Ameliorative effect of Calcium and Magnesium on growth and physiochemical attributes of *Trigonella foenum-greacum* L., under Polyethylene Glycol Induced Drought Stress

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Abstract

Drought is one of the most devastating factor that adversely affect the growth and development of plants. The aim of our study to check the exogenously applied calcium (Ca) and magnesium (Mg)'s effect on the growth and development of *Trigonella foenum-greacum* L, under drought stress conditions, *in vitro*. The experiment was Randomized Complete Block Design (RCBD), where three replicates for each treatment, one treatment was calcium and magnesium ratio 4 and PEG 0.6MPa, 2nd was calcium and magnesium ratio 4 and PEG 0.2 MPa, 3rd calcium and magnesium ratio 2 and PEG 0.6 MPa, 4th calcium and magnesium ratio 2 and PEG 0.2 MPa, 5th calcium and magnesium ratio 0.18 and PEG 0.6 MPa and last one is calcium and magnesium ratio 0.18 and PEG 0.2MPa. Results showed that growth and the physio hormonal parameters of *Trigonella foenum garaecum* L. under the PEG induced drought stress in the presence of exogenous Ca and Mg ratios. The study also showed that the Ca and Mg ratios had a positive impact on *Trigonella foenum-graecum* L. growth parameters i.e. root length, fresh weight, dry weight root moisture content, shoot length, fresh and dry weight, leaf area and primary and secondary metabolites under PEG induced drought stress. On the same way, different growth parameters like, carbohydrates, protein and chlorophyll contents were not affect in the treatment as compared to control. While, in secondary metabolites due the stress the proline content increases and the rest remain the same to the control. It is concluded that PEG induced stress affect plant's growth and development.

Key words: Calcium; Drought stress; Magnesium; Metabolites; Polyethylene Glycol

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is a medicinal plant that has been utilized for its numerous therapeutic properties as well as a food condiment since ancient times. Fenugreek seeds are golden-yellow and rhomboidal in shape, and the plant can reach a height of 60 cm (Anathan *et al*., 2022) *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 (Srinivasan, 2006). According to reports, its proteins are of higher quality than soy proteins (Feyzi *et al*. 2015). Drought is a significant abiotic stress that inhibits plant development and productivity (Riaz *et al*., 2010; Hamayun *et al*., 2010). 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 (Wery *et al*., 1994). Global warming is causing the world's water supply to diminish at an alarming rate and will get worse in the upcoming years (Lorrey, *et al*., 2007; Cook *et al*., 2007), and future population pressures are likely to make drought conditions worse (Somerville and Briscoe, 2001). Pakistan, like other nations, is suffering from a severe drought; out of the country's total area of 0.804 Mkm2, 0.563 Mkm2 are arid and receive less than 60 cm of precipitation annually (Anon., 2003). According to estimates, 4.9 million acres or one-fourth of Pakistan's entire arable land is susceptible to drought. (Khan and Qayyum, 1986). Plants also exhibit many biochemical and physiological changes under water deficit conditions. Reactive oxygen species (ROS) production causes lipid peroxidation, protein breakdown, and damage to nucleic acids (Fazel *et al*., 2007). Sweet sorghum's sensitivity to water scarcity varied according to developmental stage. The plants were extremely sensitive to drought during the vegetative and early reproductive stages. Although their water requirements were lower in the late reproductive phase, their yield was reduced due to terminal drought (Younis *et al*., 2000).

Calcium (Ca) is a necessary mineral that affects plant development and metabolism via a variety of physiological and biochemical processes. Ca is necessary for plants growth, cell wall thickness, restoration, and plant tissue formation (Hepler and Winship, 2010; Kudla *et al*., 2010). Ca takes part in cellular growth and reproduction, maintains cell pH, and acts as a regulating component in the source sink translocation mechanism via its effects on cells and cell membranes (Hirschi, 2004). These proteins recognize and interpret Ca^{2+} signals by binding to Ca^{2+} ions, which cause them to change conformation and function (Aldon *et al*., 2018). Mg is also essential for plant growth due to its direct involvement in physiological and biochemical systems. (Verma *et al*., 2019).

Magnesium (Mg) is a necessary nutrient that participates in a variety of metabolic processes during plant development and growth (Gransee and Führs, 2013). Mg is the most abundant component in chlorophyll and is responsible for photosynthesis (Cakmak and Yazici, 2010). Mg deficiency may cause programmed cell death in chloroplasts due to oxidative damage (Foyer and Noctor, 2005). The Mg bond to chlorophyll, on the other hand, accounts for a small portion of the total Mg fraction. Depending on the plant's Mg status, 20% (Gransee and Führs 2013) to 35% (Cakmak and Yazici 2010). The mechanisms of tolerance to unfavorable Ca/Mg concentrations, as well as their potential relationship to serpentinophyte resistance to metal ion excess in serpentine soils, remain unknown (Lombini *et al*., 2003). Serpentine soils have low Ca/Mg quotients, high concentrations of heavy metals (especially Ni, Cr, and Co), essential macronutrient deficiencies, and low water-holding capacity levels due to low organic matter content (Salehi *et al*., 2017).

A variety of molecular weights of the polymer polyethylene glycol (PEG) are created recently. According to a 1961 paper that appeared in the journal "Science" (Lagerwerff *et al*., 1961), 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 osmotic for use in hydroponics root medium because it did not permeate the plant (Blum, 2008) Different concentrations of PEG are present in many cases. As a result, testing at a variety of concentrations is essential (Kido *et al*., 2016). PEG has a high molecular weight (6000 or 8000 g/mol) and prevents water from penetrating the cell wall. Polyethylene glycol solution is used to control the water potential in germination experiments (Van *et al*., 2006). The present study was undertaken to check the role of calcium and magnesium in polyethylene glycol induced drought.

MATERIALS AND METHODS

Study Design and Treatment

To study the effect of calcium and magnesium concentration on *Trigonella foenium-gracium* under PEG induced drought stress, seeds were sterilized before sowing. Treatments were applied in the triplicate form based on Randomized Complete Block Design (RCBD).

