into the plant system [14]. Calcium is a secondary cationic element that serves many functions in the plant system. Its primary function is to maintain cell structure, integrity, and polarity. It is essential for plant growth and development, as well as numerous cellular metabolism and transcription factors [16].

Mg is also essential for plant growth due to its direct involvement in physiological and biochemical systems. It promotes root development, improves water and nutrient absorption, enhances carbohydrate export, and reduces ROS generation and photo-oxidative cell damage under stress conditions [17]. Mg is a necessary nutrient that participates in a variety of metabolic processes during plant development and growth [18]. Mg is the most abundant component in chlorophyll and is responsible for photosynthesis [19]. Mg deficiency may cause programmed cell death in chloroplasts due to oxidative damage [20]. The morphology of the leaves revealed a thylakoid disorder caused by Mg deficiency [21].

Drought stress can be increased by treating grains with a polyethylene glycol (PEG) solution for several days (PEG). In many cases, different concentrations of PEG are present. As a result, testing at various concentrations is critical [22]. The high molecular weight of PEG (6000 or 8000 g/mol) prevents water from penetrating the cell wall. In germination experiments, polyethylene glycol solution is used to control the water potential. It is suggested that in future experiments, the most tolerant genotypes that can germinate at this concentration be used as drought-tolerant genotypes [23]. A variety of molecular weights of the polymer polyethylene glycol (PEG) are created. According to research that appeared in the journal "Science" [24] PEG can be used to alter the osmotic potential of nutrient solution cultures and thereby cause plants to experience water deficits in a manner that is relatively controlled and appropriate for experimental protocols. Large-molecular-weight PEG was thought to be the optimum osmoticum for use in hydroponics root medium because it did not permeate the plant [25]. An increase in human number compels them to grow more food to fulfill their needs, keeping this

matter in mind, we studied the collective effects of different Ca/Mg quotients and osmotic stress produced by polyethylene glycol (PEG) on Trigonella foenum-graecum L. growth. The present study was undertaken to check the role of calcium and magnesium in polyethylene glycol-induced drought.

Objectives And Novelty Statement of The Study:

There is little study available about the plant Trigonella foenum-graecum L. drought stress, therefore the objective of the proposed study is to assess the;

- The effects of different levels of Polyethylene glycol (PEG) in Trigonella foenum-graecum L.
- The effect of Calcium and magnesium different concentrations in Trigonella foenum-graecum L. towards drought stress.
- To study the physio-hormonal attributes of Trigonella foenum-graecum L. under PEG-induced drought stress.

Materials And Methods:

To study the effect of calcium and magnesium concentrations on Trigonella foenum-graecum under PEG-induced drought stress, an experiment was conducted in the garden of AWKUM. Seeds were collected from the market and sterilized before sowing. The study included eight treatments and one control, each with three replicates, arranged in a completely randomized design. The treatment details are as follows: The Ca/Mg ratio was prepared for the experiment in three different forms, with effective values of 4, 2, and 0.18. We prepared a solution using 1000ml of water, 90mg of MgSO4, and 429mg of Ca (NO3)2. To create a solution for the 0.18 ratio, we dissolved 90 mg of MgSO4, 62 mg of Ca (NO3)2, and 260 mg of Mg (NO3)2 in 1000 ml of water. After the plants had received the Ca/Mg ratio for two weeks and one week of PEG stress, the plants were then ready for harvesting after three days. Plants were exposed to the PEG-induced osmotic stress (along with different Ca/Mg treatments) for 8 days in the experiment, with the solutions being renewed every 4 days.

To create the desired osmotic potentials, 26 mg of PEG was dissolved in 1000 mL of distilled water to achieve -0.6 MPa, while 8.6 mg of PEG was dissolved in 1000 mL of distilled water to achieve -0.2 MPa. The experiment was carried out in natural climatic conditions of the temperature range (e.g., 25°C ± 1°C), relative humidity percentage (e.g., 50% RH), and light intensity.

Table 1. Detail of Experimental Treatments

No	Treatment detail	No	Treatment detail
To	Control	T4	Ca/Mg2 ratio+PEG(0.2 MPa)
T1	Ca/Mg4 ratio+PEG(0.6 MPa)	T5	Ca/Mg0.18 ratio+PEG(0.6 MPa)
T2	Ca/Mg4 ratio+ PEG(0.2 MPa)	T6	Ca/Mg0.18 ratio+PEG(0.2 MPa)
T3	Ca/Mg2 ratio+ PEG(0.6 MPa)	T7	Ca/Mg0 ratio+PEG(0.6 MPa)
T8	Ca/Mg0 ratio+PEG(0.2 MPa)		

Flow Diagram of Methodology: The Plant growth-promoting hormones such as Ascorbic acid, Salicylic acid Indole-3-Acetic acid, and enzymatic activities can be observed under PEG-induced drought stress in the presence of calcium and magnesium concentration. For hormones and enzymatic activities, the fresh Leaf samples from all treatments were collected for the analysis, Ascorbic acid was measured using the spectrophotometric method [26], and Salicylic acid was measured using UV detection at 240nm to identify the specific wavelength of salicylic acid [27]. Using the spectrophotometric method auxin was determined by assaying 1 ml of the supernatant with 2 ml of Salkowski reagent and letting them stand for 30 minutes in a dark room before measuring the absorbance at 540 nm.

