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Differential Interactive Effects of Ca/Mg Quotients on Agro-Physiological Conditions in Prolin Variety of Sorghum Bicolor L. by Polyethylene Glycol Induced Drought Stress

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Drought is one of the factors that inversely affect the growth of plants. The present study aimed to determine the differential interactive effects of Ca/Mg quotient under PEG-induced drought in the Prolin variety of *Sorghum bicolor* L. at the vegetative stage. *Sorghum bicolor* L. variety Proline was obtained from Persabaq Nowshera, and was sown in earthen pots (lower inside diameter, 18cm upper inner diameter, 20 cm height and 2 cm thickness) filled with 2kg of air-dried soil and silt (2:1) having pH, moisture content and field capacity in triplicates in the greenhouse, provide with Ca/Mg solution properly. The designed experiment contained seven treatments each having three replicates, among these treatments first three were controlled, and the other six were treated with Ca/Mg quotient 4+PEG(0.6 MPa), Ca/Mg quotient 4+PEG(0.2 MPa), Ca/Mg quotient 2+PEG(0.6 MPa), Ca/Mg quotient 2+PEG(0.2 MPa), Ca/Mg quotient 0.18+PEG(0.6 MPa), and Ca/Mg quotient 0.18+PEG(0.2 MPa) respectively with three replicates. After 4 weeks of growth plants were then given the PEG solutions in different concentrations. It was observed in the results that in normal conditions (Control set) the selected plant gave good results however in the treatments the PEG-induced drought affected the plant growth there was a reduction in the agronomy i.e. leaf area, leaf fresh and dry weight, and the similar reduction also occurred with all other (root shoot) vegetative parts which gave minimum results as compared to the control and more reduction occur as to increase the PEG amount (0.6MPa) while in all treatment presence of Ca/Mg quotient reduce the effect of PEG by giving the plant nutritive strength. The biochemical characteristics like chlorophyll, sugar, and protein are also reduced in PEG treatment. Finally, it was concluded that the presence of Ca/Mg quotients combats the induced drought stress by PEG in the protein variety of *Sorghum bicolor* L.

Keywords: Calcium, Chlorophyll, Magnesium, Protein, Sorghum Bicolor L.

Introduction:

Sorghum bicolor and its various varieties, belonging to the Poaceae family, are widely used as a staple food and harvested for multiple purposes [1]. Over time, numerous genotypes and hybrids have been developed to address food security challenges. Sweet sorghum, originating from America and Europe, yields the best production [2]. It is considered to be well suited for hot and dry countries like Nigeria India, or Mexico [3]. As a highly efficient energy plant, sweet sorghum is increasingly chosen as a bioenergy crop [4] [5] [6].

Various environmental conditions such as drought can impact the growth and development of plants, acting as limiting factors [7] [8] [9]. The dry season or drought mainly depends on several factors including rainfall, rate of evaporation, and soil moisture [10]. Sorghum is a potential substitute for liveliness [1][11], which requires regular water supply. Disruption in water supply and its ultimate shortage can have detrimental effects on the regular production of plants contributing to the rising temperature of the earth [12]. This situation of water is extremely imperious for the development of proper plant crops [13][14][15][16], leading to many physio-biochemical changes [6][17]. Water scarcity can lead to activation of Responsive Oxygen Spp. Subsequently causing lipid peroxidation in plants [17], protein degradation or sleazing [18], and eroded nucleic drawbacks [19]. The various sorghum cultivars and varieties require soil pungency [20]. Dry spell genotypes have high epi-cuticle waxy layers over leaves to combat transpiration and infrared radiations and enable plants to withstand harsh conditions [21]. Twisting or serpentine soils impose more restrictions on plant growth and development due to their low levels of Ca/Mg nutrients, lessening the water holding capacity and reducing natural matter substance [22].

The presence of sufficient amounts of Ca/Mg nutrients in the soil helps plants maintain their life vitality under drought conditions [23]. In the present study, we selected the proline variety of sorghum to grow under polyethylene glycol drought stress conditions while properly providing the Ca./Mg nutrients continuously.

