BRAZILIAN JOURNAL OF BIOLOGY®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Exogenous Ca/Mg quotient reduces the inhibitory effects of PEG induced osmotic stress on *Avena sativa* L.

O quociente de Ca/Mg exógeno reduz os efeitos inibitórios do estresse osmótico induzido por PEG em *Avena sativa* L.

S. Bibi[a†], S. Ullah[a*], Aqsa Hafeez[b], M. N. Khan[c,d†], M. A. Javed[e], B. Ali[b], I. U. Din[f], S. A. K. Bangash[f], S. Wahab[b], N. Wahid[b], F. Zaman[d], S. K. Alhag[g,h], I. H. A. Abd. El-Rahim[i,j], A. E. Ahmed[k,l] and S. Selim[m*]

[a]University of Peshawar, Department of Botany, Peshawar, Pakistan
[b]Quaid-i-Azam University, Department of Plant Sciences, Islamabad, Pakistan
[c]The University of Agriculture, Agriculture University Public School and College (Boys), Peshawar, KP, Pakistan
[d]Islamia College Peshawar, Department of Botany, Peshawar, Pakistan
[e]Government College University, Institute of Industrial Biotechnology, Lahore, Pakistan
[f]The University of Agriculture, Institute of Biotechnology and Genetic Engineering, Peshawar, Pakistan
[g]King Khalid University, College of Science and Arts, Biology Department, Muhayl Asser, Saudi Arabia
[h]Ibb University, College of Science, Biology Department, Ibb, Yemen
[i]Umm Al-Qura University, Department of Environmental and Health Research, Makkah Al-Mukaramah, Saudi Arabia
[j]Assiut University, Faculty of Veterinary Medicine, Department of Animal Medicine, Infectious Diseases, Assiut, Egypt
[k]King Khalid University, College of Science, Department of Biology, Abha, Saudi Arabia
[l]South Valley University, Faculty of Veterinary Medicine, Department of Theriogenology, Qena, Egypt
[m]Jouf University, College of Applied Medical Sciences, Department of Clinical Laboratory Sciences, Sakaka, Saudi Arabia

[†]These authors contributed equal to this work.

Abstract

Drought is one of the most damaging abiotic stress that hinder plant growth and development. The present study aimed to determine the effects of various Ca/Mg quotients under polyethylene glycol (PEG)–induced osmotic stress on growth, uptake and translocation of Ca and Mg in *Avena sativa* (L). Plants were grown in nutrient solution supplemented with three different Ca/Mg molar quotients (0.18, 2, and 4). After 30 days plants were exposed to two different PEG (Polyethylene glycol) concentrations (0.6 MPa & 0.2 MPa) for 8 days, and solutions were renewed after 4 days. A solution containing Ca and Mg nutrients has mitigated the negative impact caused via osmotic stress on relative growth rate (RGR), absolute growth rate (AGR), crop growth rate (CGR), leaf area ratio (LAR), Leaf index ratio (LAI), root-shoot ratio (RSR), water use efficiency (WUE) and net assimilation rate (NAR). In addition, it adversely affected germination parameters, including final emergence percentage (FEP), mean germination time (MGT), Timson germination Index (TGI), germination rate index (GRI) and percent field capacity (%FC), of oat (*Avena sativa* L.). Mg and Ca in shoot and root and Ca translocation factor decreased with increasing Ca in solution, while Mg translocation factor increased with increasing Ca in nutrient solution. In this work, the combined effects of various Ca/Mg quotients and osmotic stress produced by polyethylene glycol (PEG) in different concentrations (0.6 MPa, 0.2 MPa) on the growth and element uptake of *Avena sativa* L. are examined. As a result, the Ca/Mg Quotient may naturally combat the moderate drought stress experienced by field crops.

Keywords: drought, inhibitory effects, PEG stress, translocation factors, magnesium, *Avena sativa* L.

Resumo

A seca é um dos estresses abióticos mais prejudiciais que dificultam o crescimento e o desenvolvimento das plantas. O presente estudo teve como objetivo determinar os efeitos de vários quocientes de Ca/Mg sob estresse osmótico induzido por polietilenoglicol (PEG) no crescimento, absorção e translocação de Ca e Mg em *Avena sativa* L. As plantas foram cultivadas em solução nutritiva suplementada com três diferentes quocientes molares de Ca/Mg (0,18, 2 e 4). Após 30 dias, as plantas foram expostas a duas concentrações diferentes de PEG (0,6 MPa e 0,2 MPa) por 8 dias, e as soluções foram renovadas após 4 dias. Uma solução contendo os nutrientes Ca e Mg mitigou o impacto negativo causado por estresse osmótico na taxa de crescimento relativo (RGR), taxa de crescimento absoluto (AGR), taxa de crescimento da cultura (CGR), razão de área foliar (LAR), razão de índice foliar (LAI), razão raiz-parte aérea (RSR), eficiência do uso da água (WUE) e taxa de assimilação líquida (NAR). Além disso, afetou

*e-mail: samibotany@uop.edu.pk; sabdulsalam@ju.edu.sa
Received: June 6, 2022 – Accepted: July 28, 2022

negativamente os parâmetros de germinação, incluindo porcentagem final de emergência (FEP), tempo médio de germinação (MGT), índice de germinação de Timson (TGI), índice de taxa de germinação (GRI) e capacidade de campo percentual (%FC) de aveia (*Avena sativa* L.). O Mg e o Ca na parte aérea e raiz e o fator de translocação de Ca diminuíram com o aumento de Ca em solução, enquanto o fator de translocação de Mg aumentou com o aumento de Ca em solução nutritiva. Neste trabalho, são examinados os efeitos combinados de vários quocientes de Ca/Mg e estresse osmótico produzido por PEG em diferentes concentrações (0,6 MPa e 0,2 MPa) no crescimento e na absorção de elementos de *Avena sativa* L. Como resultado, o quociente de Ca/Mg pode combater naturalmente o estresse hídrico moderado experimentado pelas lavouras.

Palavras-chave: seca, efeitos inibitórios, estresse PEG, fatores de translocação, magnésio, *Avena sativa* L.

1. Introduction

Avena sativa L. (Gramineae), commonly known as Oat, Groats, Haber, Hafer, Avena, Straw, Oatmeal, is a species of cereal grain grown for its seed. It is an annual grass about 1.5 meters high; culms tufted or solitary, erect or bent at the base, smooth. The leaves are non-articulate, green. The inflorescence is a diffuse panicle with 2–3 florets. The rachilla of the cultivated oat does not disarticulate at maturity. Its lemmas are rarely awed. The grain is tightly enclosed by the hard lemma and palea. Seed size varies with the cultivar; it is commonly about 30,000 seeds per kilogram crop (Suttie, 2004; Singh et al., 2013). Oats have numerous uses in foods; most commonly, they are rolled or crushed into oatmeal, or ground into fine oat flour (Miraj and Kiani, 2016). Oats rank around sixth in the world cereal production statistics following wheat, maize rice, barley and sorghum. Oat grain has always been an important form of livestock feed. Oats are better adapted to variable soil types and can perform better on acid soils than other small grain cereals crops. They are mostly grown in cool moist climates and they can be sensitive to hot, dry weather from head emergence through to maturity (Mushtaq et al., 2014). A wide range of chemical constituents like carbohydrates, proteins, avenanthramides, tocols, lipids, alkaloids, flavonoids, saponins, and sterols have been reported from *A. sativa*. Traditionally oats have been in use since long and are considered as stimulant, antispasmodic, antitumor, diuretic, and neurotonic. Oat possesses different pharmacological activities like antioxidant, anti-inflammatory, wound healing, immunomodulatory, antidiabetic, anticholesterolaemic, etc. A wide spectrum of biological activities indicates that oat is a potential therapeutic agent (Singh et al., 2013).