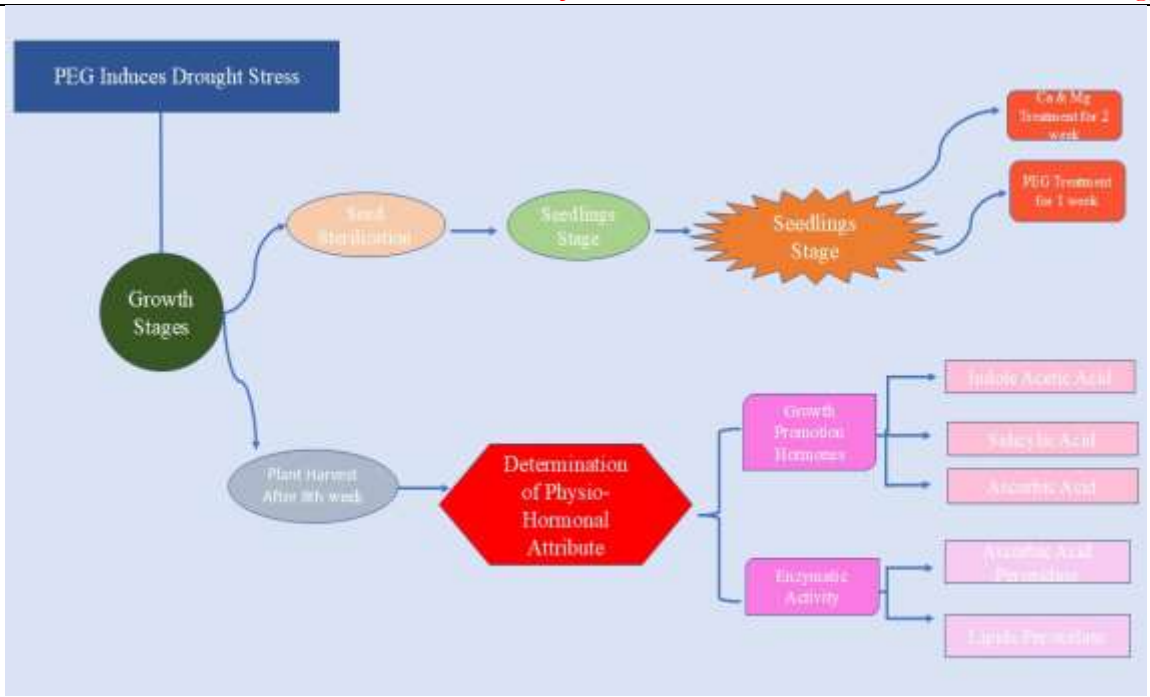

Figure 1. Flow of Study Diagram

The procedure for measuring ascorbate peroxidase (APX) activity involves homogenizing fresh plant tissue, extracting the crude enzyme, and then initiating a reaction. The reaction is monitored for a decrease in absorbance at a specific wavelength [28]. The activity of ascorbate peroxidase was measured from fresh leaves. The method used a Thio barbituric acid (TBARS) assay to determine lipid peroxidase activity. Malondialdehyde (MDA), a byproduct of lipid peroxidation, is used to assess oxidative damage [29].

Along with the several germination characteristics observed in this study, including the number and percent of germination, the length of roots and shoots, the root: shoot ratio, fresh and dry weights, and water content. At the end of the study (after 8 weeks), when plants were harvested the determination of plant growth-promoting hormones such as Ascorbic acid, Salicylic acid Indole-3-Acetic acid, and enzymatic activities lipid and ascorbic acid peroxidase were carried out. The data collected from the results was analyzed using one-way (ANOVA).

Results:

Growth Promoting Hormones:

Ascorbic acid of Trigonella Foenium-Graecium L:

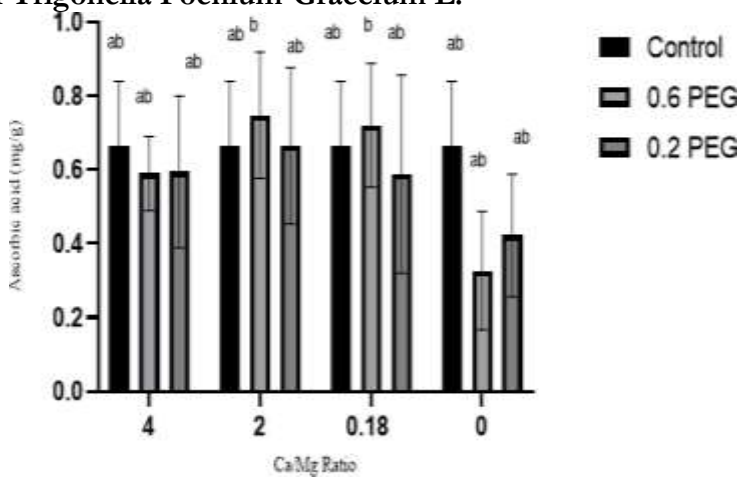

Figure 2. Effect of calcium and magnesium ratio on total Ascorbic acid content mg/g F.W under PEG (polyethylene glycol) induced drought stress.

