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Effects of Polyethylene Glycol (PEG) Simulated Drought Stress on Physio-Agronomic Characteristics in Myhco Variety of Sorghum Bicolor L

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The present study was aimed at determining the differential interactive effects of Ca/Mg quotient and PEG-simulated drought in *Sorghum bicolor* at the vegetative stage. *Sorghum bicolor* collected variety Myhco from Persabaq Nowshera were sown in earthen pots (lower inside diameter, 18cm upper inner diameter, 20 cm height and 2 cm thickness) filled with 2 kg of air-dried soil and silt (2:1) having pH, moisture content and field capacity in triplicates in the green house of the Department of Botany, University of Peshawar in 2019. The designed experiment contains seven treatments each having three replicates, among these treatments first three are control, the second three are treated Ca/Mg quotient 4+PEG0.6 Ca/Mg quotient 4+PEG0.2, Ca/Mg quotient 2+PEG0.6, Ca/Mg quotient 2+PEG0.2, Ca/Mg quotient 0.18+PEG0.6 Ca/Mg quotient 0.18+PEG0.2, while the last three treatments are treated Ca/Mg quotient 0.18+PEG0.2. Conclusions We conclude that there is a reduction in the agronomy, i.e., leaf area, leaf fresh and dry weight, and a similar reduction also occurred with all other vegetative parts. There is a clear difference between control and PEG drought, and a greater reduction is observed in 0.6 MPa drought. The biochemical characters were also affected in the same manner; a clear reduction was observed in chlorophyll, sugar, and protein, and occurred while the Ca/Mg quotient had no significant effect on *Sorghum bicolor* L.in Varity myco.

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

Introduction:

Sorghum bicolor and its varieties, belonging to the family Poaceae, are cultivated as staple foods and harvested for multiple purposes[1]. Over time, many genotypes and hybrids have been developed to address food security challenges. Among them, sweet sorghum varieties of American and European origin demonstrate the highest productivity[2]. Sweet sorghum is particularly well-suited for hot and arid regions such as Nigeria, India, and Mexico[3]. It is also recognized as an excellent energy plant and is widely cultivated as a bioenergy crop[4],[5],[6]. Various environmental conditions influence the growth and development of plants, often acting as limiting factors, with drought being one of the most significant[7],[8],[9]. Drought or dry seasons are largely determined by rainfall distribution, evaporation intensity, and soil moisture levels[10]. Under such circumstances, sorghum serves as a resilient crop and a reliable alternative for ensuring food security[1][11]. Plants also require a consistent water supply, and any disruption or shortage can severely affect their regular productivity as well as contribute to rising global temperatures[12]. This situation of water is extremely imperative for the development of proper plant crops[13],[14],[15]. Due to such situations, plants undergo many physio-biochemical changes[6],[16].

These stress conditions trigger the production of reactive oxygen species (ROS), which in turn lead to lipid peroxidation in plants[16], protein degradation[17], and damage to nucleic acids[18]. Different sorghum cultivars and varieties exhibit specific soil requirements[19]. Drought-tolerant genotypes typically possess thicker epicuticular wax layers on their leaves, which reduce excessive transpiration, reflect infrared radiation, and enhance plant survival under stress conditions[20]. Twisting or serpentine soils have more restrictions on plant growth and development as these have low levels of Ca/Mg nutrients, leading to low water holding capacity and reduced natural matter substance[21]. The presence of sufficient amounts of Ca/Mg nutrients in the soil helps plants to maintain their life under drought conditions[22]. This study focused on the Myhco variety of sorghum, cultivated under polyethylene glycol (PEG)-induced drought stress, while ensuring a continuous supply of calcium and magnesium nutrients to withstand the induced stress. 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 Mycho variety of sorghum to be cultivated in induced polyethylene glycol (PEG) drought stress conditions with continuous supply of Ca./Mg nutrients. Objectives of the Study:

In the present experiment, Sorghum bicolor variety Mycho was tested for induced-PEG drought stress. Ca and Mg nutrients were supplied to check the drought effect on the Physio-agronomic parameters of the plant. PEG-induced drought stress in the Sorghum bicolor variety Mycho was carried out for the first time.