Abiotic factors such as cold, temperature (Saeed et al., 2022), drought (Wahab et al., 2022; Farooq et al., 2022; Dola et al., 2022), heavy metals (Nawaz et al., 2022; Ma et al., 2022a, b), salt concentration (Khan et al., 2022; Hussain et al., 2022; Ali et al., 2022c, d), and nutrient deficiency (Adnan et al., 2022; Ahmad et al., 2022) drastically affect crop yield and productivity. Drought stress is known as the most influential abiotic stress or environmental factor that inhibit plant growth and production. Drought exists either due to extremely less rainfall or significant distinction in the quantity of moisture, and is considered as substantial abiotic stress hindering agriculture (Nawaz et al., 2012; Saleem et al., 2022). Drought stress individually has appeared to be the most hazardous intimidation to global food security. It cause severe effect on agricultural production particularly in the area where rainfall is confined or uncertain. Hence to improve crop production subordinated to insufficient water caused to be the prominent hindrance for the worlds' subtropical and arid regions (Nawaz et al., 2012; Faryal et al., 2022). Climate change is one of the significant challenges faced by Pakistan and adds a great deal of stress to societies and environment (Adedeji et al., 2014; Afridi et al., 2022). Climate change causes an upswing in sea level, variations in rainfall sequences, and climatic regions' movement due to increased temperatures (Solomon et al., 2007). Incidences of droughts, storms, and floods are estimated to increase due to fluctuating climatic patterns. Climate change has become an extravagant challenge for the agrarian economy of Pakistan (Shakoor et al., 2011). Different studies have demonstrated that nutrients are known to be chemical in general or chemical elements required for better production, crop development, growth as well as role in plant external activity and metabolism. The continuous droughts in Pakistan have been affecting the Indus Basin since the 19th century. In a report by the Economic Survey of Pakistan, the stagnant economic growth of the country has been due to many factors, drought being a major one. The drought of 1998-2002 was the worst drought to hit Pakistan especially province of Balochistan and Sindh were most badly affected. Thousands of acres of crops destroyed and livestock killed. This drought was estimated to have affected about a total of 3.3 million people and about 30 million livestock were affected, that included approximately 2 million deaths (Jing et al., 2007; Durrani, 2018).

Osmotic stress is produced via polyethylene glycol (PEG 6000), it also reduced rate of photosynthesis thereafter influencing chlorophyll *a* and chlorophyll *b*. different photosynthesis mechanism at cellular level containing photosystem, pigments, CO_2 reduction pathway and electron transport in plant are being affected by numerous stresses. Plant scarcity is determined by PEG i.e., seed resistance to drought by the drought index determination, seed imbibition and hydration (Landjeva et al., 2008). South Asian countries, including Pakistan, this mainly due to their geographic location as well as agricultural and water resources are heavily reliant, and their population has a limited ability to adaptation, and a weak disaster preparedness infrastructure, they are vulnerable to climate change concerns (Ali and Erenstein, 2017).

It is examined that drought stress considerably reduce root features including the length of root, root diameter, root surface, root volume and root dry weight by Junjittakarn et al. (2014). It has been reported that drought treatments reduced growth variables like relative growth rate (RGR), crop growth rate (CGR), leaf area index (LAI), and net assimilation rate (NAR) (Hossain et al., 2010). Growth parameters of two maize cultivars were reported to be affected by drought stress, including germination potential, germination rate, root and shoot length, and fresh root and shoot mass (Liu et al., 2015). Mukhtar's, research (Mukhtar, 2016) on the other hand, demonstrated the influence of drought on RGR, AGR, and net assimilation rate (NAR). Additionally due to increase in drought stress cause reduction in seedling growth and seed germination (Gharmakher et al., 2017). In accordance with Salehi-Eskandari et al. (2018) increase in Ca/MG content cause enhanced growth in *Alyssum inflatum* and *Fortuynia garcinii,* while *A. inflatum* confront drastic reduction in growth and survival. Alghabari and Ihsan (2018) studies illustrated that biomass accumulation and plant height are severely decreased under water scarcity condition, although variance in fresh biomass accumulation (45%) was comparatively high then dry biomass. Raza et al. (2017) also worked on effects of drought stress on (*Brassica napus* L.). Aslam et al. (2013) investigated the morphological and physiological response of maize hybrids under drought stress. Aslam et al. (2013) and Solanki et al. (2022) studied drought stress tolerance in crops by using plant growth promoting rhizobacteria. Arun et al. (2020) reported the mitigation of drought stress in rice crop by the application of plant growth-promoting. Population is increasing day by day and we need to increase the food production to meet this challenge. By keeping in mind this current issue, we investigated the combined effects of different Ca/Mg quotients and osmotic stress generated by polyethylene glycol (PEG) of 0.6 MPa and 0.2 MPa on *Avena sativa* L. growth and element uptake.

2. Materials and Methods

2.1. Experimental site

The research study was performed during 2018 in growing season, in the Department of Botany, University of Peshawar, Pakistan. Peshawar is situated on the Iranian plateau, which has a tropical environment. Peshawar receives 513 mm of yearly rainfall on average. The mean maximum temperature in summer is over 40 °C and the mean minimum temperature is 25 °C. The mean minimum temperature during winter is 4 °C and maximum is 18.35 °C. The relative humidity varies from 46% in June to 76% in August and precipitation amounts to 410 millimeters (16.1 inches) per year (Khan et al., 2018).

2.2. Seed material and procedures

Pure and uniform sized of same colored seeds which is smooth surfaced were preferred in current research. Surface sterilization of required seeds were performed with 70% ethanol method reported by Ali et al. (2022a).