Figure 2 illustrates that the highest ascorbic acid content was observed in the control group, while the lowest was recorded in the treatment lacking calcium and magnesium ratio under 0.6 MPa polyethylene glycol-induced stress.

Salicylic Acid Contents of Trigonella Foenium-Graecium L:

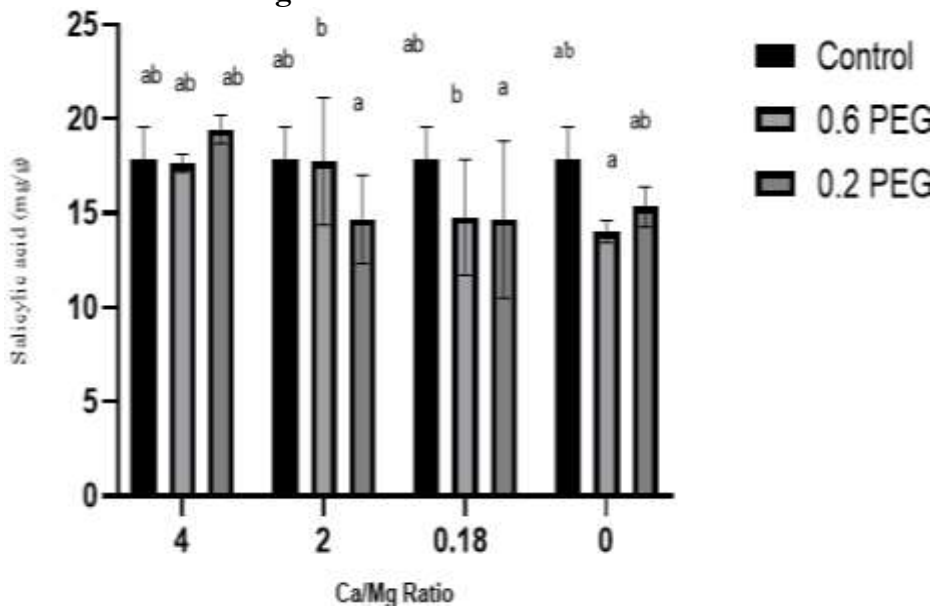

Figure 3. Effect of calcium and magnesium ratio on total Salicylic acid content mg/g F.W under PEG (polyethylene glycol) induced drought stress.

The highest salicylic acid content in Trigonella foenum-graecum L. was observed in the Ca/Mg ratio of 4 under 0.2 MPa PEG stress, while the lowest was recorded in the treatment lacking calcium and magnesium ratio under 0.6 MPa polyethylene glycol stress (Figure 3). The results showed dose-dependent activity, where a high dose of Ca/Mg produces a high content of salicylic acid in the selected plant.

IAA Content of Trigonella Foenium-Graecium L:

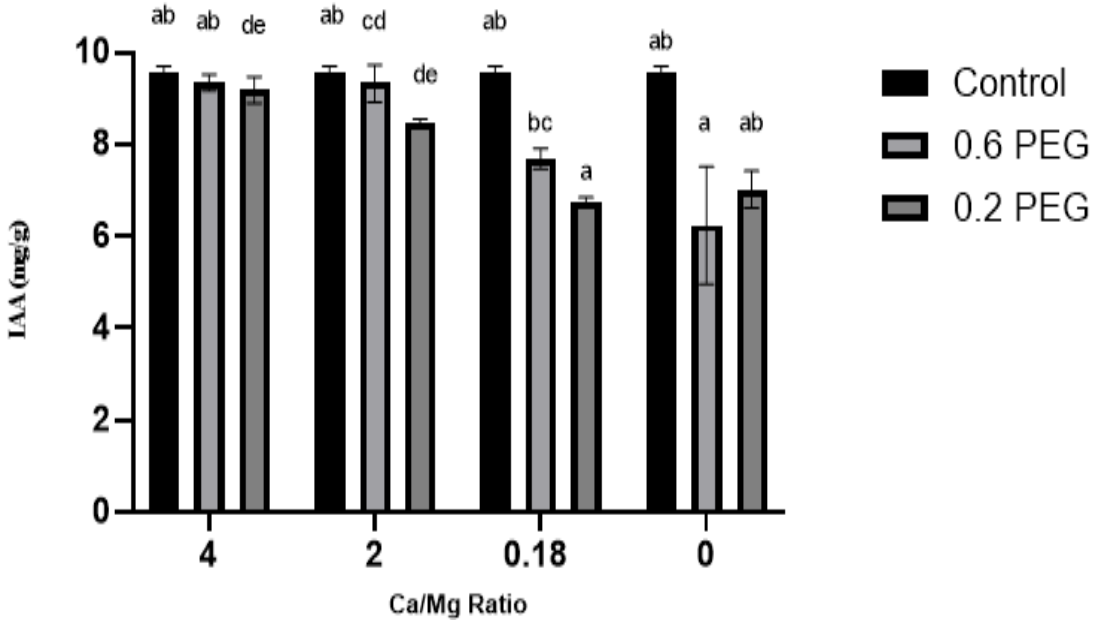

Figure 4. Effect of calcium and magnesium ratio on total IAA content mg/g F.W under PEG (polyethylene glycol) induced drought stress.