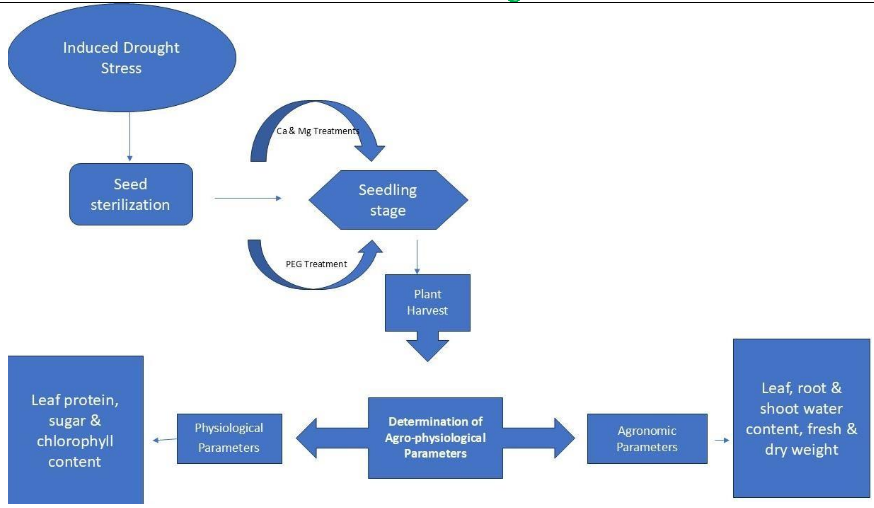
Objectives of the Study:

In the present study cereal crop Sorghum bicolor variety Prolin was selected to test against PEG-induced drought stress. The plant is provided with Ca and Mg nutrients to check the intensity of drought in the agronomic and physiologic characteristics of the crop. There is little literature available about the PEG-induced drought stress in Sorghum bicolor.

Materials and Method:

Plant Material and Growing Conditions:

The practical experiment was conducted in greenhouse conditions using seeds of the protein variety sorghum bicolor, which was obtained from peersabaq turned Peshawar. The seeds were sown in earthen pots with 20cm height with 18cm overall diameter, soil and sand ratio was 2:1. A Coplete randomize design (CRD) was used for this experiment. Plant pots were protected from intense sunlight, warm wind, and rainfall. A total of six treatments were applied, with each treatment replicated three times. Throughout the experiment, no pest or disease issues were observed and the plant pots were watered as needed. Moreover, the experiment ran for eight weeks. Seeds were sterilized using Clorox(10%) and ethanol (90%) for 5mins. Ca/Mg quotients: for the effective growth of plants, three types of Ca/Mg quotients 4, 2, and 0.18 were prepared.



Flow diagram of a methodology

Preparation of Ca/Mg Quotient:

- During experimental work, the Ca/Mg quotient was prepared. The Ca/Mg quotients are three types, 4, 2, and 0.18 for effective results.
- For 4 quotients we took 90 mg of $MgSO_4$ and 429 mg of $Ca(NO_3)_2$ dissolved in 1000 ml of water and prepared a solution.
- For 2 quotients we took 90 mg of $MgSO_4$ and 246 mg of $Ca(NO_3)_2$ dissolved in 1000 ml of water and prepared a solution.
- For the 0.18 quotient, we took 90 mg of MgSO4, 62 mg of Ca(NO3)2, and 260 mg Mg(NO3)2 dissolved in 1000ml of water and prepared a solution.
- 2 weeks After Germination Ca/Mg quotient was given to the plants and then 1 week after giving Ca/Mg quotient was given to PEG stress and then after 3 days, the plants were harvested.

Preparation of PEG:

26 mg PEG was dissolved in 1000 ml of distal water which made a potential 0.6 MPa and 8.6 mg PEG was dissolved in 1000 ml of distal water which made a potential 0.2 MPa.

Table 1. Treatments Detail.