Solution was prepared by using Ca/Mg in different proportions, three different salts such as $MgSO_4$, Mg $(NO_3)_2$, and Ca $(NO_3)_2$ were added to the solution. In addition, for the desired pot experiment different quotient of $MgSO_4$, Mg $(NO_3)_2$ and Ca $(NO_3)_2$ were used. $MgSO_4$ was used for 4, 2 and 0.18 quotients (0.75mM), while Mg $(NO_3)_2$ was used for 4 and 2 quotients (0 mM) and 0.18 quotient (1.35mM) and finally the Ca $(NO_3)_2$ was used quotient 4 (3mM), for quotients 2 (1.5mM) and for quotient 0.18 (0.38Mm). For the preparation of 4 quotients solution 90g $MgSO_4$, 492g Ca $(NO_3)_2$ were homogenously dissolved in distilled water. While 90g of $MgSO_4$, 246g of Ca $(NO_3)_2$ were dissolved in distilled water to prepare solution of 2 quotients. Finally, Mg $(NO_3)_2$ (200mg), $MgSO_4$ (90mg), and Ca $(NO_3)_2$ (62mg) were dissolved in distil water for the 0.18 quotient solution. Furthermore, continuously every four days, these solutions were aerated and replenished. After 30 days, the plants were subjected to PEG (Polyethylene glycol) induced osmotic stress. PEG with molecular weight of 6000 or above (PEG 6000) due to its inert, non-ionic, and non-absorbable cannot taken by plants (Salehi-Eskandari et al., 2018). As a result, PEG is commonly used to cause water stress and keep a constant osmotic pressure during an experiment. Three pots were supplied with PEG at the rate of 209.7 g L^{-1}, and was supplied to each Ca/Mg quotient and has a water potential of 0.6 MPa. PEG was supplied in 0.2-MPa phases with 4-hour intervals to reduce osmotic shocks. Evaporation was accounted for by adding distilled water on a regular basis to the solutions and after four days the solutions were renewed regularly. The plants were split into roots and shoots, after 8 days of PEG stress, and immediately weighed, and then dried at 70°C for 72 hours before being weighed again.

2.3. Elemental analysis

The elemental analysis of samples were performed as reported by Salehi-Eskandari et al. (2018). For elemental analysis the dried plant materials were combined in 70 percent nitric acid (v/v) in a water bath at 90°C for 4 hours. Afterwards, 2 mL hydrogen peroxide was added to the solution after it was cooled to room temperature, and it was incubated at 90°C until it became transparent. Additionally, atomic absorption spectrophotometry was used to evaluate the amounts of Mg, K, and Ca in root shoots and soil (AAS, Shimadzu Model 6200 Shimadzu, Tokyo, Japan). In addition, by dividing the accumulated concentration in the shoot by the concentration in the root, translocation factors (TFs) for Ca or Mg were computed). Summary of the experiments are given in Table 1.

Table 1. Summary of the experimental design.

Treatments	% Extract applied
T1	Control
T2	4 quotient + 0.6 MPa PEG
T3	4 quotient + 0.2 MPa PEG
T4	2 quotient +0.6 MPa PEG
T5	2 quotient + 0.2 MPa PEG
T6	0.18 quotient +0.6 MPa PEG
T7	0.18 quotient + 0.2 MPa PEG

2.4. Growth parameters measurements

2.4.1. Net Assimilation Rate (NAR)

The analysis for NAR was done in accordance with authorized method of Ahmad et al. (2020). The formula to calculate the NAR (Equation 1).

$$NAR = \left[W_2 - W_1 / t_2 - t_1 \times Loge\ A_2 - Loge\ A_1 \left(g / cm / day\right) / A_2 - A_1\right] \quad (1)$$

Whereas dry weight denoted by W_1 and W_2 at the time is denoted by t_1 and t_2, leaf surface area is denoted by A_1 and A_2 at t_1 and $t_{2.}$

2.4.2. Leaf Area Index (LAI) measurement

For the measurement of LAI we have followed the formula reported by Shah et al. (2017). The formula for calculating the LAI as given below (Equation 2):

$$LAI = \left[Leaf\ area\ \left(cm\right)^2 / Land\ area \left(cm\right)^2\right] \quad (2)$$

2.4.3. Absolute Growth Rate (AGR)

Following formula was used to estimate the absolute growth rate reported by Mukhtar (2016) (Equations 3-4).

$$AGR\left(plant\ height\right) = \left[H_2 - H_1 / t_2 - t_1\right] \quad (3)$$

$$AGR\left(dry\ matter\right) = \left[W_2 - W_1 / t_2 - t_1\right] \quad (4)$$

Whereby plant height is represented by H_1 and H_2, plant dry matter is indicated by W_1 and W_2 at t_1 and t_2 time.

2.4.4. Relative Growth Rate (RGR) measurement

Relative growth rate was measured by formula mentioned below is used to illustrate the relative growth according to Ahmad et al. (2020) (Equation 5)

$$RGR = \left[Log_e W_2 - Log_e W_1 /\ t_2 - t_1\right] \quad (5)$$

Whereas, dry mass is W_1 and W_2 at time represented as t_1 and t_2, where Log_e is noticed as natural logarithms.

2.4.5. Estimation of Mean Germination Time (MGT)

For the estimation of MGT (mean germination time) the formula reported by Sadeghi et al. (2011) was used to calculate mean germination time (MGT) (Equation 6).

$$MGT = \left[\sum Dn / \sum n\right] \quad (6)$$

Where, number of seeds for germination are denoted by Dn at day D, whereas number from start of the germination on experiments is n to day D.

2.4.6. Analysis of Crop Growth Rate (CGR)

The CGR was analyzed according to the Shah et al. (2017). The formula for calculating the CGR as follows (Equation 7):

$$Analysis\ of\ Crop\ growth\ rate = \left[W_2 - W_1 / t_2 - t_1 \times 1 / Land\ area \left(g / m^2 / d\right)\right] \quad (7)$$

Where, dry weight is indicated by W_1 and W_2 at two different time point indicated by t_1 and t_2.

2.4.7. Leaf Area Ratio (LAR) calculation

We followed the methodology of Shah et al. (2017) for calculating the leaf area ratio by combining leaf area and dry plant matter. For the purpose of calculating LAR, we utilized the following formula (Equation 8):

$$LAR = \left[Leaf\ area /\ Final\ plant\ dry\ mass\right] \quad (8)$$

2.4.8. Root to Shoot Ratio (RSR) calculation

Oven dried plant material is used to illustrate the shoot-root ratio mentioned by Chuyong and Acidri (2017) (Equation 9).

$$RSR = \left[root\ dry\ mass / shoot\ dry\ mass\right] \quad (9)$$

2.4.9. Timson Germination Index (TGI)

The Timson germination index was analyzed according to Al-Ansari and Ksiksi (2016). The formula for TGI as follows (Equation 10):

$$Timson\ germination\ index = \left[\sum G / T\right] \quad (10)$$

Where, G indicate the germinated seeds % per day and T indicate the germination time interval.

2.4.10. Analysis of Water Use Efficiency (WUE)

The oven-dried weights were summed up to obtain total biomass (TB) that was used to compute the water use efficiency (WUE) from the relation of Chuyong and Acidri (2017). The formula for the WUE is as follows (Equation 11):

$$Water\ use\ efficiency = total\ water\ used\ in\ experiment\ \left(ml\right) / total\ biomass \quad (11)$$

2.4.11. Evaluation of percent field capacity (%FC)

The %FC was measured by using formula reported by Ullah et al. (2016) for the measurement of field capacity of rhizospheric soil in percent (Equation 12).

$$Field\ Capacity\ \left(\%\right) = [\frac{W1\ \left(g\right) - W2\ \left(g\right)}{W1\ \left(g\right)} \times 100] \quad (12)$$

Where, W1 represent wet soil weight of and W2 represent dry soil weight of in grams.