Figure 4 shows that the highest IAA levels were measured in the control group without any treatment, while the lowest levels were detected in the treatment lacking calcium and

magnesium ratio and subjected to 0.6 MPa polyethylene glycol (PEG) stress. It shows that the presence of Ca/Mg is important to alleviate the effect of PEG-induced drought stress in the selected plant.

Enzymatic Activity:

Lipid peroxidase content of Trigonella frenum-francium L:

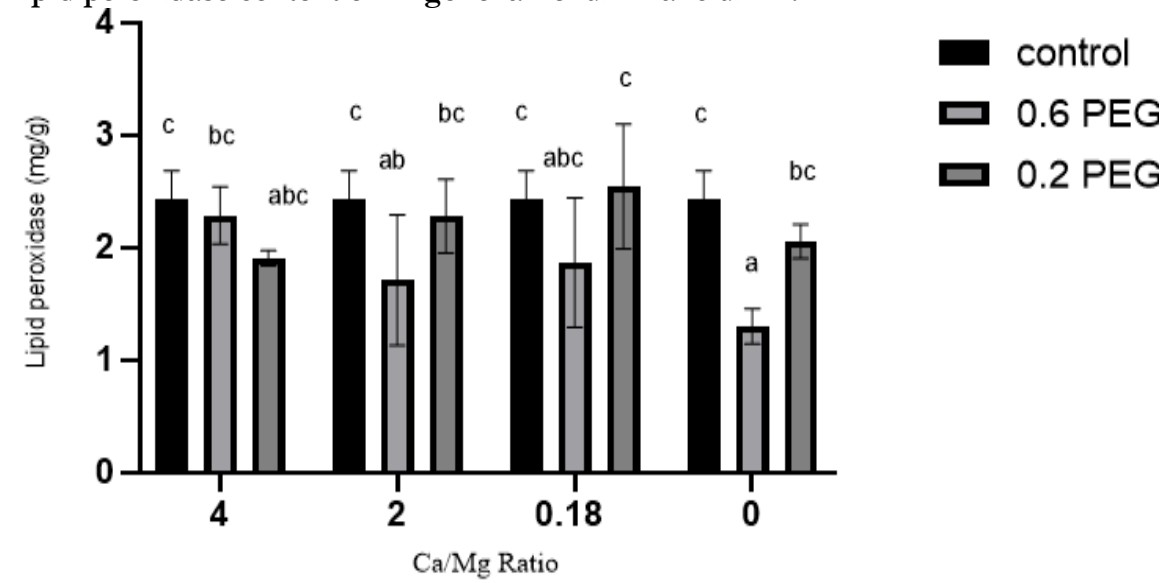

Figure 5. Effect of calcium and magnesium ratio on total Lipid peroxidase content mg/g F.W under PEG (polyethylene glycol) induced drought stress.

Figure 5 illustrates that the highest lipid peroxidase content was observed in the treatment with a Ca/Mg ratio of 0.18 and 0.2 MPa polyethylene glycol (PEG) stress. This indicates that Ca/Mg has positive effects on the growth and development of plants reducing the effect of PEG. However, the lowest content was recorded in the treatment lacking calcium and magnesium and subjected to 0.6 MPa PEG stress.

Ascorbic acid peroxidase content of Trigonella frenum-francium L:

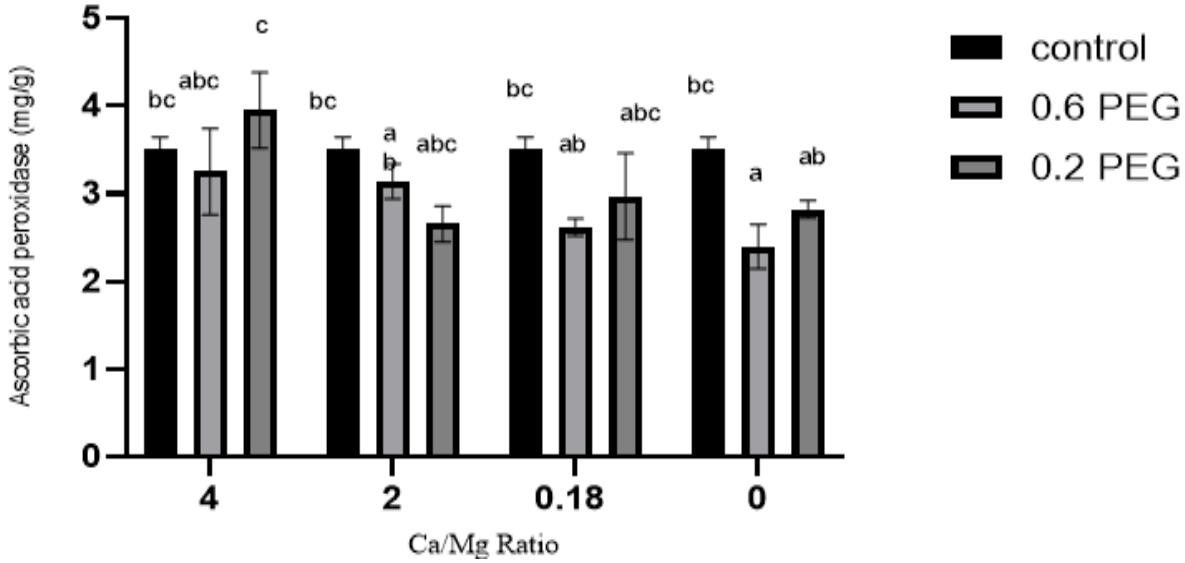

Figure 6 Effect of calcium and magnesium ratio on total Ascorbic acid peroxidase content mg/g F.W under PEG (polyethylene glycol) induced drought stress.