Materials and Methods:

Plant material and growing conditions:

The experiment was carried out under greenhouse conditions. Seeds of the Myhco variety of Sorghum bicolor, obtained from Persabaq, Nowshera, were sown in earthen pots measuring 20 cm in height and 18 cm in diameter. A soil-to-sand ratio of 2:1 was used for pot filling. The experiment was arranged in a Completely Randomized Design (CRD). Pots were maintained under controlled conditions, protected from direct sunlight, warm winds, and rainfall. A total of six treatments were applied; each replicated three times. No pest or disease incidence was observed during the experiment. Pots were watered as needed, and the study was conducted over a period of eight weeks. Seeds were surface-sterilized with 10% Clorox and 90% ethanol for 5 minutes. For effective plant growth, three Ca/Mg quotients (4, 2, and 0.18) were prepared. The treatment details are as follows: The Ca/Mg ratio was prepared for the experiment in three different forms, with effective values of 4, 2, and 0.18. We prepared a solution using 1000ml of water, 90mg of $MgSO_4$, and 429mg of $Ca(NO_3)_2$. To create a solution for the 0.18 ratio, we dissolved 90 mg of MgSO4, 62 mg of Ca (NO3)2, and 260 mg of Mg (NO3)2 in 1000 ml of water. After the plants had received the Ca/Mg ratio for two weeks and one week of PEG stress, the plants were then ready for harvesting after three days. Plants were exposed to the PEG-induced osmotic stress (along with different Ca/Mg treatments) for 8 days in the experiment, with the solutions being renewed every 4 days.

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

Table 1. Treatment Details.

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:

The following parameters were determined using the formulas below.

Relative Water Content:

RWC (%) = [(FW – DW) / (TW – DW)] × 100.

FW: stands for the sample's fresh weight.

TW: is the sample's turgid weight, obtained after fully rehydrating it.

DW: is the sample's dry weight, determined after drying it in an oven

Root shoot ratio Root-Shoot Ratio = Dry weight of roots / Dry weight of shoots

Leaf area index =Total Leaf Area (m^2) / Ground Area (m^2

Seedling Vigor Index I: Germination (%) × Mean seedling length (cm)

Seedling Vigor Index II: Germination (%) × Mean seedling dry mass (g or mg)

Physiological Parameters to Study:

Leaf protein content:

Protein content was determined using the fresh plant leaves, which were crushed and added to a phosphate buffer solution of pH 7.5. The crushed sample was then centrifuged, and the absorbance of the supernatants was recorded after 30 minutes of incubation at 650nm using a 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.

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. Supernatant 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].

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

The data collected from the results were analyzed using one-way analysis of variance (ANOVA).

Results:

The results indicated that maximum leaf fresh weight at $P < 0.05$ was reported in T1(control). The minimum leaf fresh weight was reported in T2(Ca/Mg quotient 4+PEG 0.6 MPa). The highest amplitude for leaf dry weight has been reported in T1(control). The lowest amplitude reported in T3(Ca/Mg quotient 4+PEG 0.2 MPa. The leaf moisture content in T1(control) was the lowest in T2(Ca/Mg quotient 4+PEG 0.6 MPa) (Table 2).

Table 2. Effect of Ca/Mg quotient on agronomical characteristics of Sorghum bicolor L. under simulated stress.

treatment	Leaf fresh weight	Leaf dry weight	Leaf moisture content
T1	1.35 ± 0.66^{a}	0.46 ± 0.07^{a}	52.66 ± 48.09^{a}
T2	0.35 ± 0.15^{b}	0.16 ± 0.03^{b}	15.02 ± 11.76^{a}
T3	0.50 ± 0.49^{b}	0.13 ± 0.11^{b}	16.41 ± 17.99^{a}
T4	1.08 ± 0.39^{ab}	0.18 ± 0.10^{b}	45.74 ± 10.70^{a}
T5	0.76 ± 0.30^{ab}	0.17 ± 0.09^{b}	44.99 ± 24.59^{a}
T6	1.12 ± 0.67^{ab}	0.19 ± 0.09^{b}	45.22 ± 15.30^{a}
T7	0.81 ± 0.31^{ab}	0.26 ± 0.11^{b}	32.79 ± 15.86^{a}

The results showed that the maximum root fresh weight ($P < 0.05$) was recorded in T1 (control). The minimum was observed in T5 (Ca/Mg quotient 2 + PEG 0.2 MPa). Similarly, the highest root dry weight was also found in T1 (control), whereas the lowest was reported in T5. In contrast, root moisture content was greatest in T3 (Ca/Mg quotient 4 + PEG 0.2 MPa). The lowest was observed in T1 (control) (Table 3).