2.4.12. Final Emergence Percentage (FEP)

The FEP was measured according to Babar et al. (2014). The formula for final emergence percentage as follows (Equation 13):

$$Final\ emergence\ \left(\%\right) = no.\ of\ seeds\ emerged / total\ no.\ of\ seeds\ sown\ \times 100$$

2.4.13. Germination Rate Index (GRI)

Kader (2005) give the formula which is validated to calculate the germination % at every day during germination which is shown via germination rate index (Equation 14).

$$GRI = G_1 / + G_2 / 2 + \ldots\ldots\ldots\ldots + G_X / X \quad (14)$$

G1 represents germination % on day one, G2 represents germination % on day two, and GX represents germination % on day X.

2.4.14. Soil percent moisture content (%MC)

To calculate dry weight, soil is oven dried at 70°C for 72 hours till the constant weight. The %MC was calculated as suggested by Ullah et al. (2016). The following formula was used to measures the moisture content of soil (Equation 15):

$$\%MC = [\frac{W1 - W2}{W1} x100] \quad (15)$$

Where, W1 represent fresh soil weight of and W2 represent dry soil weight of in grams.

2.4.15. Evaluation of Seed Vigor Index –I (SVI)

The measurement of SVI for seedling length, Bina and Bostani (2017) methods used for measuring the seed vigor index. The formula for SVI as follows (Equation 16):

$$SVI = \left[L\ (cm)\ x\ G\ (\%)\right] \quad (16)$$

Where L represent the length of seedling in cm, and G represent the germination of seeds in percent.

2.4.16. Evaluation of Seed Vigor Index-II (SVII)

Al-Ansari and Ksiksi (2016) gave the formula to describe seed vigor index for dry matter (Equation 17).

$$Seed\ vigor\ index - II = \begin{bmatrix} Seedling\ dry\ weight\ (mg)\ x \\ Seed\ germination\ \%\ age \end{bmatrix} \quad (17)$$

2.4.17. Analysis of plant height stress index (%)

Ellis and Roberts (1981) reported formula to calculate plant height stress index (PHSI). Therefore, in the current study we used the following formula for calculating PHSI in percent (Equation 18):

$$PHSI\ (\%) = \left[(PS - 1\ /\ PS - 2)\ x\ 100\right] \quad (18)$$

Where, PS-1 represent plant height of stressed plant, and PS-2 represent plant height of control plants.

2.4.18. Measurement of Mean Emergence Time (MET)

Using Ellis and Roberts' methodology, the mean emergence time was recalculated (Ellis and Roberts, 1981). The formula for MET was as follows (Equation 19)

$$MET = \left[\sum Dn / \sum n\right] \quad (19)$$

Where D denotes the number of days since the first emergence of the seed, the number of seeds that appeared on day D is denoted by the letter n.

2.4.19. Evaluation of Emergence Index (EI)

For the analysis of emergence index the formula of the Association of Official Seed Analysis (AOSA) was used.

2.4.20. Analysis of germination percentage

Formula described by Gharmakher et al. (2017) was used to calculate the germination % (Equation 20).

$$Germination\ percentage\ (GP) = \left[\sum G / N \times 100\right] \quad (20)$$

Where N is the number of total seeds, while G is the number of germinated seeds.

2.4.21. Analysis of Emergence Energy (EE)

Basra et al. (2005) proposed the formula to measure emergence energy. It's the proportion of the total number of seeds sown to the number of seeds that appeared after the fourth day after sowing. It is expressed as a percentage (Equation 21).

$$Emergence\ energy\ (\%) = \begin{bmatrix} seeds\ emerged\ after\ 4\ days\ of\ sowing\ / \\ total\ no.\ of\ seeds\ sown \times 100 \end{bmatrix} \quad (21)$$

2.4.22. Analysis of Leaf Water Content (LWC)

The water content of the leaf was calculated from the fresh (FW) and dry weight (DW) according to Ali et al. (2022b) (Equation 22):

$$LWC = FW - DW / FW \times 100 \quad (22)$$

2.4.23. Statistical analysis

Experimental data was calculated by computing standard errors and means values. The Statistix 8.1 software was used to analyze all the collected data by applying analysis of variance (ANOVA) and pairwise comparison between all mean values was computed by using LSD test ($p = 0.05$).

3. Results

3.1. Effects of Ca/Mg quotients and PEG induced osmotic stress on plant fresh and dry matter yield

The study revealed that total Shoot length (Figure 1) was observed maximum at 0.6 MPa in Ca/Mg = 0.18 quotient, followed by highest root length in the absence of PEG as shown in Figure 1. The shoot fresh weight was highest in the absence of PEG (Figure 1). In the Ca/Mg = 0.18 quotients, the lowest PEG treatment (0.2 MPa) resulted in the highest dry weight of the shoot and root, whereas Ca/Mg = 4 quotients,