Ca/Mg ratio was made ready for the experimental task. Ca/Mg quotients come in three different forms, with effective values of 4, 2, and 0.18 (Table 1). We prepared a solution using 1000mL of water, 90mg of $MgSO_4$, and 429mg of $Ca(NO_3)_2$. We prepared a solution using 1000mL of water, 90mg of $MgSO_4$, and 246mg of $Ca(NO_3)_2$. In order to create a solution for the 0.18 ratio, we dissolved 90mg of $MgSO_4$, 62mg of $Ca(NO_3)_2$, and 260mg of $Mg(NO_3)_2$ 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. On the same way, 26mg PEG was dissolved in 1000mL of distal water which make a potential of 0.6 MPa and 8.6mg PEG was dissolved in 1000mL of distal water which make a potential of 0.2 MPa.

Table 1. Treatment detail.

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)		

Determination of agronomic parameters For agronomic characteristics the plant was harvested six months into the experiment and various parameters such as shoot, root length, fresh weight of shoot and root, shoot and root dry weight were measured. On the same way, plants were dried in the sun after being weighed fresh, and the dry weight of root and shoot was calculated. The relative water content of all three relocated plants' fresh leaves was determined using the method of (García-Mata and Lamattina, 2001).

By using the following formula and computed the RWC,

RWC (%) = (FW-DW) / (TW-DW) * 100

Primary and secondary metabolites determination

For primary and secondary metabolites fresh Leaf samples from all treatments were collected for the analysis of primary metabolites.

Total sugar, chlorophyll, protein, total lipids, and prolines, phenols, and flavonoids are all different measurements using the following method

Total carbohydrates were determined using the Anthrone method (Yemm and Willis, 1954) which analyzed each repeated fresh leaf. (Maclachlan and Zalik 1963) were used to calculate chlorophyll levels from fresh leaves and the flowing equation were used to determine the chlorophyll a, chlorophyll b, total chlorophyll and carotenoids

Equation:

Chlorophyll a. (mg/g) = 12.3D663 – 0.86 D645 / d* 1000*w

Chlorophyll b (mg/g) = 12.3D645 – 0.86 D663 / d* 1000*w

Total Chlorophyll = Chl a + Chl a

Carotenoids (mg/g) = (7.6 D480 – 1.49 D510 / D* 1000*w) * V

To extract and estimate soluble proteins, the Bradford's Assay reagent was used (Jones *et al*., 1989).

For total lipids were determined using spectrophotometric method (Van Handel, 1985). Bates *et al*., (1973) assisted in determining proline.

The method was used to measure phenol in fresh leaves (Chung *et al*., 2016). Mervet *et al*., (2009) method was used to calculate complete and total flavonoids.

Data analysis

Data was recoreded in the form of triplicates and analyzed by one way ANNOVA by applying multiple Duncan range test, using SPSS software.

RESULTS

Agronomic parameters

The results showed that different agronomic traits were affected significantly, under various treatment conditions (Table 2). It was found that root length, root fresh weight, root dry weight and moisture contents were significantly reduced under drought condition, while, recovery was taken under consideration with the applications of Ca and Mg treatments, respectively. On the same way, maximum root length wass found in TO (control), while the least growth was observed in the T7, which is treatment of polyethylene glycol (0.6 MPa). Similarly root fresh weight and maximum root dry weight was indicated in T4, which is treatment of calcium and magnesium ratio 2 and polyethylene glycol 0.2 MPa and the minimum is in T7 (polyethylene glycol 0.6 MPa). With the same context, root moisture contents was higher $p>0.05$ in control and least was found in calcium and magnesium ratio 0.18and polyethylene glycol 0.6 MPa stress.

Table 2. Effect on root length, root fresh weight, root dry weight and root moisture content of *Trigonella foenun-graecum*

Treatment	**Root length**	**Root fresh weight**	**Root Dry weight**	**Moisture content**
T0	9.11 ± 0.61^{cd}	0.39 ± 0.08^{a}	0.07 ± 0.01^{d}	0.393 ± 1^{d}
T1	9.80 ± 0.38^{d}	0.34 ± 0.11^{a}	0.05 ± 0.01^{ab}	0.374 ± 1^{bcd}
T2	9.13 ± 0.65^{cd}	0.38 ± 0.04^{a}	0.06 ± 0.01^{c}	0.391 ± 1^{cd}
T3	9.60 ± 0.06^{d}	0.36 ± 0.06^{a}	0.07 ± 0.03^{c}	0.361 ± 0.88^{cd}
T4	9.84 ± 0.08^{d}	0.37 ± 0.09^{a}	0.07 ± 0.03^{d}	0.371 ± 1^{cd}
T5	6.10 ± 0.97^{b}	0.25 ± 0.05^{a}	0.05 ± 0.02^{d}	0.27 ± 0.99^{bcd}
T6	8.44 ± 0.42^{c}	0.26 ± 0.03^{a}	0.048 ± 0.01^{bc}	0.30 ± 1.00^{abc}
T7	3.90 ± 0.20^{a}	2.14 ± 0.15^{b}	0.036 ± 0.01^{a}	3.28 ± 3.74^{a}
T8	4.53 ± 0.21^{a}	2.67 ± 0.21^{c}	0.041 ± 0.01^{a}	3.77 ± 9.71^{ab}

megapascal To(Control), T1(Ca/Mg ratio4+PEG 0.6 MPa),T2 (Ca/Mg ratio 4 +PEG 0.2 MPa),T3(Ca/Mg ratio2+PEG 0.6 MPa),T4(Ca/Mg ratio2+PEG 0.2 MPa),T5(Ca/Mg ratio0.18+PEG 0.6 MPa),T6(Ca/Mg ratio0.18+PEG 0.2 MPa),T7(PEG 0.6 MPa),T8(PEG 0.2 MPa).

It was taken under consideration that maximum shoot length wass found in T1 (Ca/Mg4 ratio+PEG 0.6 MPa) and the minimum is found in T7 (polyethylene glycol 0.6 MPa).Whereas, maximum shoot fresh weight is found at $p<0.05$ in T2 which is calcium magnesium ratio 2 and PEG 0.6 MPa stress and minimum is indicated in T5(Ca/Mg ratio0.18+PEG 0.6 MPa) which has no calcium and magnesium ratio and has a PEG stressed 0.2 MPa; therefore the maximum shoot dry weight are indicated T2 which is treatment of calcium and magnesium ratio 4 and polyethylene glycol 0.2 MPa and the minimum are in T7 which is treatment of polyethylene glycol 0.6 MPa Similarly shoot moisture contents maximum at p 0.05 in T3 which is Ca/Mg ratio 4 and PEG 0.2 MPa stress and minimum are found in calcium and magnesium ratio 0.18 and polyethylene glycol 0.2 MPa stress.