The maximum ascorbic acid peroxidase of Trigonella frenum-francium L. was found at least $p>0.05$ in Ca/Mg ratio 4 and at 0.2 MPa PEG stress and the minimum was noted in

treatment which had no calcium and magnesium ratio and polyethylene glycol of 0.6 MPa stress (Figure 6).

The code utilized for estimation of the Effects of Exogenous Calcium and Magnesium on Physio-Hormonal Attributes of Trigonella Foenum-Graecum l using AI is as under:

```
import pandas as PD
import numpy as np
import matplotlib.pyplot as plt
import seaborn as sns
From Scipy import stats
from sklearn.preprocessing import MinMaxScaler
from sklearn.ensemble import RandomForestRegressor
From sklearn.model_selection import train_test_split
from sklearn.metrics import r2_score
# Load dataset
df = pd.read_csv("fenugreek_data.csv")
# Handle missing values
df = df.drop ()
# Normalize data
scaler = MinMaxScaler()
df.iloc[:, 1:] = scaler.fit_transform(df.iloc[:, 1:])
# ANOVA test for significant differences
f_stat, p_value = stats.f_oneway(df["Control"], df["Low_Ca"], df["High_Ca"], df["Low_Mg"], df["High_Mg"])
print(f"F-statistic: {f_stat}, P-value: {p_value}")
if p_value < 0.05:
   print("Significant effect of treatment on attributes.")
Else:
print("No significant effect of treatment.")
# Data visualization
plt.figure(figsize=(10, 6))
sns.boxplot(data=df)
plt.title("Effect of Calcium and Magnesium on Fenugreek Attributes")
plt. label("Treatment")
plt. label("Normalized Values")
plt.show()
# Machine learning model
X = df.iloc[:, 1:]
y = df["Growth"]  # Target variable
X_train, X_test, y_train, y_test = train_test_split(X, y, test_size=0.2, random_state=42)
model = RandomForestRegressor(n_estimators=100, random_state=42)
model.fit(X_train, y_train)
y_pred = model.predict(X_test)
print(f"R² Score: {r2_score(y_test, y_pred)}")
# Correlation analysis
corr_matrix = df.corr()
plt.figure(figsize=(8,6))
sns.heatmap(corr_matrix, annot=True, cmap="cool warm")
plt.title("Correlation Between Attributes")
plt.show()
```

Discussion:

According to Xu et al., [30] the endogenous levels of ABA, IAA, and JA in wheat plants were interestingly regulated differentially by Si application during water-deficit stress compared to PEG treatment alone. According to this study, exogenous Si enhances plant development by regulating phytohormone levels and nutrient (Na, Mg, and Si) uptake in wheat under water-deficit stress. A similar result was also obtained by authors in [31], where the ascorbic acid levels were found to be improved by minerals under PEG stress. Our results also indicate that the maximum ascorbic acid contents were found in the control and the minimum are observed in treatment which has no calcium and magnesium ratio and polyethylene glycol stress. The same trend was observed for auxin levels in our study. The findings of [32] demonstrated that ascorbic acid and its immediate precursor helped shield plants from oxidative damage caused by various stresses. Additionally, increases in endogenous jasmonic acid (JA), salicylic acid (SA), and abscisic acid (ABA) concentrations during drought stress suggest that these hormones play a role in conferring resistance to drought-induced stress, as noted in [33]. According to these findings, endogenous SA levels and signaling played a protective function in the Arabidopsis plant's reaction to the PEG-simulated drought [34]. Drought stress brought on by polyethylene glycol (PEG). The P. simonii seedlings showed reduced growth, reduced aquaporin expression levels, activated auxin (IAA) and abscisic acid (ABA) signaling pathways, and decreased net photosynthetic rate as they adapted to drought [35].

A crucial enzyme controlling ROS levels, ascorbate peroxidase acts in various cellular components. Varying abiotic stressors have different effects on the expression of the APX-coding genes in different plant species [36]. The high concentration of Na+ in the nutrient solution had a significant impact on the nutritional needs of the plant, particularly on the uptake of Ca2+ and K+, which were constrained due to competition. By increasing NaCl stress levels, a rise in proline accumulation, ascorbic peroxidase activity, and lipid peroxidase activity were observed. However, the addition of PEG 6000 to NaCl treatment had a more significant effect on enhancing these enzyme activities. These findings imply that maize plants may need to have more proline to sustain osmotic adjustment and more antioxidant enzyme activity to better combat active oxygen species (AOS) when exposed to salt and water stress [37].

The results of this study illustrate that high lipid peroxidase contents were present in calcium and magnesium with a ratio of 0.18 and polyethylene glycol, while the minimum is observed in treatments that have no calcium and magnesium ratio and polyethylene glycol stress. Similar results were observed for ascorbic acid peroxidase levels.