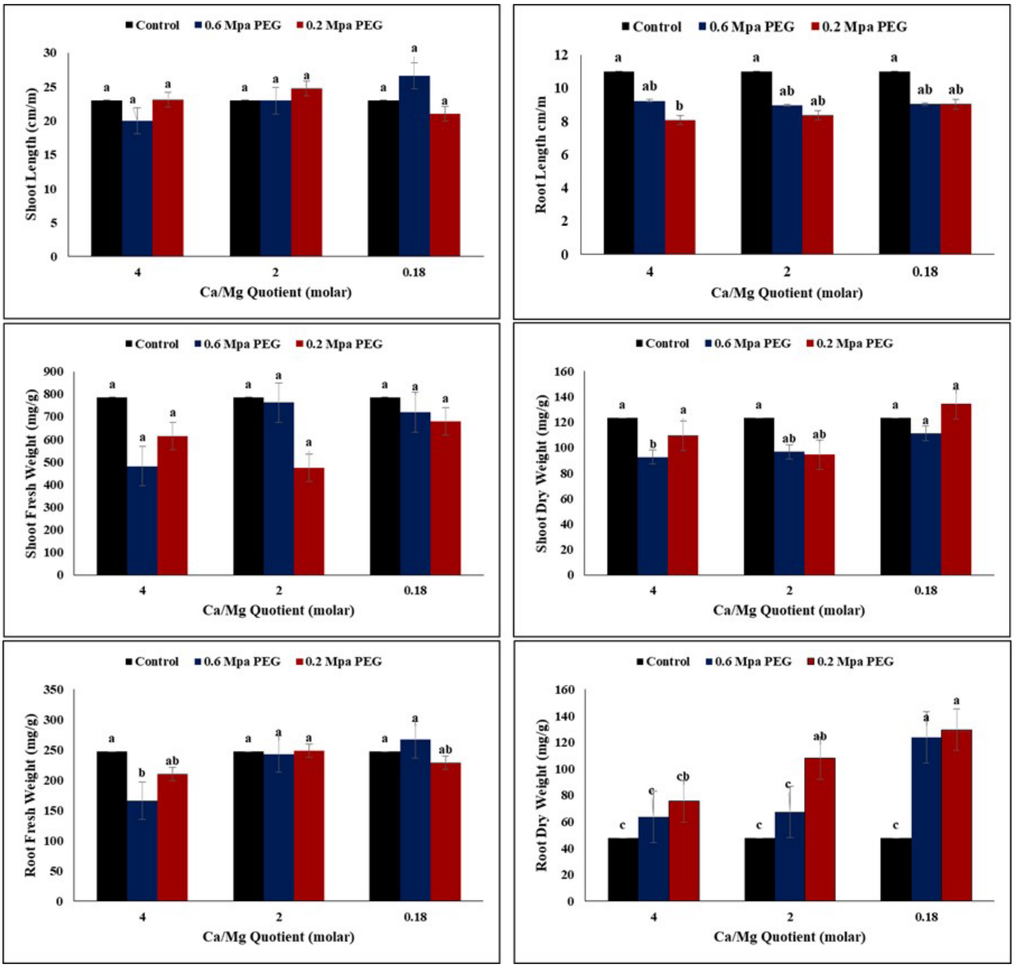

Figure 1. Effects of different Ca/Mg quotients on growth and biomass of *Avena sativa* L. under PEG-imposed osmotic stress. Statistically significant differences ($p<0.05$) between means using LSD test are indicated with different letters.

shoot dry weight (Figure 1) was lower at 0.6 MPa PEG treatment. However, at 0.6 MPa PEG generated osmotic stress, shoot and root fresh weight were lower in Ca/Mg = 4 quotients, while root fresh weight was highest in Ca/Mg = 0.18 quotient (Figure 1) while, root dry weight was lower in the absence of PEG (Figure 1). The experiment showed the highest absolute growth rate in terms of plant height when PEG was not present. The minimum absolute growth rate was reported at 0.2 MPa PEG simulated drought stress, indicating that the absolute growth rate decreased with PEG. While subjecting to plant weight, the absence of PEG resulted in the lowest absolute growth rate. Different biochemical and physiological processes like growth, reproduction, development, metabolism etc. under drought stress therefore plant development is affected (Jaleel et al., 2008). Additionally it is also determined that drought stress has numerous effect in processes of development and growth of plant like germination, number of leaves, leaf area, leaf size, plant height, stem ratio, dry matter distribution and production, fruit, flower maturity and production (Anjum et al., 2017).

3.2. Effects of Ca/Mg quotients and PEG-induced osmotic stress on Ca/Mg concentrations and translocation factors

At 0.2 MPa PEG stress level, the shoot Ca concentration was maximum in Ca/Mg = 0.18 treatment, while the shoot Ca concentration was lowest in Ca/Mg = 4 quotients at 0.6 MPa PEG induced stress level (Figure 2). By applying Ca/Mg = 0.18 quotients, the root Ca concentration was lowest at 0.6 MPa PEG; however, highest at 0.2 MPa PEG (Figure 2). With increasing Ca and Mg nutrients in the solution, the Ca content in the shoots and roots increased. At 0.6 MPa PEG induced stress, the shoot Mg concentration was maximum in Ca/Mg = 2 quotients (Figure 2), while the root Mg concentration was maximum in Ca/Mg = 2 quotients at 0.2 MPa PEG induced stress (Figure 2). The shoot and root Mg concentrations were lowest at 0.6 MPa PEG induced stress. As the PEG induced stress increased, the shoot and root Mg concentrations decreased (Figure 2). The Ca translocation factor was highest in the presence of 0.2 MPa PEG in the Ca/Mg = 0.18 treatment (Figure 2), whereas the Mg translocation factor was highest in the presence of 0.6 MPa PEG in the Ca/Mg = 2 treatment (Figure 2). In the

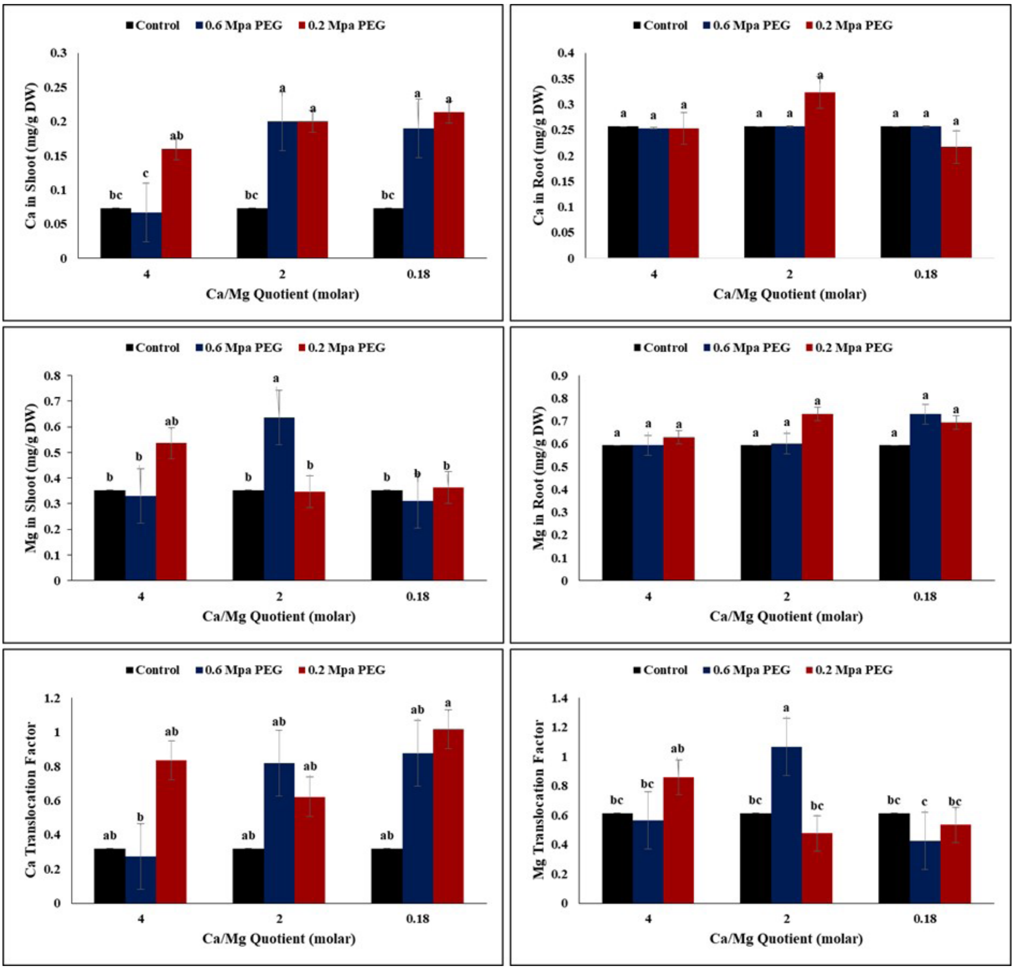

Figure 2. Effects of different Ca/Mg quotients on Ca/Mg concentrations and translocation factors of *Avena sativa* L. under PEG-imposed osmotic stress. Statistically significant differences (p<0.05) between means using LSD test are indicated with different letters.

presence of PEG (0.6 MPa) in Ca/Mg = 4 and 0.18 treatment Ca and Mg translocation factor was minimum.