Table 2 Effect on shoot length, shoot fresh weight, shoot dry weight and root moisture content of *Trigonell foenum-graecum*

Treatment	Shoot length	Shoot fresh weight	Shoot dry weight	Shoot moisture content
To	15.340±1.830bc	1.358±0.122^{cd}	1.258± 0.122^{b}	0.157± 0.0100^{b}
T1	17.404 ±1.141^{d}	1.344±0.109^{cd}	1.244± 0.109^{b}	0.147± 0.030^{b}
T2	15.390 ±1.951^{bc}	1.377±0.039^{d}	1.277± 0.039^{b}	0.169± 0.0120^{b}
T3	16.800 ±0.167^{cd}	1.355±0.061^{cd}	1.255± 0.061^{b}	0.174± 0.100^{b}
T4	17.522 ±0.248^{d}	1.367± 0.088^{d}	1.267± 0.088^{b}	0.167± 0.09^{b}
T5	12.808 ±0.755^{a}	1.201± 0.090^{b}	1.201± 0.046^{b}	0.132± 0.03^{b}
T6	13.663 ±1.203ac	1.219± 0.080^{b}	1.019± 0.149^{a}	0.133± 0.20^{a}
T7	12.007 ±0.211^{a}	1.219± 0.080^{a}	0.935± 0.040^{a}	0.108± 0.285^{a}
T8	12.260 ±0.209^{a}	1.028± 0.011^{ab}	0.956± 0.040^{a}	0.121± 0.073^{a}

Ca=calcium, Mg=magnesium, PEG=polyethylene glycol, MPa= megapascal To(Control), T1(Ca/Mg ratio4+PEG 0.6 MPa),T2 (Ca/Mg ratio 4 +PEG 0.2 MPa),T3(Ca/Mg ratio2+PEG 0.6 MPa),T4(Ca/Mg ratio2+PEG 0.2 MPa),T5(Ca/Mg ratio0.18+PEG 0.6 MPa),T6(Ca/Mg ratio0.18+PEG 0.2 MPa),T7(PEG 0.6 MPa),T8(PEG 0.2 MPa).

Results showed that maximum leaf area at p>0.05 is found in TI(Ca/Mg4 ratio PEG 0.6 MPa) and the minimum is found in T7 (polyethylene glycol 0.6 MPa) **Table 3.** Whereas, the leaf fresh weight at p>0.05 was found in control and minimum are found in T5 (Ca/Mg 2 ratio and stress of PEG is 0.2 MPa). Similarly, maximum leaf dry weight was indicated in T5 (Ca/Mg 2 ratio and stress of PEG is 0.2 MPa), while, least dry weight was taken under consideration in T7 (polyethylene glycol 0.6 MPa). On the same context, leaf moisture contents was higher at p>0.05 in control and least in T5 (Ca/Mg 2 ratio and stress of PEG is 0.2 MPa).

Table 3 Effect on leaf area, leaf fresh weight, leaf dry weight and leaf moisture content of *Trigonella foenum-graecum.*

Treatment	Leaf area(cm)^2	Leaf fresh weight	Leaf dry weight	Leaf moisture content
To	21.033±0.372a	0.284±0.015a	0.039±0.020a	32.931±1.000a
T1	27.854±0.145^a	0.231±0.080a	0.030± 0.013a	29.276±1.216a
T2	21.187 ±0.423^a	0.261±0.061a	0.041± 0.010a	31.474±1.695a
T3	24.920 ±0.014^a	0.240±0.061a	0.048± 0.016a	27.746 ±1.000a
T4	23.273±0.007^a	0.241±0.120a	0.039± 0.019a	28.582 ±1.023a
T5	9.198±0.082^a	0.148±0.052a	0.057± 0.029a	13.489 ±1.179a
T6	15.892 ±0.120^a	0.195±0.006a	0.068± 0.082a	20.255 ±1.000a
T7	6.700±0.015^a	0.153±0.020aa	0.032± 0.009a	19.697 ±3.493a
T8	9.052±0.020^a	0.185±0.110a	0.049± 0.007a	24.289 ±0.867a

Ca=calcium, Mg=magnesium, PEG=polyethylene glycol, MPa= megapascal. To (Control), T1(Ca/Mg ratio4+PEG 0.6 MPa),T2 (Ca/Mg ratio 4 +PEG 0.2 MPa),T3(Ca/Mg ratio2+PEG 0.6 MPa),T4(Ca/Mg ratio2+PEG 0.2 MPa),T5(Ca/Mg ratio0.18+PEG 0.6 MPa),T6(Ca/Mg ratio0.18+PEG 0.2 MPa),T7(PEG 0.6 MPa),T8(PEG 0.2 MPa).

Primary and Secondary metabolites

Total carbohydrates contents

Results indicated maximum carbohydrates contents at $p<0.05$ in Ca/Mg ratio 0.18 and PEG 0.6 MPa stresses conditions. While, minimum contents were taken under consideration at $p<0.05$ in 0 Ca/Mg ratio and PEG 0.2 MPa stress level (Figure 1).