Table 3. Effect on root fresh weight, root dry weight, and root moisture content of Sorghum bicolor L.

Treatment	Root fresh weight	Root dry weight	Root moisture content
T1	0.95 ± 0.15^{a}	0.53 ± 0.09^{a}	2.08 ± 0.98^{b}
T2	0.36 ± 0.06^{b}	0.33 ± 0.09^{ab}	4.30 ± 3.07^{ab}
T3	0.30 ± 0.20^{b}	0.17 ± 0.24^{b}	10.52 ± 9.23^{a}
T4	0.40 ± 0.18^{b}	0.26 ± 0.09^{ab}	5.09 ± 2.50^{ab}
T5	0.23 ± 0.05^{b}	0.16 ± 0.12^{b}	6.58 ± 0.41^{ab}
T6	0.53 ± 0.45^{b}	0.32 ± 0.06^{ab}	4.13 ± 2.77^{ab}
T7	0.47 ± 0.26^{b}	0.41 ± 0.24^{ab}	2.85 ± 1.54^{b}

The results indicated that the maximum shoot fresh weight ($P < 0.05$) was recorded in T1 (control). The minimum was observed in T3 (Ca/Mg quotient 2 + PEG 0.6 MPa). The highest shoot dry weight was found in T7 (Ca/Mg quotient 0.18 + PEG 0.2 MPa), whereas the lowest was reported in T5 (Ca/Mg quotient 2 + PEG 0.2 MPa). Shoot moisture content was greatest in T5. The lowest was observed in T7 (Table 4).

Table 4. Effect on shoot fresh weight, shoot dry weight, and shoot moisture content of Sorghum bicolor L.

Treatment	Shoot fresh weight	Shoot dry weight	Shoot moisture content
T1	6.50 ± 0.79^{a}	0.64 ± 0.08^{ab}	6.63 ± 0.66^{a}
T2	4.20 ± 0.87^{bc}	0.37 ± 0.10^{bc}	9.18 ± 1.75^{a}
T3	1.82 ± 1.06^{d}	0.35 ± 0.25^{c}	10.16 ± 7.19^{a}
T4	3.29 ± 0.73^{bc}	0.39 ± 0.11^{bc}	10.75 ± 0.74^{a}
T5	2.74 ± 1.09^{cd}	0.34 ± 0.20^{c}	11.66 ± 3.80^{a}
T6	4.55 ± 1.38^{b}	0.64 ± 0.22^{ab}	7.51 ± 1.86^{a}
T7	4.67 ± 0.61^{b}	0.76 ± 0.12^{a}	6.06 ± 0.10^{a}

From the findings, it is clear that the maximum root shoot ratio at $P < 0.05$ was reported in T2 (Ca/Mg quotient 4+PEG 0.6 MPa). The minimum root shoot ratio in T6(Ca/Mg quotient 0.18+PEG 0.6 MPa). The highest amplitude for leaf area has been reported in T7. The lowest amplitude reported in T2(Ca/Mg quotient 2+PEG 0.2 MPa) is in the Table.

Table 5. Effect on root-shoot ratio and leaf area of Sorghum bicolor L.