No.	**Treatment Detail**	**No.**	**Treatment Detail**
T1	Control	T5	2 quotient +PEG0.2 MPa
T2	4 quotient +PEG0.6 MPa	T6	0.18quotient+PEG0.6 MPa
T3	4 quotient +PEG0.2 MPa	T7	0.18quotient+PEG0.2 MPa
T4	2 quotient +PEG0.6 MPa		

Agronomic Parameters to Study:

For agronomic characteristics, the plant was harvested and various parameters such as fresh weight of leaves, shoot and root, shoot and root dry weight were measured. Plants were dried after being weighed fresh, and then the dry weight of leaves, roots, and shoots was calculated. The relative water content of all three parts was determined using the method of (Garcıa-Mata and Lamattina, 2001). By using the following formula and computing the RWC, RWC (%) = (FW-DW) / (TW-DW) * 100

Physiological Parameters to Study:

Leaf Protein Content:

Protein content was determined using the fresh plant leaves which were crushed and added with a phosphate buffer solution of pH 7.5. The crushed sample was then centrifuged and absorbance of supernants was recorded after 30 mins of incubation at 650nm by spectrophotometer. Bovine serum albumin (BSA) was used as a standard to determine the unknown protein content.

Sugar Estimation:

Fresh leaves were crushed in distilled water using a pestle and mortar, and the mixture was centrifuged for 5 minutes. To 0.1 mL of the supernatant, 1 mL of phenol was added, followed by the addition of sulfuric acid. The absorbance was then measured at 420 nm. Glucose was used as the standard for determining the concentration [24].

Chlorophyll Content of Leaves:

Fresh leaves were mixed with 4ml of acetone and centrifuged for 5 min at 2000rpm. Supernant was collected and used for chlorophyll determination. The absorbance of 'chlorophyll a was measured at 645nm and 'chlorophyll b' at 663nm using a spectrophotometer [25].

The following formula was used to measure the total chlorophyll content [26].

Total chlorophyll (mg/g) = (20.2 x A645) + (8.02 x B663)

Results:

Effects of Ca/Mg Quotient on Agronomical Parameters of Sorghum Bicolor Variety Prolin Nder Peg Induced Drought Stress

The results indicated that the leaf fresh weight in T1 (control) was the highest compared to the treated sets. Minimum leaf fresh weight was recorded in the T2 set (Ca/Mg quotient 4 + PEG 0.6MPa). It indicated that the negative effect of PEG negative effects was reduced by the Ca/Mg quotient in other treatments which showed moderate effects. The highest amplitude for leaf dry weight was recorded in the T1 set and the lowest was in PEG-induced stress sets (T5). However, the moisture content of the Leaf was highest in the T3 set and lowest in the T4 set. Results are presented in Table 2.

Table 2. Effect proline variety of *Sorghum bicolor* L.

Treatment	Leaf fresh weight	Leaf dry weight	Relative water content
T1	1.45 ± 0.12^{a}	0.51 ± 0.03^{a}	0.52 ± 0.10^{a}
T2	0.69 ± 0.09^{ab}	0.34 ± 0.09^{ab}	0.36 ± 0.13^{a}
T3	1.01 ± 0.47^{b}	0.26 ± 0.15^{b}	0.63 ± 0.42^{a}
T4	0.89 ± 0.20^{b}	0.22 ± 0.04^{b}	0.36 ± 0.05^{a}
T5	0.71 ± 0.09^{b}	0.21 ± 0.02^{b}	0.44 ± 0.11^{a}
T6	0.90 ± 0.36^{b}	0.27 ± 0.18^{b}	0.40 ± 0.20^{a}
T7	1.21 ± 0.47^{ab}	0.34 ± 0.10^{ab}	0.45 ± 0.11^{a}

The results highlighted that maximum root fresh weight in a variety of Proline ($P>0.05$) was reported in T1 (control), while minimum root fresh weight was observed in T4 (Ca/Mg quotient 2+PEG 0.6 MPa). Whereas; the highest amplitude for root dry weight has been reported in T1 (control), while the lowest amplitude was reported in Prolin in T5 (Ca/Mg quotient 2+PEG 0.2 MPa). Additionally, the highest root moisture content was observed in

T7 (Ca/Mg quotient 0.18 + PEG 0.2 MPa), while the least root moisture content was noted in T4 (Ca/Mg quotient 2 + PEG 0.6 MPa), as shown in Table 3.