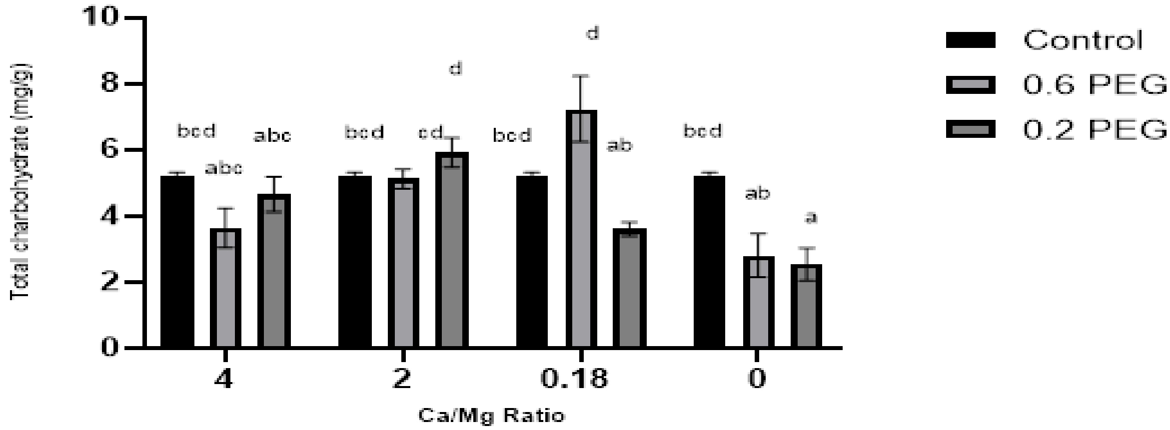

Figure 1 Effect of Ca/Mg ratio on total carbohydrates content mg/g (F.W) of *Trigonella foeanum-gracum* under polyethylene glycol (PEG) induced drought stress.

Total protein contents

The results indicated that maximum protein contents was found in control and the minimum were found in all those treatments with no calcium and magnesium ratio and PEG stress in 0.6 MPa, respectively (Figure 2).

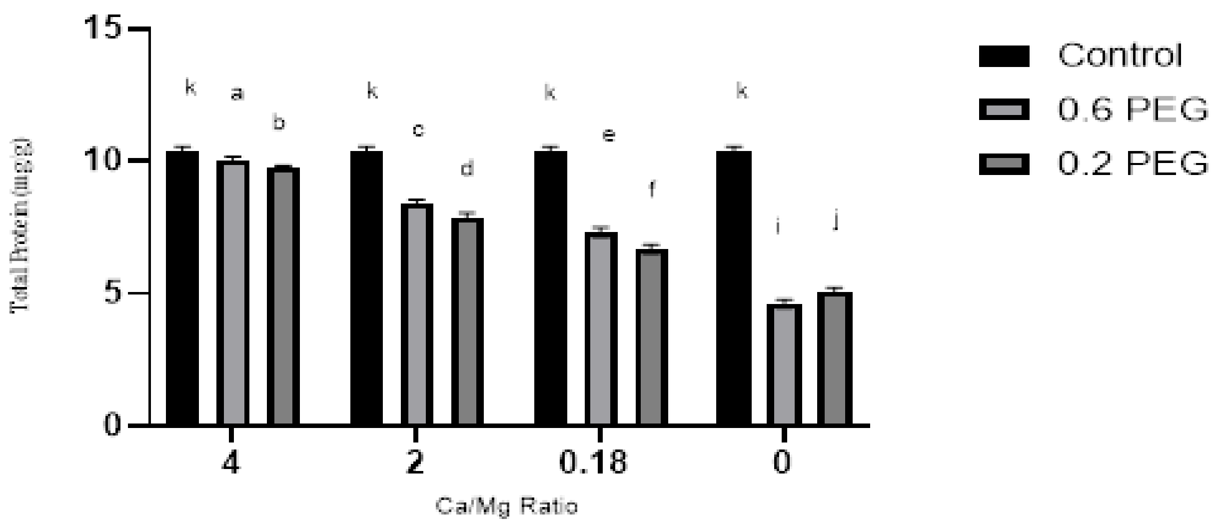

Figure 2 effects of Ca/Mg ratio on protein content mg/g (F.W) under polyethylene glycol (PEG) induced drought stress.

Chlorophyll "a" contents of *Trigonella foenum-graecum*

It was found that maximum chlorophyll a contents were found control plants, while, minimum contents were found in drought treatment i.e. polyethylene glycol of 0.6 MPa. It was also taken under co nsideration that the application of calcium and magnesium ratio recovering the chlorophyll contents.

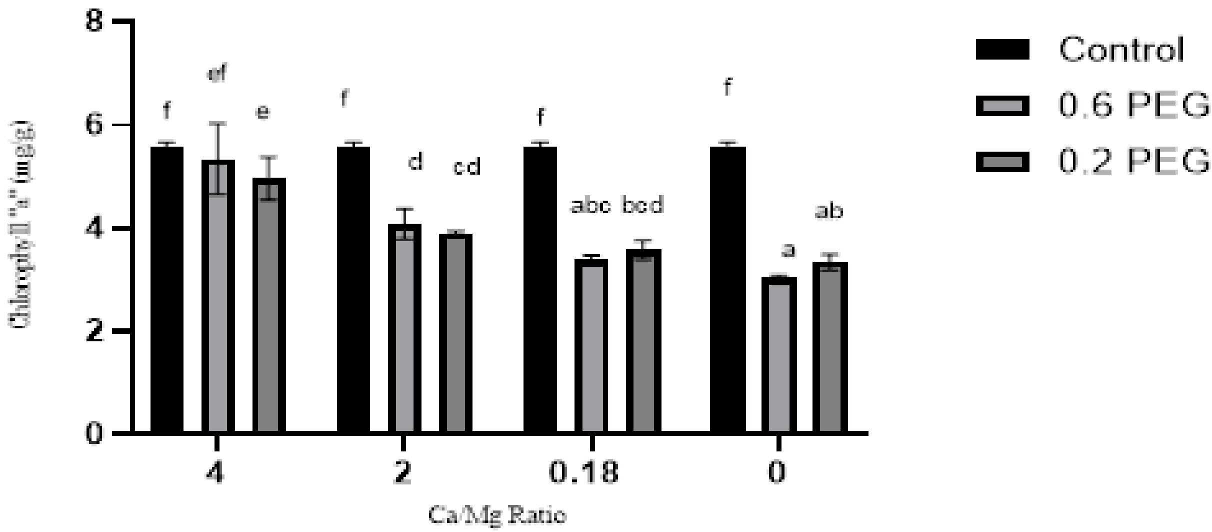

Figure 3. effects of Ca/Mg ratio on chlorophyll ´a¸ content mg/g F.W under polyethylene glycol (PEG) induced drought stress.