Results revealed that germination rate index (GRI) and Timson germination index (TGI) were lowest in Ca/Mg = (2 quotients) at 0.6 Mpa PEG, indicating that PEG stress influenced Timson germination index and germination rate index (Table 1). Our findings are consistent with those of Qayyum et al. (2011), who discovered that increasing osmotic stress lowers germination characteristics. Furthermore, Hossain et al. (2010) proposed that the growth specification such as crop growth rate (CGR), relative growth rate (RGR), and net assimilation rate (NAR) were decreased when treated under drought stress; the lowest CGR, NAR, and RGR were found in Ca/Mg = 4 quotients at 0.6 Mpa, according to the findings. It has been reported that seed germination and seedling growth were hampered by water scarcity (Batool et al., 2014).

3.3. Effects of Ca/Mg quotients and PEG induced osmotic stress on plant growth

It was observed that the absolute growth rate of height (AGRH) and leaf area ratio (LAR) was highest in the absence of PEG, and crop growth rate (CGR), relative growth rate (RGR), absolute growth rate of weight (AGRW) and net assimilation rate (NAR) were high in Ca/Mg = 0.18 treatment in the presence of 0.2 MPa PEG (Table 2). Leaf area index (LAI) was maximum in Ca/Mg = 4 treatment in the presence of 0.6 MPa PEG stress, while crop growth rate (CGR) relative growth rate (RGR), absolute growth rate of plant weight (AGRW), and net assimilation rate (NAR) were minimum at that treatment, mean germination time was utmost in Ca/Mg = 2 treatment at 0.6 MPa PEG. The absolute growth rate of height (AGRH) and leaf area index (LAI) was lowest in Ca/Mg = 4 quotients in the presence of 0.2 MPa PEG stress, leaf area ratio (LAR), and mean germination time was lower in Ca/Mg = 0.18 quotient at 0.2 MPa PEG stress (Table 2).

Ca/Mg = 0.18 treatment in the presence of 0.2 MPa PEG showed highest Timson germination index (TGI), germination rate index (GRI), percent field capacity (% FC), final emergence percentage (FEP). Root to shoot ratio (RSR) was highest, while seed vigor index I was lowest in Ca/Mg = 4 quotients in the presence of 0.6 MPa PEG. Seed

Table 2. Interactive effect of Ca/Mg quotient on different parameters under PEG simulated drought stress of 0.6 MPa and 0.2 MPa.

Parameters	Control	Ca/Mg 4Q+0.6Mpa PEG	Ca/Mg 4Q+0.2Mpa PEG	Ca/Mg 2Q+0.6Mpa PEG	Ca/Mg 2Q+0.2Mpa PEG	Ca/Mg 0.18Q+0.6Mpa PEG	Ca/Mg 0.18Q+0.2Mpa PEG
Absolute growth rate of plant height (AGRH)	4.008±3.320	1.683±0.699	1.141±0.752	2.166±0.212	2.416±0.412	2.991±0.837	2.625±0.254
Absolute growth rate of plant weight (AGRW)	7.770±2.328	4.850±2.033	14.44±10.72	7.941±3.227	20.72±10.02	34.15±4.840	45.14±18.04
Relative growth rate (RGR)	344.7±67.28	303.4±35.31	371.2±76.61	316.4±35.59	417.9±48.22	514.1±25.33	586.5±166.3
Net assimilation rate (NAR)	17.89±5.360	11.16±4.681	33.25±24.70	18.28±21.25	47.71±23.09	78.65±11.14	103.9±41.55
Crop growth rate (CGR)	0.431±0.129	0.269±0.112	0.802±0.596	0.441±0.179	1.536±0.557	1.897±0.268	2.507±1.002
Leaf area index (LAI)	0.399±0.059	0.401±0.070	0.201±0.045	0.256±0.040	0.224±0.026	0.356±0.055	0.175±0.062
Leaf area ratio (LAR)	0.038±0.007	0.042±0.010	0.019±0.006	0.025±0.002	0.019±0.003	0.025±0.004	0.011±0.003
Mean germination time (MGT)	4.659±0.102	4.721±0.149	4.504±0.096	4.785±0.132	4.689±0.205	4.572±0.101	4.485±0.056
Timson germination index (TGI)	50.00±3.628	44.07±4.655	49.62±2.283	43.33±3.271	44.07±3.777	50.74±4.996	54.44±2.400
Root-shoot ratio (RSR)	0.936±0.266	1.072±0.220	0.963±0.212	0.993±0.192	0.993±0.192	0.470±0.044	0.708±0.101
Water use efficiency (WUE)	0.102±0.033	0.148±0.026	0.119±0.028	0.101±0.032	0.150±0.062	0.098±0.021	0.117±0.036
Percent field capacity (%FC)	9.105±1.276	9.504±3.869	10.371±5.941	10.354±2.580	10.010±2.995	10.629±1.049	17.91±3.934
Final emergence percentage (FEP)	100.0±0.000	88.88±3.142	86.66±0.000	95.55±6.285	91.11±6.285	95.55±6.285	100.0±0.000
Seed vigor index I (SVI- I)	3400±1033	2582±119.1	2698±125.0	3048±311.4	3040±540.8	3426±626.9	3006±420.5
Seed vigor index II (SVI- II)	13813±2868	13458±239.9	11460±2652	15300±3593	11151±4858	9707±2270	8576±1078
Germination rate index (GRI)	71.29±7.351	61.70±9.450	74.07±5.582	59.51±6.682	62.59±8.995	74.370±9.861	81.962±4.797
Mean emergence time (MET)	1.313±0.268	1.127±0.513	1.778±0.285	1.001±0.344	1.298±0.539	1.566±0.410	1.899±0.114
Germination energy (GE)	60.00±5.443	53.33±5.443	57.77±3.142	51.11±3.142	53.33±5.443	62.22±3.142	64.44±3.142
Emergence energy (EE)	61.70±3.558	54.40±4.918	59.60±2.298	54.61±3.527	54.78±2.901	61.87±5,727	66.16±2.348
Emergence percent (EP)	100.0±0.000	88.88±3.142	86.66±1.421	95.55±6.285	91.11±6.285	95.55±6.285	100.0±0.000
Leaf water content (LWC)	86.29±0.953	86.90±2.546	81.27±3.531	85.95±0.449	84.65±2.397	86.74±0.714	81.42±2.992
Emergence index (EI)	10.36±1.524	9.255±1.417	11.11±0.837	8.927±1.002	9.388±1.349	11.15±1.479	12.29±0.719
Germination percent (GP)	100.0±0.000	88.88±3.142	86.66±0.000	95.55±6.285	91.11±6.285	95.55±6.285	100.0±0.000
Percent moisture content (%MC)	8.333±1.065	8.566±3.189	9.133±4.902	9.333±2.104	9.033±2.439	8.966±1.297	15.10±2.785

vigor index I was increased in Ca/Mg = 0.18 quotient in the presence of highest PEG stress level, however, Water use efficiency (WUE) and seed vigor index II was highest in Ca/Mg = 2 treatment. Germination rate index (GRI), Timson germination index (TGI) and final emergence percentage (FEP) were reduced in Ca/Mg = 2 treatment in the presence of 0.6 MPa PEG stress. In the absence of PEG, the % field capacity (% FC) also reduced. Root to shoot ratio (RSR) and water use efficiency (WUE) was lower in Ca/Mg = 0.18 quotient in the presence of 0.6 MPa PEG, while seed vigor index II was lower in the presence of 0.2 MPa PEG (Table 2).