Table 3. Effect proline variety of *Sorghum bicolor* L.

Treatment	Root fresh weight	Root dry weight	Relative water content
T1	0.95±0.15[a]	0.53±0.09[a]	0.24±0.05[a]
T2	0.36±0.06[b]	0.33±0.09[ab]	0.30±0.49[a]
T3	0.30±0.20[b]	0.17±0.24[b]	0.22±0.24[a]
T4	0.40±0.18[b]	0.26±0.09[ab]	0.10±0.04[a]
T5	0.23±0.05[b]	0.16±0.12[b]	0.23±0.10[a]
T6	0.53±0.45[b]	0.32±0.06[ab]	0.18±0.07[a]
T7	0.47±0.26[b]	0.41±0.24[ab]	0.48±0.39[a]

The results showed that maximum shoot fresh weight at P>0.05 was reported in T1 (control) along with minimum shoot fresh weight in T2 (Ca/Mg quotient 4+PEG 0.6 MPa). However, the highest amplitude for shoot dry weight has been reported in T1(control) along with the lowest amplitude reported in T2 (Ca/Mg quotient 4+PEG 0.6 MPa).

The highest root moisture content was observed in T2 (Ca/Mg quotient 4 + PEG 0.6 MPa), while the lowest root moisture content was recorded in T1 (control), as shown in Table 4.

Table 4. Effect proline variety of *Sorghum bicolor* L.

Treatment	Shoot fresh weight	Shoot dry weight	Relative water content
T1	6.06±0.12[a]	0.87±0.03[a]	7.38±0.10[b]
T2	2.97±0.09[b]	0.32±0.09[b]	11.52±0.13[a]
T3	4.45±0.47[ab]	0.61±0.15[ab]	8.71±0.42[ab]
T4	3.80±0.20[ab]	0.52±0.04[ab]	8.37±0.05[ab]
T5	4.48±0.09[ab]	0.61±0.02[ab]	7.83±0.11[ab]
T6	4.46±0.36[ab]	0.53±0.18[ab]	9.49±0.20[ab]
T7	5.21±0.47[ab]	0.66±0.10[ab]	8.53±0.11[ab]

Effect of Ca/Mg quotient on the physiological characteristic of protein variety of *Sorghum bicolor* L. under simulated stress.

Effect on Chlorophyll "a" Content (µmg/g) of Proline Variety of *Sorghum Bicolor* L:

Figure 1 shows that the maximum chlorophyll "a" content was reported at P>0.05 in quotient 2 under drought stress 0.2 MPa. Whereas; minimum chlorophyll "a" content was reported at P>0.05 at quotient 0.18 under drought stress 0.6 MPa.

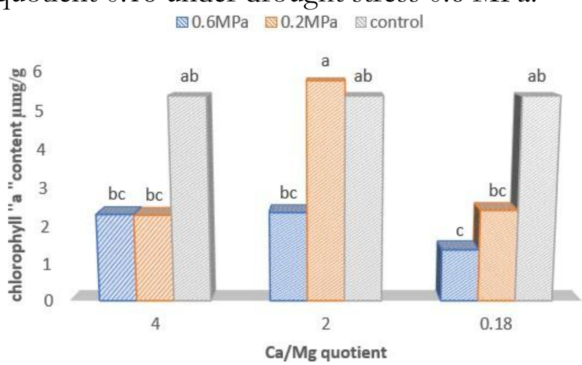


Figure 1. Effect on chlorophyll "a" content (µmg/g) of protein variety of *Sorghum bicolor* L.