Effect on chlorophyll "b" contents

Highest value of chlorophyll b contents is found at $p<0.05$ in control no treatment and the lowest value are in treatment which have only PEG 0.6 MPa and not have calcium and magnesium ratio

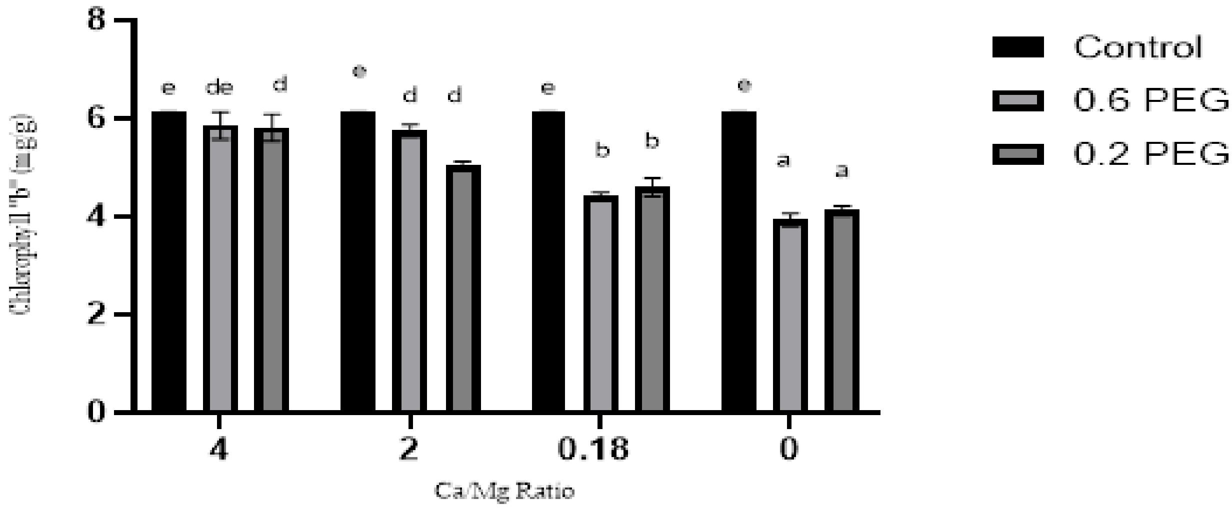

Figure 4 effects of Ca/Mg ratio on chlorophyll "b" content mg/g F.W under polyethylene glycol (PEG) induced drought stress.

Total chlorophyll contents

Maximum total chlorophyll a contents are found at $p<0.05$ in control and the minimum are found in treatment which have only polyethylene glycol of 0.6 MPa and not have calcium and magnesium ratio.

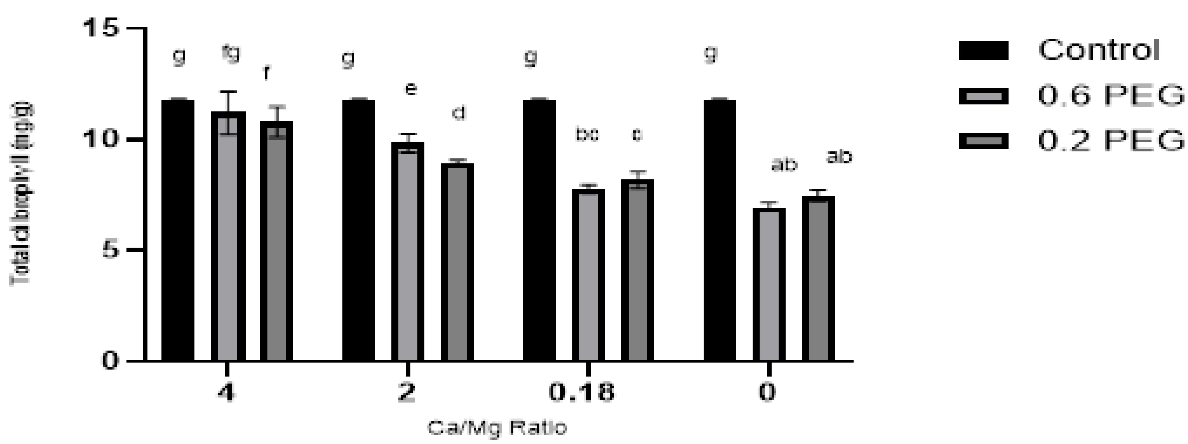

Figure 5 effects of Ca/Mg ratio on total chlorophyll contents mg/g (F.W) under polyethylene glycol (PEG) induced drought stress.

Total carotenoid contents

Maximum carotenoid contents were found at $p<0.05$ in control and the minimum are found in treatment which have only polyethylene glycol of 0.6 MPa and not have calcium and magnesium ratio.

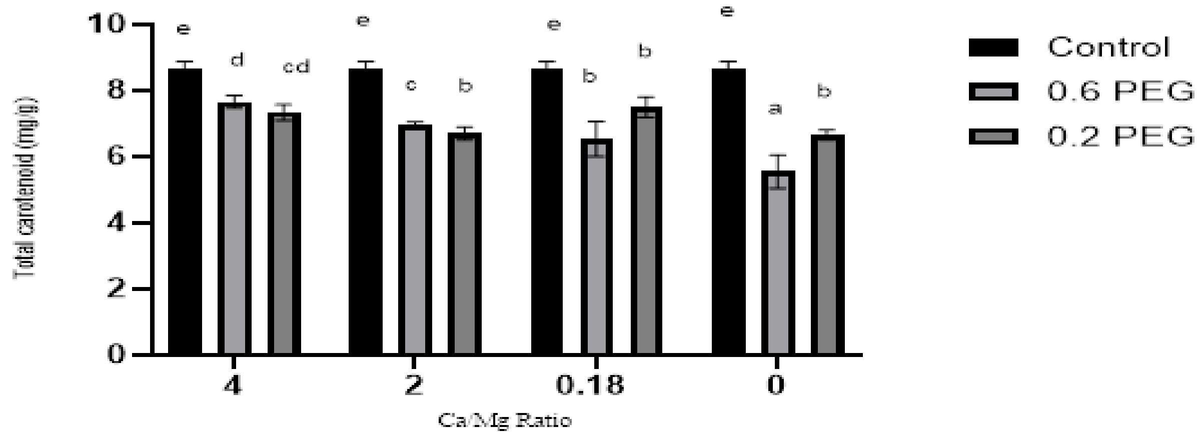

Figure 7 effect of Ca/Mg ratio on total carotenoid content mg/g (F.W) under polyethylene glycol (PEG) induced drought stress.