Ca/Mg = 0.18 quotient at lowest PEG stress level (0.2 MPa) showed highest Mean emergence time (MET), emergence energy (EE), germination energy (GE), germination percentage (GP), emergence percentage

(EP), emergence index (EI), and percent moisture content (%MC), while leaf water content (LWC) was lowest in this treatment. Mean emergence time (MET), emergence energy (EE), and emergence index (EI) was lowest in Ca/Mg = 2 treatment at 0.6 MPa PEG. However, emergence percentage (EP), water content (WC) and germination percentage (GP) were decreased in Ca/Mg = 4 treatment in the presence of low-stress level (0.2 MPa) as given in Table 2. In nutritional solution, different Ca/Mg quotients had a considerably favourable influence on relative growth rate (RGR), absolute growth rate (AGRW), net assimilation rate (NAR), crop growth rate (CGR) leaf area index (LAI), and germination rate index at (<0.001***) neglect the adverse effect of PEG, while PEG adversely effected leaf area ratio (LAR) at (<0.002***) (Table S1). PEG induced drought stress showed significantly positive effect of Ca/Mg quotient on germination energy (GE), emergence energy (EE) and germination percentage (GP), Timson germination index (TGI), root to shoot ratio (RSR), in the presence of, however, plant highest stress index (PHSI) and leaf water content (LWC) were adversely affected by PEG at (<0.01** & <0.05*) level (Table S2).

Germination rate index (GRI), and Timson germination index (TGI), relative growth rate (RGR), absolute growth rate (AGRW), net assimilation rate (NAR), had a substantial positive association, while there was significant negative correlation between leaf area ratio (LAR), mean germination time (MGT) and root to shoot ratio (RSR). There was negative correlation between water use efficiency (WUE), leaf area index (LAI) and absolute growth rate (AGRH) (Table S3). Table S4 indicated positive correlation between percent field capacity (%FC), germination percentage (GP) and between % moisture content (%MC), however, there was negative correlation between germination energy (GE), mean emergence time (MET), final emergence percentage (FEP), emergence percentage (EP), between leaf water content (LWC), energy index (EI), and seed vigor index I, seed vigor index II. Results listed in (Table S5; Figures 3 and 4) PC_1 explained 50.529% of the total variation and was substantially correlated, according to principal component analysis, these analysis were on the basis of 12 stated traits germination rate, mean germination time, Thimson germination index, crop growth rate, absolute growth rate (weight), relative growth rate, and shoot root ratio. At the same time with regard to water use efficiency PC_2 is considered to 17.157%. Though PC_3 is accounted 11.069% of the total variance that includes leaf area index, PC_4 is considered as 9.017% of total difference along with absolute growth rate. Essential constituents was conditioned on twelve certain traits. PC_4 first elucidated that 39.731% of total difference was notably associated with emergence time, mean emergence time, germination energy and energy index. Accompanied by PC_1, PC_2 examined 28.024% of total variation addition to final emergence %, germination %, emergence %, seed vigor index I and seed vigor index II. PC3 accounted 11.352% to its total difference together with plant height stress index, percent field capacity and leaf water content (Table S6).

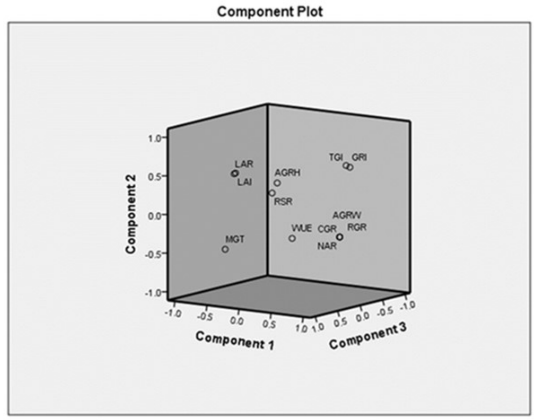

Figure 3. Principle component analysis based on correlation matrix of measured traits under various levels of PEG drought stress.

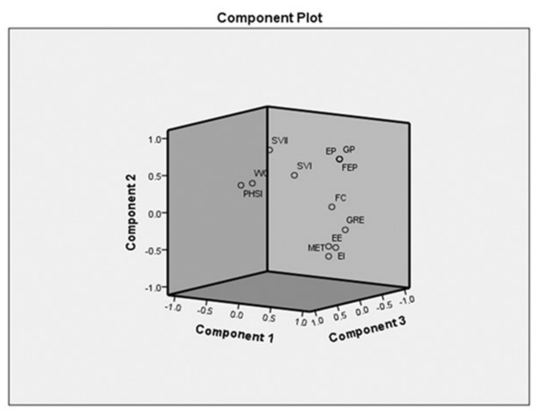

Figure 4. Principle component analysis based on correlation matrix of measured traits under various levels of PEG drought stress.

4. Discussion

Sustainable agriculture needs crop yield and quality while ameliorating abiotic constraints (Amna et al., 2021; Mehmood et al., 2021; Zainab et al., 2021; Ali et al., 2022a). Nowadays, food security is a critical issue owing to the increasing population (Fróna et al., 2019; Ali et al., 2022b). Keeping food security and climatic changes in mind, the current work aims to investigate the combined effects of different Ca/Mg quotients and osmotic stress generated by polyethylene glycol (PEG) of 0.6 MPa and 0.2 MPa on *Avena sativa* L. growth and element uptake. The present findings revealed a decrease in mean emergence time and Timson germination index as a result of drought severity is enhanced with the increase in PEG, although Piwowarczyk et al. (2014) studied that three variable concentrations of PEG 6000 (like 50, 100, and 150 g/L in MB media), increase multiple levels of water stress. Enhanced PEG level (0.6 Mpa) in Ca/Mg = quotient 4, cause increase in leaf area index, whereas LAI reduced at reduced level of PEG (0.2Mpa) in Ca/Mg= 0.18 quotient recommended that Ca/MG quotient nullifies PEG influence of leaf area index. Thus, both leaf area ratio and leaf area index were severely affected by PEG induced osmotic stress. While Hossain et al. (2010) reported in their study that during drought treatment LAI is reduced. In the present research work absolute growth rate, relative growth rate, and net assimilation rate were decreased under drought stress as reported by Mukhtar (2016). Highest and lowest root to shoot ratio was accounted at 0.6 MPa PEG, based on the Ca/Mg quotient, i.e. maximal at 4%. In contrast, root-shoot ratio was promoted by the low value at 0.18 fraction clarifies that high Ca/Mg proportion. Our present study is in agreement with that of Zeid and Shedeed (2006), wherever hypocotyl height, germination capacity, and root/shoot fresh and dry weight were decreased by polyethylene glycol (PEG)- caused water stress in alfalfa plant (*Medicago sativa*). In contrast, root length was increased. The recent study concluded that *Avena sativa* L. root length of was not considerably influenced via PEG; similarly in Alghabari and Ihsan (2018) estimated that water stress decreased freshness and dry root biomass accumulation over time, whereas root length increased.