Effect on Chlorophyll "b" Content (µmg/g) of Protein Variety of *Sorghum Bicolor* L:

Figure 2 clearly shows that the maximum chlorophyll "b" content was observed in the control group, with statistical significance at P>0.05. Whereas; minimum chlorophyll "b" content was reported at P>0.05 in quotient 0.18 under drought stress 0.6 MPa.

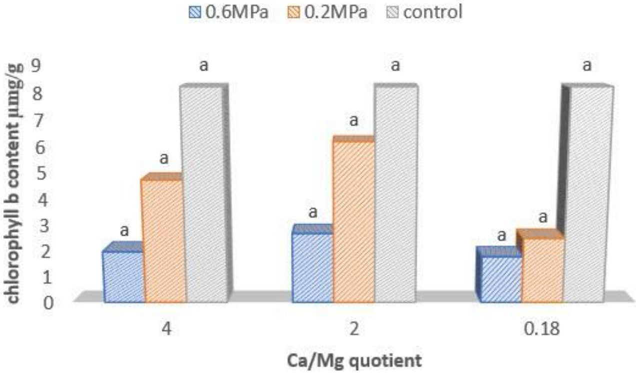


Figure 2. Effect on chlorophyll "b" content (µmg/g) of protein variety of *Sorghum bicolor* **L.**

Effect on carotenoid content (µmg/g) of protein variety of *Sorghum bicolor* L:

As shown in Figure 3, the highest carotenoid content was recorded in the control group, with a significant difference at P<0.01. The minimum carotenoid content showed a significant difference of P<0.01 in quotient 0.18 under drought stress 0.6 MPa.

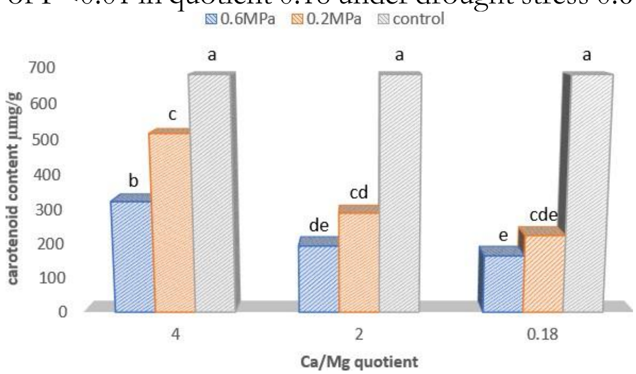


Figure 3. Effect on carotenoid content (µmg/g) of protein variety of *Sorghum bicolor* L.

Effect on Sugar Content (µmg/g) of Protein Variety of *Sorghum Bicolor* L:

Figure 4 indicated that the maximum sugar content was reported nearer to P <0.05 in the control. The lowest sugar content was observed in the Ca/Mg quotient 4 under drought stress (0.6 MPa), with a significant difference at P<0.05.

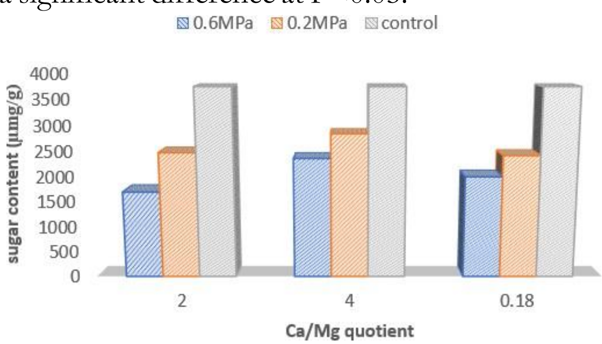


Figure 4. Effect on sugar content (µmg/g) of protein variety of *Sorghum bicolor* **L.**

Effect on protein content (mg/g) of protein variety of *Sorghum bicolor* L.

Figure 5 illustrates that the maximum protein content was reported at a significant difference of <0.05 in the control. However, the minimum protein content had a significant difference of $P<0.05$ in quotient 4 under drought stress 0.6 MPa.