Total lipid contents

It was found that lipids contents were not affected in either treatments (Figure 8). However, maximum lipid contents reported in control and minimum in 4 Ca/Mg ratio and PEG 0.6 MPa stress, but the data was not statistically significant.

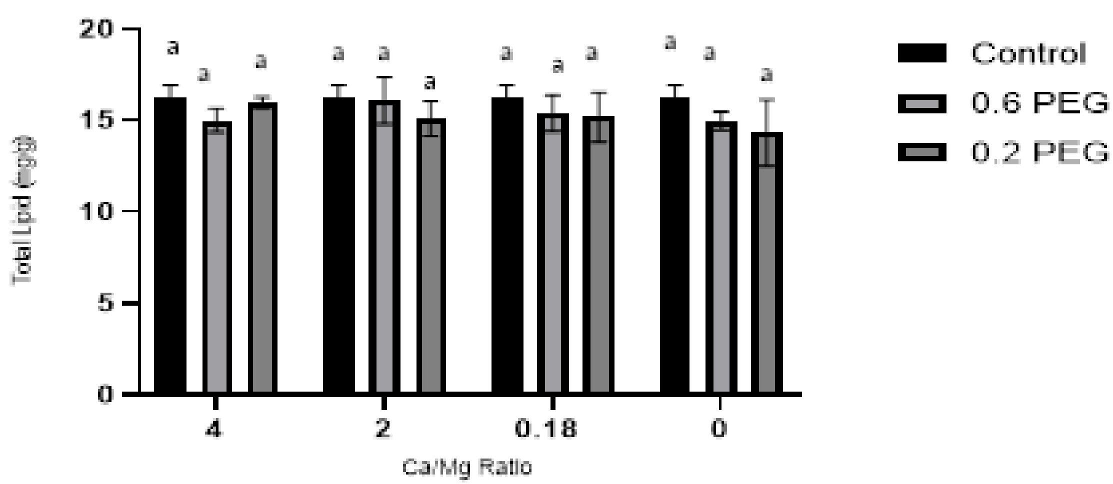

Figure 8 Effects of Ca/Mg ratio on lipid content mg/g (F.W) of *Trigonella foenum-greacum* under polyethylene glycol (PEG) induced drought stress.

Secondary metabolites

Total phenol of *Trigonella foenum-graecum*

It was taken under consideration that maximum phenol contents was found in control which have no treatment and the minimum are found in treatment which have no calcium and magnesium ratio and stress have 0.6 MPa polyethylene glycol PEG.

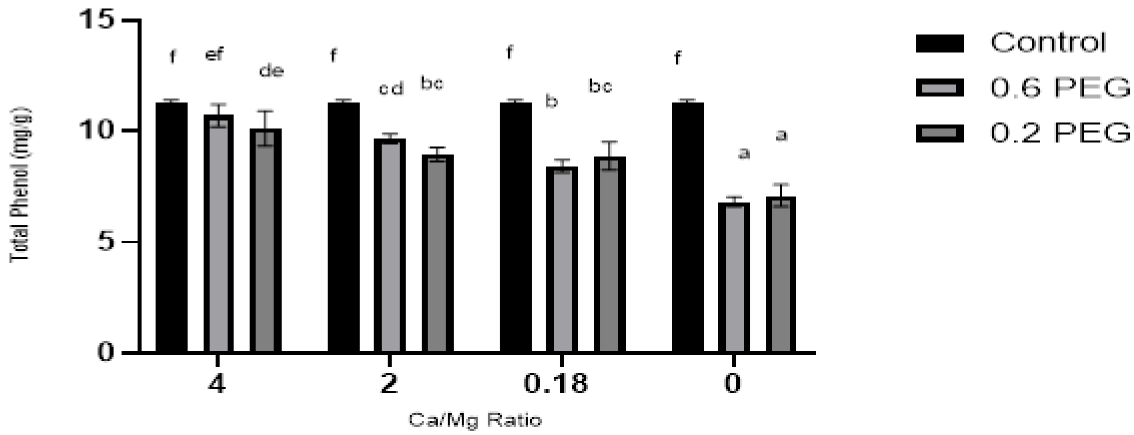

Figure 9 effect of Ca/Mg ratio on total lipid content mg/g (F.W) under polyethylene glycol (PEG) induced drought stress.

Flavonoid contents of *Trigonella foenum-graecum*

Maximum chlorophyll a contents are found at $p>0.05$ in Ca/Mg ratio 4 and at PEG 0.6 MPa l and the minimum are found in treatment which have 0.18 calcium and magnesium ration and polyethylene glycol of 0.2MPa.

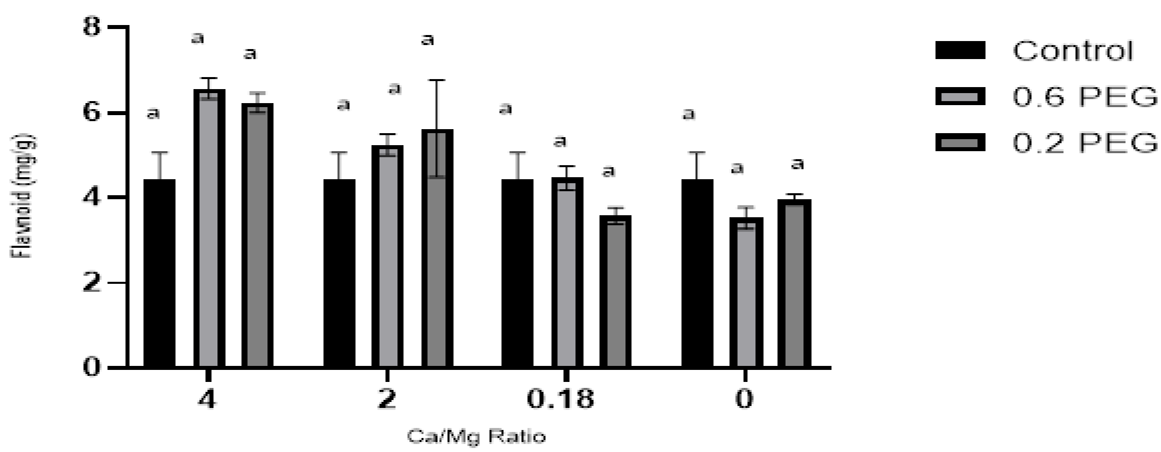

Figure 10 effect of Ca/Mg ratio on flavonoid content mg/g (F.W) under polyethylene glycol (PEG) induced drought stress.