Treatment	RSR	LA
T1	0.81±0.17[a]	146.17±29.05[ab]
T2	0.84±0.39[a]	66.99±18.12[c]
T3	0.48±0.08[a]	94.34±47.54[bc]
T4	0.65±0.15[a]	94.19±29.41[bc]
T5	0.56±0.21[a]	91.52±25.12[bc]
T6	0.47±0.19[a]	98.53±31.08[bc]
T7	0.54±0.19[a]	167.60±35.51[a]

Effect of Ca/Mg Quotient on the Physiological Characteristics of Sorghum bicolor L. (Var. Myhco) under Simulated Drought Stress

Effect on chlorophyll "a" content (µmg/g) of Sorghum bicolor L. (Myhco):

Figure 1 shows that the maximum chlorophyll a content ($P < 0.05$) was observed in the Ca/Mg quotient 4 under 0.6 MPa drought stress, whereas the minimum was recorded in the quotient 0.18 under 0.2 MPa drought stress.

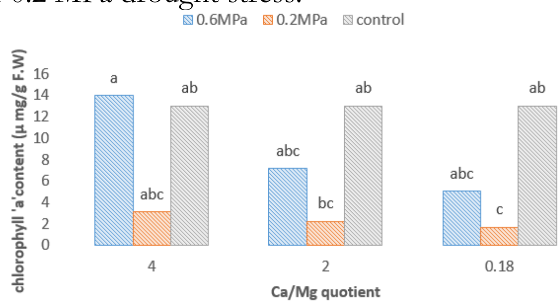

Figure 1. Effect on chlorophyll "a" content (µmg/g) of Sorghum bicolor (Myhco)

Effect on chlorophyll "b" content (µmg/g) of Sorghum bicolor L.:

From Figure 2, it's clear that the maximum chlorophyll "b" at $P < 0.05$ was reported in the control, and the minimum chlorophyll b content was reported at $P < 0.05$ in quotient 0.18 under drought stress 0.2 MPa.

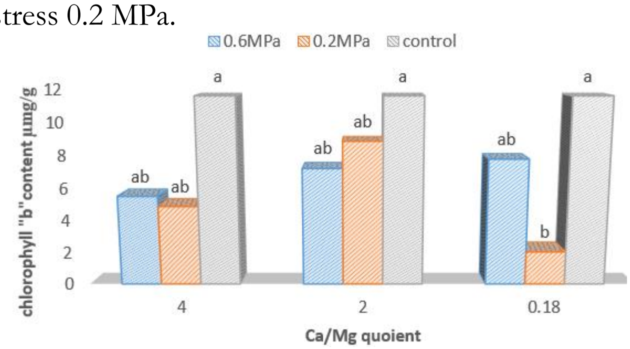

Figure 2. Effect on chlorophyll "b" content (µmg/g) of Sorghum bicolor (Myhco)

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

Figure. 3 indicated that maximum carotenoid content was reported at $P < 0.05$ in the control. Whereas, the minimum carotenoid content was reported at $P < 0.05$ at a quotient of 4 under drought stress 0.2MPa.

Effect on sugar content (µmg/g) of Sorghum bicolor L:

Figure 4 indicates that the maximum sugar content ($P < 0.05$) was recorded in the control, while the minimum was observed in Ca/Mg quotient 4 under 0.2 MPa drought stress.

Effect on protein content (mg/g) of Sorghum bicolor L:

Figure 5 showed that maximum protein content was reported at a significance of P < 0.05 in the control, and minimum protein content was reported at P < 0.05 in quotient 4 under drought stress 0.6 MPa.

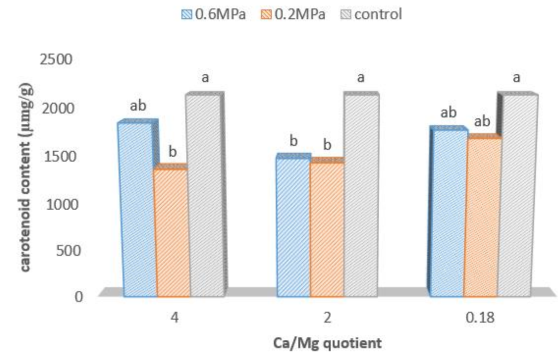

Figure 3. Effect on carotenoid content (μmg/g) of Sorghum bicolor L. (Myhco)