Two wheat genotypes subjected to mild water deficit conditions showed significant alterations in the relative levels of APX (ascorbate peroxidase) transcripts. While cytosolic APX2 was solely up-regulated in the drought-tolerant genotype, cytosolic APX1 expression levels are increased in both genotypes. Thylakoid APX transcript levels rose in the drought-tolerant genotype, whereas stromal APX2 expression levels were higher in the drought-sensitive cultivar [38]. The same results also indicated an increase in ascorbic peroxidase levels in the leaves of P. acutifolius under stress conditions. Furthermore, P. acutifolius showed greater constitutive levels of Ascorbic peroxidase activity [39].

Consistent with our findings, [40] observed that in drought-adapted wheat genotypes, lower levels of lipid peroxidation were associated with elevated constitutive and induced APOX activity. In P. acutifolius, it appears that greater levels of constitutive CAT activity and both constitutive and induced levels of APOX activity largely removed the result of the SOD reaction, H_2O_2, under osmotic stress conditions [41][42].

Conclusion:

It is concluded from the present study that the calcium and magnesium ratio alleviated the adverse effects of polyethylene glycol on Trigonella foenum-graecum L. growth-promoting hormones and enzymatic activities parameters such as ascorbic acid, salicylic acid, and auxin

under PEG induced drought stress. The present study suggests that calcium and magnesium are very crucial for proper growth and development of plants. The study examining the effects of exogenous calcium and magnesium on fenugreek (Trigonella foenum-graecum L.) under PEG-induced drought stress could have limitations related to the artificial nature of PEG-induced stress, potential inconsistencies in application methods, needs open field validation, and it needs for further exploration of specific hormonal pathways and genetic factors involved in the plant's response to drought stress on a molecular level.

Conflict of Interest:

The authors have no conflict of interest.

References:

[1] P. H. Ester Anon, Xavier Serra-Picamal, "Cell crawling mediates collective cell migration to close undamaged epithelial gaps," *Proc. Natl. Acad. Sci.*, vol. 109, no. 27, pp. 10891–10896, 2012, doi: https://doi.org/10.1073/pnas.1117814109.

[2] N. Ashraf, D. Karlan, and W. Yin, "Tying Odysseus to the Mast: Evidence From a Commitment Savings Product in the Philippines," *Q. J. Econ.*, vol. 121, no. 2, pp. 635–672, May 2006, doi: 10.1162/QJEC.2006.121.2.635.

[3] A. Y. I. Cakmak, "Magnesium: A forgotten element in crop production," Better crops. Accessed: Mar. 02, 2025. [Online]. Available: https://www.researchgate.net/publication/291869977_Magnesium_A_forgotten_element_in_crop_production

[4] A. S. Chandio, T. S. Lee, and M. S. Mirjat, "The extent of waterlogging in the lower Indus Basin (Pakistan) – A modeling study of groundwater levels," *J. Hydrol.*, vol. 426–427, pp. 103–111, Mar. 2012, doi: 10.1016/J.JHYDROL.2012.01.017.

[5] K. Foster, H. Lambers, D. Real, P. Ramankutty, G. R. Cawthray, and M. H. Ryan, "Drought resistance and recovery in mature Bituminaria bituminosa var. albomarginata," *Ann. Appl. Biol.*, vol. 166, no. 1, pp. 154–169, Jan. 2015, doi: 10.1111/AAB.12171.

[6] G. N. CHRISTINE H. FOYER, "Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context," *Plant. Cell Environ.*, 2005, doi: https://doi.org/10.1111/j.1365-3040.2005.01327.x.

[7] A. G. H. Führs, "Magnesium mobility in soils as a challenge for soil and plant analysis, magnesium fertilization and root uptake under adverse growth conditions," *Plant Soil*, vol. 368, pp. 5–21, 2013, doi: https://doi.org/10.1007/s11104-012-1567-y.

[8] W. Grzebisz, "Crop response to magnesium fertilization as affected by nitrogen supply," *Plant Soil*, vol. 368, no. 1–2, pp. 23–39, Jul. 2013, doi: 10.1007/S11104-012-1574-Z/METRICS.

[9] K. C. Wanli Guo, Shaoning Chen, Nazim Hussain, Yuexi Cong, Zongsuo Liang, "Magnesium stress signaling in plant: Just a beginning," *Plant Signal. Behav.*, vol. 10, no. 3, 2015, doi: https://doi.org/10.4161/15592324.2014.992287.

[10] A. L. K. Muhammad Hamayun, Sumera Afzal Khan, Zabta Khan Shinwari, "Effect of polyethylene glycol induced drought stress on physio-hormonal attributes of soybean," Pakistan Journal of Botany. Accessed: Mar. 02, 2025. [Online]. Available: https://www.researchgate.net/publication/230845375_Effect_of_polyethylene_glycol_induced_drought_stress_on_physio-hormonal_attributes_of_soybean

[11] M. T. Melanie Hauer-Jákli, "Critical Leaf Magnesium Thresholds and the Impact of Magnesium on Plant Growth and Photo-Oxidative Defense: A Systematic Review and Meta-Analysis From 70 Years of Research," *Front. Plant Sci.*, vol. 10, 2019, doi: https://doi.org/10.3389/fpls.2019.00766.