Proline contents of *Trigonella foenum-graecum*

maximum proline content at p>0.05 is indicated in Ca/Mg ration 0.18 at PEG 0.2 MPa stress and the minimum are found in a treatment Ca/Mg ratio 4 at PEG 0.6 MPa stress(fig.10).

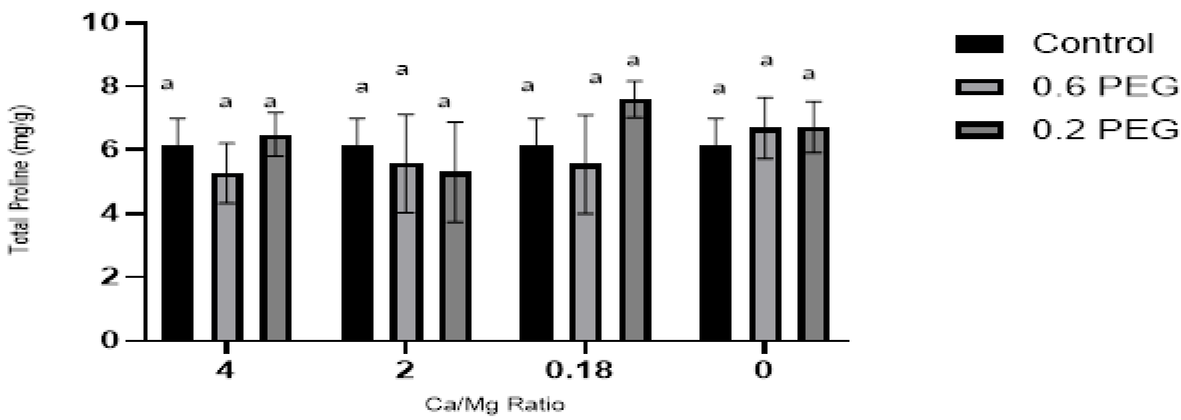

Figure 10 effect of calcium and magnesium ratio on total proline content mg/g (F.W) under polyethylene glycol (PEG) induced drought stress.

DISCUSSION

Our result shows that calcium and magnesium ratio have a positive effect on root parameters of trigonella under PEG induced stress. If we see to table 1, the root length in control and the calcium and magnesium have no difference, but in the treatment there is no calcium and magnesium a clear difference in table 1 at p<0.05 The highest root length in T1 that is calcium and magnesium ratio 4 and PEG is 0.6 MPa And the lowest value in T7 which have no calcium and magnesium ratio but having PEG induced drought stress. The similar result find out with (Kinji *et al.*, 2012) And similarly result was found in root fresh weight that inT2 which has a height value that is treatment of calcium and magnesium ratio 4 and PEG induced stress is 0.2 MPa and the low value is found in treatment T7 which has PEG induced drought stress and does not have calcium and magnesium. parallel result also found with Murat Ali Turan, *et al.*, (2010) whereas in root dry weight there is also a significant results at p<0.05 and a clear difference in a treatment which have high ratio of calcium and magnesium and have a not calcium and magnesium ratio that is T7 which have only PEG stress and also clear from the results of root moisture content calcium magnesium ratio have a positive effect that show in T2 which have a high calcium and magnesium ratio and the T7 which have only PEG induced drought stress and not have calcium and magnesium ratio (Awasthi, *et al*., 2016).

From our experiments, if we see in table 2, it is clear that calcium and magnesium ratio have a significant effect on shoot parameters that we see to shoot length in a treatment which has a high value in T5 which is calcium and magnesium ratio 2 and polyethylene glycol-induced stress is 0.2 MPa and whereas the lowest value in a shoot length in a treatment of T7 which is PEG stress of 0.6 MPa and have no calcium and magnesium ratio The inability to modify and relocate photosynthetic output effectively may be the reason of the decrease in shoot length (Xiong and Zhu, 2002). Therefore and look at shoot dry weight also determine that a treatment in which there are high calcium and magnesium ratio have a high value of shoot dry weight and a treatment which there is no calcium and magnesium ratio and have a PEG stress have a lower shoot dry weight And it also clear from shoot fresh weight that in a treatment in which there is calcium and magnesium ratio high and have a high value of fresh weight i.e. in T3 and a treatment in which no calcium and magnesium have a low value of shoot dry weight i.e. T7 (Kumari *et al*.,2011). Relative water content (RWC) content was significantly reduced under water stress condition And if we see to shoot moisture content it also clear that in a treatment which have a high calcium and magnesium ratio also have high level of shoot moisture content and a treatment which have no calcium and magnesium ratio and have a high PEG stress in that treatment the shoot moisture content are low. According to a research calcium and magnesium have a greater impact on shoot (Qureshi *et al*., 2013).

From our experiments, it is evident from Table 2 that calcium and magnesium ratios have a significant impact on leaf parameters that we observe to leaf length in a treatment that has a high value in T5 (calcium and magnesium ratio of 2) and polyethylene glycol-induced stress is 0.2 MPa, whereas the lowest value in a leaf length in a treatment of T7 (PEG stress of 0.6 MPa and have no calcium and magnesium ratio) and have no calcium and magnesium ratio. The treatment with a high calcium and magnesium ratio will therefore have a high value of shoot dry weight, while the treatment with a low calcium and magnesium ratio will have a lower value of shoot dry weight. When wheat seedlings were exposed to polyethylene glycol (PEG), their leaf growth and shrinking abruptly stopped, as observed using a highly sensitive growth sensor (Veselov *et al*., 2002).