The germination index, root length, shoot length, germination length, and seed vigor have all been dramatically lowered as a result of the water scarcity (Khodarahmpour, 2011). Root parameters including dry root mass and fresh root weight reduced under drought stress treatments caused by polyethylene glycol (PEG) at 0.6Mpa also at 0.2 Mpa. However, other root specification were increased through magnesium sulphate, magnesium nitrate, and calcium nitrate. Likewise Junjittakarn et al. (2014) reported that the root parameters including root length, weight, level, volume and diameter were noticeably decreased because of drought, on the other hand Chegah et al. (2013) mentioned that the bulk dry and fresh weight of roots and shoots were observed in control (100% FC). The longest and lowest shoot lengths were seen in the current experiment at increased levels of water stress caused by PEG. Moreover, increase in plant height was because of the applied plant nutrients together with $MgSO_4$, $Ca(NO_3)_2$, and $Mg(NO_3)_2$ correlates with results of Pervez et al. (2009) that noticed plant height was considerably affected under water stress. Gharmakher et al. (2017) reported that increase in drought levels cause decrease in seedling growth and seed germination. In our study, we recoded lowest germination index at 0.6 Mpa PEG, while at low levels, germination index raised due to the existence of Ca/Mg proportion, and seed vigor index reduced with drought stress stimulated by PEG which were similar to the results of Batool et al. (2014) who illustrated significant reduction in the, the seed vigor index germination index, the dry and fresh weight of the seedling. Water limitations effects the shoot lengths, and seedling but indicate a minor increase in root length in accordance to stress.

The current study explained that so that in shoot, higher level of magnesium was set at high and low Ca/Mg percent, i.e., 2 and 4 fraction at 0.6 and 0.2MPa PEG, suggesting that an increase Ca proportion, the level of Magnesium is marked up. However, lower concentration of Mg was noticed in Ca/Mg = 0.18 proportion at 0.6MPa signifying that minimum Ca concentration in the solution decreased the level of Mg in shoot. Lower concentration of calcium was reported in Ca/Mg 4 percent at 0.6 Mpa in shoot, although high concentration of calcium was present in Ca/Mg 0.18 percent at 0.2 MPa. As reported by Grant and Racz (1987) increased Mg concentration ameliorated mg uptake in plant as well as its concentration in shoots. With the increase in Ca concentration in the solution, the magnesium content in the stem declined while the magnesium absorption in the root still got higher. It is noticed that Ca interrupted Mg translocation which is evidenced by a decreased concentration of Mg in stem and enhanced concentration of Mg in roots. It is indicated that magnesium and calcium solution can enhance Ca concentration in root whereas higher level of Ca in shoot were 0.18% at 0.2MPs.

Reports by Salehi-Eskandari et al. (2018) suggested that the decrease in Ca concentration was observed in roots rather than an increase in Ca concentration correlated to average Mg/Ca proportion. Whereas in Mg/Ca extract, enhanced Mg concentration in shoot and root translocation was interlinked with inhibited root growth of PEG treated plants, but there is no considerable effect was observed in Ca migration. However the expectation were validated by Goodwin-Bailey et al. (1992) was that in this course of application root deficiency obligatory grown-up via Mg deficiency. Whereas it could not be neglected that root function and its growth is at once damaged via toxins that can decrease Mg- containing nutrients concentration. Highest concentration of MG in root was noticed in Mg/Ca = 2 and 0.18% at 0.2MPa and 0.5MPa individually implying that Mg level in roots was enhanced with increased Mg in solution.

Increased Ca content in nutritional solution, on the other hand, was observed at control and Ca/Mg= 4% at 0.6 MPa, implying that increased Ca content in nutritional solution inhibited Ca accumulation in root. As described by Ghasemi et al. (2015b) cell death and membrane leakage in root is caused by increased Ca/Mg concentration in Nutritional Solution Inflammation. Although, 10mm concentration of Ca show much effect as compared to that of 3mm Ca concentration. In any case, death is visible at extremely low Ca concentrations (2 mM) when plants are also exposed to other stressors, such as the increased ammonium concentration in nutrients (Ghasemi et al., 2015a). An increase in Ca concentration in a nutrient solution leads to the increase in root Ca concentration which is evidenced by increased root Ca amount for Mg/Ca= 2% at 0.2 MPa. Simultaneously it is noticed that enhanced Mg concentration in nutrient solution can cause decrease in Ca concentration in roots. Therefore Mg migrating factors were measured in Mg/Ca% accompanied by the nutrient solution which contain high amount of Ca, as a consequence in high and low Ca concentration in nutrient solution Mg translocation factor were found to be decreased. It can be illustrated that low Ca concentration nutrient solution was applied to the plant contain high amount of Ca translocation factor whereas higher content of Ca nutrient solution was applied to lower Ca translocation factors.

5. Conclusion

In conclusion, we looked at the impacts of high Ca/Mg quotients and PEG-induced osmotic stress separately and in combination; PEG had adverse effects on oat's seedling, vegetative and physiochemical traits. The reduction in growth characters of oat by application of PEG and augmentation by application of Mg and Ca nutrients indicated that above mentioned minerals cause increase in oat plant growth which as a result mitigate PEG.

Acknowledgements

The authors would like to express their gratitude to the Deanship of Scientific Research at King Khalid University for funding this work through Research Group Project under grant number (R.G.P 1- 100/43). Also, the authors extend their appreciation to the Deanship of Scientific Research at Umm Al-Qura University for supporting this work by Grant Code: (22UQU4320609DSR03).

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Supplementary Material

Supplementary material accompanies this paper.

Table S1. Analysis of variance of measured traits treated with nutrients under various levels of PEG simulated drought stress of 0.6 and 0.2 MPa.

Table S2. Analysis of variance of measured traits treated with nutrients under various levels of PEG simulated drought stress of 0.6 MPa and 0.2 MPa.

Table S3. Pearson correlation coefficients between measured traits.

Table S4. Pearson correlation coefficients between measured traits.

Table S5. Eigen values, variation explained (%), cumulative variance (%), coefficients of determination of first four principle component based on correlation matrix of measured traits under various levels of PEG drought stress of 0.6 MPa and 0.2 MPa.

Table S6. Eigen values, variation explained (%), cumulative variance (%), coefficients of determination of first three principle component based on correlation matrix of measured traits under various levels of PEG drought stress of 0.6 MPa and 0.2 MPa.

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