[12] L. J. W. Peter K. Hepler, "Calcium at the Cell Wall-Cytoplast Interface," *J. Integr. Plant Biol.*, 2010, doi: https://doi.org/10.1111/j.1744-7909.2010.00923.x.

[13] C. Hermans, G. N. Johnson, R. J. Strasser, and N. Verbruggen, "Physiological characterisation of magnesium deficiency in sugar beet: Acclimation to low magnesium

differentially affects photosystems I and II," *Planta*, vol. 220, no. 2, pp. 344–355, Dec. 2004, doi: 10.1007/S00425-004-1340-4/METRICS.

[14] T. M. Hildebrandt, "Synthesis versus degradation: directions of amino acid metabolism during Arabidopsis abiotic stress response," *Plant Mol. Biol.*, vol. 98, no. 1–2, pp. 121–135, Sep. 2018, doi: 10.1007/S11103-018-0767-0/METRICS.

[15] A. Caverzan, G. Passaia, M. Margis-Pinheiro, "Plant responses to stresses: Role of ascorbate peroxidase in the antioxidant protection," *Genet. Mol. Biol*, vol. 35, 2012, doi: 10.1590/S1415-47572012000600016.

[16] S. Javad *et al.*, "Hydrogen sulphide alleviates cadmium stress in Trigonella foenum-graecum by modulating antioxidant enzymes and polyamine content," *Plant Biol.*, vol. 24, no. 4, pp. 618–626, Jun. 2022, doi: 10.1111/PLB.13393.

[17] J. Z. Jingjing Jiang, Shenghui Ma, Nenghui Ye, Ming Jiang, Jiashu Cao, "WRKY transcription factors in plant responses to stresses," *J. Integr. Plant Biol.*, 2016, doi: https://doi.org/10.1111/jipb.12513.

[18] A. Khan, A.R., Qayyum, "Management of rainfed farming," *Progress. Farming*, 1986.

[19] Khushboo, K. Bhardwaj, P. Singh, M. Raina, V. Sharma, and D. Kumar, "Exogenous application of calcium chloride in wheat genotypes alleviates negative effect of drought stress by modulating antioxidant machinery and enhanced osmolyte accumulation," *Vitr. Cell. Dev. Biol. - Plant*, vol. 54, no. 5, pp. 495–507, Oct. 2018, doi: 10.1007/S11627-018-9912-3/METRICS.

[20] É. A. Kido, J. R. C. Ferreira-Neto, V. Pandolfi, A. C. de Melo Souza, and A. M. Benko-Iseppon, "Drought Stress Tolerance in Plants: Insights from Transcriptomic Studies," *Drought Stress Toler. Plants, Vol 2 Mol. Genet. Perspect.*, pp. 153–185, Jan. 2016, doi: 10.1007/978-3-319-32423-4_6.

[21] S. Köşkeroğlu and A. L. Tuna, "The investigation on accumulation levels of proline and stress parameters of the maize (Zea mays L.) plants under salt and water stress," *Acta Physiol. Plant.*, vol. 32, no. 3, pp. 541–549, Jan. 2010, doi: 10.1007/S11738-009-0431-Z/METRICS.

[22] J. Kudla, O. Batistič, and K. Hashimoto, "Calcium Signals: The Lead Currency of Plant Information Processing," *Plant Cell*, vol. 22, no. 3, pp. 541–563, Apr. 2010, doi: 10.1105/TPC.109.072686.

[23] Z. N. Malyukova LS, Nechaeva TL, Zubova MYu, Gvasalia MV, Koninskaya NG, "Physiological and biochemical characterization of tea (Camellia sinensis L.) microshoots in vitro: The norm, osmotic stress, and effects of calcium," *Sel'skokhozyaistvennaya Biol.*, vol. 55, no. 5, pp. 970–980, 2020, doi: 10.15389/agrobiology.2020.5.970eng.

[24] L. S. LS Malyukova, ZV Pritula, NV Kozlova, AV Velikiy, EV Rogozhina, VV Kerimzade, "Effects of calcium-containing natural fertilizer on Camellia sinensis (L.) Kuntze," *Bangladesh J. Bot.*, vol. 50, no. 1, 2021, doi: https://doi.org/10.3329/bjb.v50i1.52686.

[25] M. Naeem, J. R. Traub, H. ur R. Athar, and W. Loescher, "Exogenous calcium mitigates heat stress effects in common bean: a coordinated impact of photoprotection of PSII, up-regulating antioxidants, and carbohydrate metabolism," *Acta Physiol. Plant.*, vol. 42, no. 12, pp. 1–13, Dec. 2020, doi: 10.1007/S11738-020-03171-4/METRICS.

[26] X.-X. Z. Li-Jun Ou, Zhou-Bin Liu, Yu-Ping Zhang, "Effects of exogenous Ca 2+ on photosynthetic characteristics and fruit quality of pepper under waterlogging stress," *Chil. J. Agric. Res.*, vol. 77, no. 2, 2017, doi: http://dx.doi.org/10.4067/S0718-58392017000200126.