As a result, by examining the leaf dry weight as well, it can be determined that treatments with high calcium and magnesium ratios result in high shoot dry weight values, while treatments with low calcium and magnesium ratios and PEG stress result in lower leaf dry weight values. And it is also evident from shoot fresh weight that a treatment with a high calcium to magnesium ratio and a high value for fresh weight, such as T3, and a treatment with a low value for leaf dry weight, such as T7, are in contrast. And if we look at the moisture content of the shoots, it is also evident that a treatment with a high calcium to magnesium ratio is effective. And if we look at the amount of moisture in the leaves, it is also evident that treatments with high calcium and magnesium ratios also have high levels of moisture in the leaves, whereas treatments with low calcium and magnesium ratios and significant PEG stress have low moisture in the leaves (Mastoidis and Koroneiki). Both cultivars of plants were subjected to drought stress, which led to a reduction in the size of the epidermal and mesophyll cells and an increase in cell density (Bosabalidis and kofidis 2002).

Chlorophyll are the main essential pigment for photosynthesis which converts light energy into chemical energy (Chen, *et al.*, 2011). Current results show that the calcium and magnesium ratio have a significant effect on chlorophyll content if we see Figures 3,4,5 and 6 in that in treatment which have a high value of calcium and magnesium ratio have a high value of chlorophyll and the treatment which have no calcium and magnesium ratio and have a PEG induced drought stress in that treatment the contents of all chlorophyll are low. Rambabu *et al*., (2016) investigated the effect of drought Stress on Chlorophyll content and Anti-Oxidant Enzymes of Green Gram Genotypes (*Vigna Radiata* L.) and resulted that the total chlorophyll content decreased due to water stress and it was positively and significantly correlated with the seed yield. Baghbani *et al*., (2017) evaluated how ultraviolet radiation (abiotic stress) influenced the chlorophyll, carotenoid, protein, and proline concentrations of several annual desert plants. They found that increased UV radiation had an impact on the chlorophyll contents.

Carotenoid are the main accessory pigment for photosynthesis which converts light energy into chemical energy from Figure 6 it clear that the maximum carotenoid contents are found at $p<0.05$ in control and the minimum are found in treatment which have only polyethylene glycol of 0.6 MPa and not have calcium and magnesium ratio, it clear that calcium and magnesium have a positive effect on carotenoid contents under PEG induced drought stress. Liatile *et al*., (2021) experimented with changing the photosynthetic pigment of water-stressed cowpea, and the results showed that daily watering had the greatest concentration of all photosynthetic pigments, including chlorophyll a, chlorophyll b, and carotenoid content.

Getachew *et al.*, (2000) also studies that the efficiency of microbial protein synthesis, measured as mol purine/mol SCFA, was significantly ($P < 0.05$) decreased by the addition of PEG. And from current study that the calcium and magnesium have a best role in protein content under a PEG induced drought stress if we see in figure 1 that clear that in treatment which have no calcium and magnesium have low protein content Clashing results have been described from our result slight but significant decrease in protein level was also observed under the PEG induce drought from research of Benhabbour, *et al.*, (2008) results showed that protein adsorption decreased with increasing PEG.

Since lipids make up the majority of membranes, they contribute to plant cells' resilience to environmental challenges. A severe water shortage causes a perturbation of the interaction between proteins and membrane lipids as well as to a reduction in the transport and activity of the enzyme the bilayer's capacity (Yordanov et al., 2003). Drought Stress caused significant lipid alterations in metabolism (Parvaneh *et al.*, 2012), in our result that Figure 7 indicated that the maximum lipid contents at $p<0.05$ reported in control and minimum at $p<0.05$ in 4 Ca/Mg ratio and PEG 0.6 MPa stress it mean in treatment which have only PEG induce stress and not have Ca/Mg ratio in which a low lipid was detected.

From the result of Puente *et al.*, (2017) it is clear that there was no observable difference in total phenolic chemicals across treatments. In vitro-grown *Agave salmiana* L. plants responded to PEG-induced drought stress by increasing their saponin content and antioxidant activity. And from our studies we find that in figure 8 it is indicated that at $p<0.05$ the maximum phenol is found in control which have no treatment and the minimum are found in treatment which have no calcium and magnesium ratio and stress have 0.6 MPa polyethylene glycol PEG.
Tawfik (2008) examined the impact of water stress in addition to calcium treatment on plant and discovered that plants exposed to water stress produced more osmo-protectants including total soluble sugars, proline, and glycine betaine. That result is in parallel with our result that in our experiment that the PEG induced stress have a positive impact on proline i.e., reduced in a treatment which have a calcium and magnesium concentration high and have increased value in treatment have no calcium and magnesium ratio and only PEG induced stress. Hashem *et al.*, (2019) have also a result same to our Proline showed improvement across all cultivars, with

G179 recording the highest enhancement value and Sk101 the lowest increase in comparison to control plants. These findings were in line with those made by Purbajanti *et al.*, (2017) who claimed that the proline content of the rice cultivars under study's leaves rose during drought stress.

The buildup of proline and soluble sugars, along with increased levels of total phenolics, total flavonoids, and total antioxidant capacity, may be major factors in faba bean drought resistance, improving osmotic adjustment and free radical resistance (Abid *et al*.,2021). From our results it is clear that the maximum chlorophyll contents are found at $p>0.05$ in Ca/Mg ratio 4 and at PEG 0.6 MPa l and the minimum are found in treatment which have 0.18 calcium and magnesium ration and polyethylene glycol of 0.2MPa. as same to Alsayed et al., (2012) on the other hand, the methanolic extract without any additives had the maximum flavanol level (control).

CONCLUSION

It is concluded from the present study that in the presence of calcium and magnesium plant resist the drought for a limited time. When available amount of Ca and Mg either finished or became low then plant affected by drought. Continuously addition of Ca & Mg might reduce the drought stress in plants. Application of ca/mg quotient to plants exposed to drought stress regulates its metabolites, increase dry matter and also chlorophyll contents. So the application of Ca/Mg quotient enabled drought stressed *Trigonella foenum-graecum* plant to cope with the prevailing stress environment by maintaining its proper root & shoot growth.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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AUTHOR CONTRIBUTION

Hussan Ara Begum and Syed Hidayat Yar designed the project and performed the experiment. Asif Khan, Basit Ali and Behram Ali prepared the very first draft of manuscript. Hussan Ara Begum and Asif Khan review the article and approve for final submission.

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