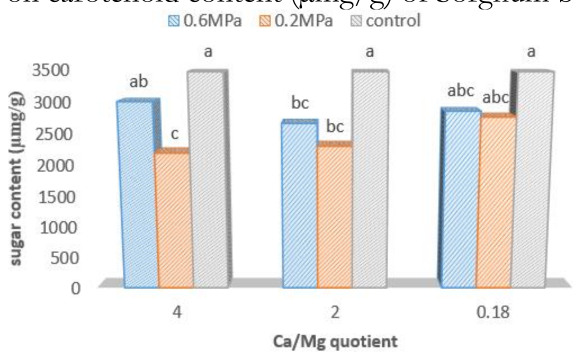

Figure 4. Effect on sugar content (μmg/g) of Sorghum bicolor (Myhco).

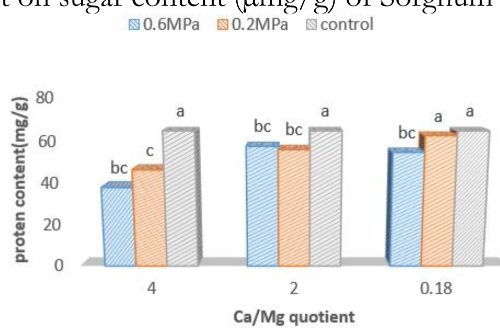

Figure 5. Effect on protein content (mg/g) of Sorghum bicolor (Myhco)

Discussion:

In addition to abiotic factors, nutrient deficiencies can also act as stress-inducing agents in sorghum. For instance, inadequate levels of calcium and magnesium reduce plant vigor and tolerance to drought. Drought is one of the major stresses that severely affects plant growth. It can be classified into two main types: terminal and intermittent. Terminal drought occurs when soil moisture declines toward the end of the growing season, whereas intermittent drought results from irregular rainfall patterns during the season. The present investigation reveals that PEG-induced drought stress reduces fresh and dry weight, moisture content, root shoot ratio, and leaf area. While plants with Ca/Mg nutrients have sufficient fresh and dry weight, root shoot ratio, and moisture content. This suggests that Ca/Mg supplementation enhances the plant's ability to withstand PEG-induced drought stress. Our findings are consistent with those of. Tourian et al. [31] and several other studies.

Agronomic Parameters:

Presently, the study showed that shoot length, fresh weight, and dry weight of the myco variety of Sorghum bicolor remarkably reduced due to drought stress at the vegetative stage. Our study went in line with Thakur and Rai [32], who found that drought stress decreases the length and fresh weight of shoots of crops studied. The study also showed that an increase in drought stress causes a reduction in root fresh and dry weight. Similar results were reported by[17]. Some researchers have reported that water scarcity can lead to an increase in root length[26], a response more commonly observed in arid or xerophytic plants. The present work showed that high quotients of Ca/Mg produce high shoot fresh and dry weight and reduced root fresh and dry weight. While reducing the Ca/Mg quotients and increasing the PEG ratio brings a slight change in the shoot low weight, roots slightly increase in weight. At this stage, our findings were in agreement with those of [25]. PEG-induced osmotic stress negatively affected both root and shoot growth, with the effects being more pronounced at higher concentrations. However, the effects became moderate at higher Ca/Mg ratios. Similar findings were also reported by [25].

Physiological Parameters:

As the drought affects the growth and development of a variety of Sorghum bicolor, this morphological hindrance is an indication of physiological stress of the plant, where harmful activity is activated and causes growth in stress. The present experiment showed that increasing the drought stress duration at the vegetative stage not only reduces the total chlorophyll content but also solely the chlorophyll a and b, both of which were negatively affected. This decrease in the chlorophyll a content occurs due to the inhibition of biosynthesis of precursors for chlorophyll a due to reduced moisture content, as reported by[27]. Similar results were reported by[3], who noted that the reduction in chlorophyll content is consistent under stress conditions. In the present experiment, protein, proline, and sugar were reduced significantly due to the induced drought by PEG and were reduced further with an increase in drought duration during the vegetative growth of the Myhco variety of the Sorghum bicolor. These results are in accordance with the plant physiologist who revealed that carbohydrates play a positive role in plant resistance to drought stress[28]. Similar results were reported by[15].

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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