[27] G. S. Premachandra, D. T. Hahn, and R. J. Joly, "Leaf Water Relations and Gas Exchange in Two Grain Sorghum Genotypes Differing in Their Pre- and Post-Flowering Drought Tolerance," *J. Plant Physiol.*, vol. 143, no. 1, pp. 96–101, Jan. 1994, doi: 10.1016/S0176-1617(11)82103-6.

[28] P. Kumar, "Measurement of Ascorbate Peroxidase Activity in Sorghum," *Bio-protocol*, vol. 12, no. 20, Oct. 2022, doi: 10.21769/BIOPROTOC.4531.

[29] Y. W. Kaiyu Qin, Shuaiqun Fan, Fenguo Zhang, "Effects of Exogenous Calcium on Datura

Seed Germination under Drought Stress," *J. Bot. Res*, vol. 1, no. 2, 2019, doi: https://doi.org/10.30564/jrb.v1i2.862.

[30] C. S. Victor Hugo Ramírez-Builes, Jürgen Küsters, Thais Regina de Souza, "Calcium Nutrition in Coffee and Its Influence on Growth, Stress Tolerance, Cations Uptake, and Productivity," *Front. Agron*, vol. 2, 2020, doi: https://doi.org/10.3389/fagro.2020.590892.

[31] A. K. Atif Riaz, Adnan Younis, Asif Riaz Taj, "Effect of drought stress on growth and flowering of marigold (Tagetes erecta L.)," Pakistan Journal of Botany. Accessed: Mar. 02, 2025. [Online]. Available: https://www.researchgate.net/publication/259484411_Effect_of_drought_stress_on_growth_and_flowering_of_marigold_Tagetes_erecta_L

[32] A. Sahu, K. Kishore, R. K. Nayak, S. N. Dash, S. C. Sahoo, and S. Barik, "Influence of potassium on mineral content, yield and quality attributes of dragon fruit (Selenicereus monacanthus) in acidic soil of Eastern tropical region of India," *J. Plant Nutr.*, vol. 46, no. 11, pp. 2621–2636, 2023, doi: 10.1080/01904167.2022.2160744.

[33] D. C. S. R.K. Sairam, D.S. Shukla, "Stress induced injury and antioxidant enzymes in relation to drought tolerance in wheat genotypes," *Biol. Plant.*, vol. 39, pp. 357–364, 1997, doi: 10.1023/A:1001009812864.

[34] K. Srinivasan, "Fenugreek (Trigonella foenum-graecum): A Review of Health Beneficial Physiological Effects," *Food Rev. Int.*, vol. 22, no. 2, pp. 203–224, Apr. 2006, doi: 10.1080/87559120600586315.

[35] I. Türkan, M. Bor, F. Özdemir, and H. Koca, "Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant P. acutifolius Gray and drought-sensitive P. vulgaris L. subjected to polyethylene glycol mediated water stress," *Plant Sci.*, vol. 168, no. 1, pp. 223–231, Jan. 2005, doi: 10.1016/J.PLANTSCI.2004.07.032.

[36] Y. J. Z. L. van den Berg, "Response of South African indigenous grass species to drought stress induced by polyethylene glycol (PEG) 6000," *South African J. Bot.*, vol. 72, no. 2, pp. 284–286, 2006, doi: https://doi.org/10.1016/j.sajb.2005.07.006.

[37] G. Verma, D. Srivastava, P. Tiwari, and D. Chakrabarty, "ROS Modulation in Crop Plants Under Drought Stress," *React. Oxyg. Nitrogen Sulfur Species Plants Prod. Metab. Signal. Def. Mech.*, pp. 311–336, Jul. 2019, doi: 10.1002/9781119468677.CH13.

[38] S. V. Theysshana Visuvanathan, Leslie Thian Lung Than, Johnson Stanslas, Shu Yih Chew, "Revisiting Trigonella foenum-graecum L.: Pharmacology and Therapeutic Potentialities," *Plants*, vol. 11, no. 11, p. 1450, 2022, doi: https://doi.org/10.3390/plants11111450.

[39] J. Wery, S. N. Silim, E. J. Knights, R. S. Malhotra, and R. Cousin, "Screening techniques and sources of tolerance to extremes of moisture and air temperature in cool season food legumes," pp. 439–456, 1994, doi: 10.1007/978-94-011-0798-3_26.

[40] X. Du Huayan Yin , Fan Yang, Xiaoyan He, Ping Mu, and W. Ma, "Advances in the functional study of glutamine synthetase in plant abiotic stress tolerance response," *Crop J.*, vol. 10, no. 4, pp. 917–923, 2022, doi: https://doi.org/10.1016/j.cj.2022.01.003.

[41] C. Yue *et al.*, "Effects of cold acclimation on sugar metabolism and sugar-related gene expression in tea plant during the winter season," *Plant Mol. Biol.*, vol. 88, no. 6, pp. 591–608, Aug. 2015, doi: 10.1007/S11103-015-0345-7/METRICS.

[42] Jian-Kang Zhu, "Abiotic Stress Signaling and Responses in Plants," *Cell*, vol. 167, no. 2, pp. 313–324, 2016, [Online]. Available: https://www.cell.com/cell/fulltext/S0092-8674(16)31080-7?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS0092867416310807%3Fshowall%3Dtrue