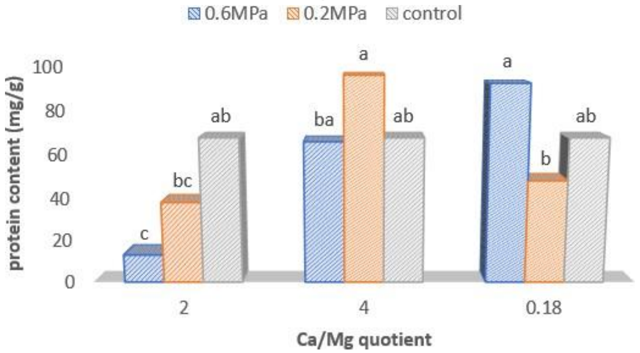


Figure 5. Effect on protein content (mg/g) of protein variety of *Sorghum bicolor* L.

Discussion:

In addition to various abiotic factors that stress the sorghum plant, certain nutrient deficiencies can also act as stressors. For instance, insufficient calcium and magnesium levels can reduce the plant's vigor and its ability to withstand drought conditions. Drought is a significant challenge that affects plant growth seriously. There are two primary types of drought including terminal and intermittent drought. In case of terminal drought, soil moisture decreases toward the end of the season, while intermittent is based on varying intervals of rainfall. A current study revealed that PEG-induced drought reduces fresh and dry weight, moisture content, root shoot ratio, and leaf area. While plant having Ca/Mg nutrients have sufficient fresh and dry weight, root shoot ratio, and moisture content because they provide support for plants to withstand the prevailing drought conditions of the PEG. The results of this study align with the research of Tourian et al., [27] and many others.

Agronomic Parameters:

This research illustrated that shoot length, fresh weight, and dry weight of the Prolin variety of Sorghum bicolor remarkably reduced due to drought stress at the vegetative stage. The results are consistent with the findings of Thakur and Rai [28], who reported that drought stress leads to a reduction in shoot length and fresh weight in the crops studied. The current study also demonstrated that increasing levels of drought stress result in decreased root fresh and dry weight, supporting similar observations made by Krouma [29]. While some researchers reported that due to water scarcity root length increases [30]. This might be in the case of arid or xeric plants.

The current study showed that high quotients of Ca/Mg produce high shoot fresh and dry weight and reduced root fresh and dry weight. Reducing the Ca/Mg quotient while increasing the PEG concentration caused a slight decrease in shoot weight and a slight increase in root weight. At this stage, our study is in agreement with Salehi-Eskandari et al., [31]. PEG-induced osmotic stress showed undesirable growth of root and shoot, while its effect was more noticeable at high concentrations.

However, it becomes moderate with high ratios of CA/Mg. Similar results were presented by Salehi, et al., [10].

Physiological Parameters:

As the drought affects the growth and development of the Prolin variety of Sorghum bicolor, so this morphological hindrance is the indication of physiological stress of the plant where harmful activity was initiated and causes growth in stress. The present experiment demonstrated that as drought stress duration increased during the vegetative stage, there was a noticeable decline in total chlorophyll content. Both chlorophyll a and b were negatively affected, indicating that prolonged drought stress significantly impairs the photosynthetic capacity of the plant. This decrease in the chlorophyll content occurs due to the inhibition of the biosynthesis of precursors for chlorophyll due to reduced moisture content as reported by Makhmudov [32]. Similar findings were also reported by Anjum et al., [33], highlighting that the reduction in chlorophyll is dependable.

The results of this study follow the plant physiologist who revealed that carbohydrates play a positive role in plant resistance to drought stress [34]. Similar results were reported by Jnandabrahim and Sailen [35].

Conclusion:

The present study showed that the presence of proper nutrients plays a vital role in combating any kind of stress faced by plants from the environment. Ca/Mg quotients withstand the plant with prevailing PEG-induced drought stress. High ratios of PEG reduce the vegetative growth but high quotients of Ca/Mg reduce its effect in the treatment sets.

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